REFERENCE RANGES FOR TRIAGE ROOM, BIOCHEMICAL, HEMATOLOGICAL AND TUMOUR MARKER PARAMETERS FOR TAITA-TAVETA COUNTY, KENYA

RICHARD GITIMU

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DECLARATION

I, Richard Gitimu, duly declare that this thesis is my original work and has not been presented for a degree or any other award in any other university

Signature _____Date_____

RICHARD GITIMU
Reg. No: I84/31987/2015

Supervisors

We confirm that the candidate carried out the work reported in this thesis under our supervision

Signature _____ Date_____

Prof Eliud NM Njagi
Department of Biochemistry and Biotechnology
Kenyatta University
Po Box 43844-00100
Nairobi

Signature_____ Date_____

Prof Joseph K Gikunju
Department of Medical Laboratory Sciences
Jomo Kenyatta University of Agriculture and Technology
Po Box 62000-00100
Nairobi

Signature_____ Date_____

Dr. Stanley K Waithaka
Department of Medical Laboratory Sciences
Mount Kenya University
Po Box 342-01000
Thika

DEDICATION

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ABBREVIATIONS AND ACRONYMS

µl	Microlitre
AIDS	Acquired Immunodeficiency Syndrome
ALB	Albumin
ALP	Alkaline Phosphates
ALT	Alanine Aminotransferase
AMY	Amylase
AST	Aspartate Aminotransferase
BAS	Basophil
D-BIL	Direct Bilirubin
T-BIL	Total Bilirubin
BMI	Body Mass Index
Ca	Calcium
Cl	Chloride
CLSI	Clinical Laboratory Standard Institute
CR	Creatinine
CV	Coefficient of Variation
EOS	Eosinophil
GGT	Gamma-glutamyl Transpeptidase
H_2O	Water
H_2SO_4	Sulfuric acid
HB	Hemoglobin
HBsAg	Hepatitis B surface Antigen
HCl	Hydrochloric Acid
HCT	Hematocrit / Packed Cell Volume
HCV	Hepatitis C Virus
HDL	High-Density Lipoprotein
HIV	Human Immunodeficiency Virus
IFCC	International Federation of Clinical Chemists
ISE	Ion Selective Electrode
K	Potassium
LDH	Lactate Dehydrogenase
LDL	High Low Lipoprotein
LFT	Liver Function Test
LYM	Lymphocyte
MCH	Mother Child Health
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin Concentration
MCV	Mean cell volume
mmol/L	Millimole per Litre
MON	Monocyte
MPV	Mean Platelet Volume
NA	Sodium
NCCLS	National Committee for Clinical Laboratory Standards
NEU	Neutrophil
nm	Nanometres
ºC	Degrees Celcius
P	Inorganic Phosphorous

PCT	plateletcrit
PDW	Relative width of the distribution of platelets in a volume
pH	Hydrogen Ion Concentration
PLT	Platelet
PSA	Prostatic specific antigen
QC	Quality Control
QCA	Quality Control Assessment
RBC	Red blood cells
RDW-CV	Relative Distribution Width of red blood cells by volume, standard deviation
RDW-SD	Relative Distribution Width of red blood cells by volume, standard deviation
SD	Standard Deviation
T-CHOL	Total Fasting Cholesterol
T3	Triidoxine
T4	Thyroxine
TP	Total Protein
TG	Fasting Triglycerides
TSH	Thyroid Stimulating Hormone
U	Urea
UA	Uric Acid
VDRL	Venereal Disease Research Laboratories
WBC	White blood celL

ABSTRACT

Medical diagnosis is based on information from findings such as the medical history of the client, physical examination, self-report and interviews with the patient's relative or both clinical reports from laboratory tests and radiologic studies. To assess one's health status, different parameters are analysed progressively, and the generated reports are used to make a medical decision on the health status of the patients. Thus vital signs and laboratory report test results must be reported along with reference ranges. Little has been done to establish reference ranges for different populations in Kenya. Laboratory interpretation of test results with appropriate precision and accuracy for diagnostic purposes requires reference ranges or cutoff values. This study was a comprehensive determination of reference ranges for vital signs, hematology, clinical chemistry; cancer markers apparently from a healthy volunteer coastal population; vital parameters (for ages 4-93 years were 191 males and 317 females), body mass index (125 males and 127 females) pregnant women (296), infants, children, adolescents (males 129 and 132 females) and the geriatric (males 153 and 177 females), random blood sugar (131 males and 175 females), thyroid profile (124 males and 120 females) from which a total of 2390 subjects were recruited. Biochemistry analysis for liver, kidney function tests, lipid profile and electrolytes were analyzed using intergra 400 chemistry autoanalyzer. Tumour marker samples for the elderly population were analyzed using Chemwell auto analyzer (Biomerieux, Lyon, France), a closed system machine used to analyze tumor makers. The glucometer (On-call Plus, glucose meter from Roche Diagnostics GmbH, Mannheim, Germany) was used for random blood glucose analysis. All data were expressed as median and 2.5-97.5 %interval. Subsequently, independent-sample t-tests were performed to identify significant differences between the data of the males and the females. An analysis of variance (ANOVA) was performed for clinical chemistry, hematology and special chemistry parameters, with sex as a factor and age as covariate. Statistical significance was reached in the case of $\rho < 0.05$. The results were expressed as mean ± SD. Statistical Package for Social Science version 20 statistical software was used for data analysis.

The study established combined male and female reference ranges for vital signs, biochemical, and hematological for children, adolescents, pregnant mothers and the geriatric population of the Taita Taveta community. The study reported statically differences in EOS (%), NEU ($x10^9$/L), BAS ($x10^9$/L), HB (g/dL), MCV (fL), PLT and CA for infants, children and adolescents by gender. ALP (U/L), D-BIL, CREAT, NA, UA and PVC for pregnant mothers per trimester. For geriatric population, the difference was recorded by gender in NEU (%), LMP (%), ALT (U/L), T-BIL, CREAT, CA19-9 and CEA. The study also compared established reference ranges by age and what is published in medical books and similar research works where differences were reported in ALP (U/L), AST (U/L), HB (g/dL), PVC (%) and MCV (fL) by age. At the same time, PSA, CA15-3 and CA 125 were recorded by gender. The present study was the first to report hematology analysis, serum biochemistry, tumor/ cancer markers and vital signs reference ranges for selected categories in Kenya and one of very few such studies in Africa different from those in use in the local health facility. Since there was a statistical difference in gender and age, both the sex and age of a patient must be considered when analyzing different patient samples. This will help improve on management and can be adapted for caregiving to the people of Taita-Taveta County in addition to setting a bench for future research works

CHAPTER ONE

Introduction

1.1 Preface

Restorative determination is based on data from discoveries such as a therapeutic history of the client, physical examination, self-report, interviews with the patient's relative or both and clinical reports from research facility tests and radiologic considerations. In some cases, a combination of the patient's history and a clinical examination by an essential-level doctor is sufficient to choose whether restorative treatment is required and what treatment ought to be given. In any case, frequent research facility examinations or symptomatic imaging strategies are required to affirm a clinically suspected determination or to get more exact data. Diverse parameters can be utilized to survey one's wellbeing status, which are performed continuously. Imperative signs ought to be taken on a normal premise and are the starting and significant examination parameters that allow knowledge in working of the body and are taken when the person is "at rest".

Clinical research facility requires a test that comes about to be surveyed. Hence, is a decision on a patient's prosperity "normal," or does the subject have a few neurotic or pathological impairments? Hence, it's basic that crucial signs and research facility report tests that come about are detailed alongside reference ranges, within the past, ordinarily called "normal ranges" (Ilcol *et al*., 2018). Which ought to be locally be set up. Setting up reference interims has continuously been a challenge as significance yielding may exist in infection

frequencies; organic variety in analytes due to hereditary and ethnic statement, sexes, nourishment, ages, topographical components, biorhythmic changes, pregnancy, physical workout, utilize of drugs and conventional solutions, sample collection strategies; test performance; test interpretation; and other factors (Yin *et al*.,2018). Physicians depend on the accessibility of suitable and dependable reference interims to precisely translate research facility tests comes about combined with information collected amid restorative meet and clinical examination (Cook *et al.*,2017). Even though well-being experts understand the significance of reference interims, numerous research facilities still don't have comprehensive information, particularly ranges specific for their commonplace understanding of a typical patient's population. There proceed to be significant gaps within the accessible reference intervals as frequently interims cited within literature were obtained utilizing more seasoned techniques and instrumented and cover a constrained extend of age bunches or a moderately little number of tests (Lo and Armbruster 2012, Ozadra *et al.*,2019) a previous chair of the IFCC Committee for Reference Interims and Choice Limits (CRIDL), noted that "The hypothesis of reference values was created more than 30 years back, but its application in most clinical research facilities is still fragmented to date. "This is due to the reality that getting a 'good' reference interim could be an exceptionally requesting movement, in terms of time, cash and knowledge" (Lo and Armbruster, 2012). Cerriotti also noted, "The time, effort and money required to establish reference intervals are large and clinical laboratories are disinclined to modify

reference intervals as this is a demanding task also requiring education of clinicians and patients. Large multicenter studies are needed to make real progress in this field and bridge the gaps between a very nice theory (IFCC and CLSI documents). The Clinical and Laboratory Standards Institute (CLSI, previously NCCLS) was first published the C28 Guideline (Defining, Establishing, and verifying reference intervals in the clinical laboratory) in 1995 and the current edition of the guideline, C28-A3 in 2008 (Huma and Waheed, 2013 ,Ghazizadeh *et al*.,2020).

Generally, clinically studied considerations and scheduled clinical patient management in most African nations have depended on European-generated mechanized instrument values, US built-up reference interims or the U.S. NIH division of Helps (DAIDS,) toxicity reviewing tables in evaluating clinical parameters in study participants. The US-established reference interims are gotten from the Massachusetts General center for reference values and serve as the standard reference interim comparison for most studies (Adeli *et al.,* 2017) for Research Reference Intervals in Africa.

Previous research works have illustrated those findings from one population when compared with a different group. It is clear that what is typical for one ponder bunch is diverse from another group. Pregnancy perspectives of the body's chemistry, so pregnant ladies, children and the elderly have their posse have their possess

The physiological changes in liver functioning in pregnancy are transient and rarely permanent. Disorders arising in pregnancy, such as pre-eclampsia and

eclampsia, acute fatty liver of pregnancy (AFLP), hemolysis, elevated liver enzyme and low platelets (HELLP) syndrome, cholestasis, hyperemesis gravidarum and isolated cases of raised liver enzymes can have serious implications (Das *et al.*, 2013, Nabi *et al.*,2019).

The medical laboratory comprises several departments comprising phlebotomy, microbiology,hematology/immunohematology,histology,parasitology,Immunology and clinical biochemistry, which all play a vital role in lowering the patient suffering, morbidity and mortality. Both hematological and chemical biochemistry have been used for the longest time for their accessibility in both developed and developing countries.

This has been made possible by the fact that it's easy to conduct most of the routine test with ease but there are challenges in the sense that there exists uniformity in how the tests are performed, leading to negative adverse effects on care and management of patients. To overcome this problem, to ensure that quality results and Standard Operating Procedures (SOPs) are achieved, each laboratory must develop SOPs and use them in their settings. In the absence of standard operating procedures (SOPs), it becomes a challenge for many clinical laboratories to give reliable results which the World Health Organization has advocated. To achieve this objective, a series of subject-specific guidelines to develop SOPs suiting the requirements of particular laboratories have been developed **(**Harris *et al.*,1980 , Rapport *et al*., 2013). This proves that medical laboratory work forms an integral part in health care, where by 60-70% of clinical decision rely on laboratory investigations (Bae,2014 ,Cook *et al*

2017,) Due to the increased utilization of laboratory guided management, this has created influx of multiple companies supplying variety of testing reagents to the ever demanding market. In addition, this is also accompanied by technology changes.

Results from different tests can be reported as qualitative or quantitative. The quantitative the results can be reported as negative or positive, whereas qualitative is reported in figures that are followed by a reference range which is defined from a health population for comparison (Ilcol *et al.*,2018). When results have been compared between populations, there have been noted statically centrally contrast differences in pregnancy conditions which are physiological differences from those not pregnant and adult biochemical ranges from children reference ranges (Waithaka *et al.,* 2009 , Gitimu *et al.*, 2016 Kainyu *et al.*,2018).

Reference ranges are affected by factors such as instruments, geographical location, analytical method, race, diet, type of sample, age and sex. Age is a key factor in categorizing the human population. As per (Bogin, 2013), four categories have been determined: geriatric, adult, pre-pubertal and newborn population. Gender indicated the difference in adults' creatinine kinase, gamma-glutamyl transferase, uric acid, creatinine, lactate dehydrogenase, and fertility hormones (Gitimu *et al.*,2016) in males testosterone values decreased as one aged, thus making it important to indicate the age of subject under study. Common changes were noted with advancement in age, both female and males, where there was a decrease in total protein and serum albumin and

increase in glucose, urea, alkaline phosphate, creatinine and urea, Creatinine, urea and uric acid was lower in healthy females than healthy males. Males who are less than sixty years of age have higher serum cholesterol, triglycerides, calcium and sodium levels (Zhang *et al.,* 2020).

Reference ranges are constructed to include values between 2.5- 95 % derived from a health population. Currently, most laboratory investigations are compared with ranges from America and Europe (Amitrani *et al.*,2020). To establish reference levels, the following procedure is followed:

After sample collection, partitioning the right groups as per age or gender, inspection of how each group is distributed, outlier identification and statically determination of reference range (Alghushairy *et al.,* 2021). Due to varying circumstances that vary from technology, personnel, target population and ecological factors, the international federation of clinical chemistry recommends that each laboratory set up its ranges (Milinković *et al*.,2018). This can be done directly or indirectly. Indirectly involves using secondary data obtained from the patients, with assumptions that the obtained data is compared with normal results of the health population (Ferre *et al.,* 1999). The direct method applies where healthy groups access health facilities for instance, those who go for blood donation (Ilcol *et al.,* 2018).

Direct method was used to establish normal reference values for coastal populations. Samples were collected from Taita Taveta County (Taveta, Wundanyi, Voi and Mwatate constituencies) whereby different groups were targeted; pregnant women, geriatric, children and adolescents who were in their

normal health state at the time of recruitment. Routinely requested and analysed biochemical and hematological profiles in the medical laboratory medicine includes; full blood count, liver function tests, pancreatic function tests, renal function tests, cardiac tests, lipid profiles, thyroid function test, glucose metabolism, bone chemistry, fertility hormones, tumor(cancer) markers, and body fluids chemistry. Other procedures that fall in laboratory science established in the study were body mass index (BMI) and vital signs, which are key parameters in phlebotomy.

Normal Reference ranges that were established in the current study included; Vital signs (Temperatures, pulse rate. Oxygen concentration in the tissues (SPO_2), blood pressure (BP), and body mass index (BMI,). Hematology parameters were basophils (BAS), eosinophils (EOS); hematocrit (HCT); hemoglobin (HB); lymphocytes (LYM); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); mean corpuscular volume (MCV); (MON), monocytes (MON); neutrophils (NEU); platelet count (PLT); white blood cell count (WBC) and red blood cell count (RBC). Biochemical analytes included total fasting cholesterol (CHOL), fasting triglycerides (TG), low density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol high density ratio, albumin (ALB), alanine aminotransferase (ALT), direct bilirubin (D-BILI), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (T-BILI), creatinine (CREAT), random blood glucose (RBG), potassium (POT), phosphorus (PHOS), sodium (SOD), uric acid (UA), blood urea nitrogen (BUN), total

protein (TP), and alpha-amylase (AMY). Tumour markers tests included total prostatic specific antigen (TPSA), carcinoembryonic antigen (CEA), Ca 125, Ca 15-3, Ca 19-9; Thyroid function tests included, thyroid stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3) and free tri-idothyroxine (FT3). The analytical work was done using clinical chemistry automated / intergra 400), Chemwell for special chemistry (Hitachi), and glucose meter (on-call plus) instruments. (BMI) was done with a digital weighing scale and a tape measure (stadiometer) while mindray monitor was used to measure and record all vital sign parameters.

1.2 Problem Statement

Clinical health and medical research facilities within the Coastal Clinics (Kenya) have been utilizing manufacturers' reference values to help in decesion making with respect to clinical conclusion and treatment and they are not locally established. This can be disasterous in where a clinician may consider a health patient as ill or an ill patient as normal, as the clinician utilizes patient results compared to reference intervals established with a different population from the one that is utilizing the results.

Justification

Since most reagents used are from Europe, America or Asia; reference values based on healthy infants, children under the age 17 years (infants, children and adolescents), pregnant mothers and geriatric population (56-100 years) from the Kenyan coast are different genetically, the nutritional factors, the machine used in the evaluation and also the geographical location that have been known

to affect diiferent biochemical results. Taita Taveta is inhabited by local population of Wataita and Wataveta in addition to immigrants from different parts of the world, hence making it a cosmopolitan study area with reference ranges gaps which the current study was meant to address. Continuous use of reference ranges established elsewhere can result to mismanagement of local population. Thertefore there is an urgent need to have local established reference values for good clinical practice and research.

1.3 Research Questions

i. Are vital signs reference ranges for the Kenyan coastal region's Kenyan population different from those found in literature?
ii. Are there biochemical and hematological reference ranges for the infants, children, expectant mothers and the elderly population of the Kenyan coastal region?
iii. Are there significant age-related differences in biochemical and hematological reference ranges for infants, children, expectant mothers and the elderly population of the Kenyan coastal region?
iv. Are the biochemical and hematological reference ranges for the infants, children, expectant mothers and the elderly population of the Kenyan coastal region different from those found in the literature?
v. Are there tumor reference ranges for the geriatric population of the Kenyan coastal region?
vi. Are the tumor reference ranges for the geriatric population of the Kenyan coastal region different from those found in literature?

1.4 Hypothesis

No significant sex and age-related differences exist in biochemical, hematological, tumor reference and vital signs normal range for Kenyan infants, children, expectant mothers and the elderly population residing at the Kenyan coastal region and those found in the literature.

Significant differences exist between the reference range values of biochemical, hematological, tumor and vital signs normal reference ranges for infants, children, expectant mothers and the elderly population of the Kenyan coastal region and those found in the literature.

1.5 Study Objectives

1.5.1 Main objectives

To establish vital signs, hematological, biochemical and tumor markers reference ranges for different population of Taita Taveta County in the Kenyan coastal region.

1.5.2 Specific Objective

i) To establish age and sex-related reference ranges of five vital signs of healthy population of Kenyan coastal region.

ii) To compare established age and sex-related reference ranges of five vital signs healthy population of Kenyan coastal region with those in literature.

iii) To establish age and sex-related reference ranges of twenty-three hematological parameters and fourteen biochemical parameters for infants, children, expecting mothers and the elderly population of the Kenyan coastal region.

iv) To compare established reference ranges for infants, children, expectant mothers and the elderly population of Kenyan coastal region with those found in the literature.

v) To establish age and sex-related reference ranges of common tumor markers of geriatric population of the Kenyan coastal region.

vi) To compare established age and sex-related reference ranges of common tumor markers of healthy geriatric population of Kenyan coastal region with those in literature.

1.6 Impact of results

The study has established coastal reference ranges for imperative vital signs and routinely analyzed hematological and biochemical tests for children, adolescents, pregnant and geriatric population. This will form a base of more research work in the related laboratory work, recommendations to the routine users on utilization of locally established normal reference ranges for the Kenyan coastal population.

CHAPTER TWO
2 Literature review

2.1 Physical examination

2.1.1 Vital signs

For over 100 years, medics have performed this surveillance using the same vital signs; temperature, pulse, blood pressure, respiratory rate, and oxygen saturation in recent years (Cedar,2017). Prompt detection and reporting of changes in these vital signs are essential as delays in initiating appropriate treatment can detrimentally affect the patient's outcome (Vincent *et al*., 2018). For proper care in a patient's physiology, vital signs should be assessed accurately (Prgomet *et al.*,2016). Increased patient survival rates, have resulted to an increasingly complex and older patient population (James *et al.*,2010 ,Hanlon *et al.*,2018). Elderly clients of 65 years and above have twice the risk of developing peri-operative complications than younger adults in addition to being admitted as emergencies and those who are to undergo emergency surgeries (Van Holstein *et al.*,2018). Diminished reserves in renal, hepatic and cognitive renal function also contribute to older patients being at higher risk of adverse events (Thornlow *et al.*, 2009 , Roller-Wirnsberger,2020). For this reason, the five traditional vital signs are of greater importance in one's health care to be evaluated before any acute change in client's physiology is recognized (Kramer *et al.,* 2019).

2.1.2 Temperature

The body's temperature is the balance between heat generated by the body and heat lost, also known as thermoregulation. In a clinical setup, body temperature

is affected by factors such as age, skin exposure (in the operating theatre) or pathophysiology (sepsis). Inaccurate measurement may be attributed to factors such as the consumption of cold or hot fluids before the measurement of sublingual temperatures. Clinically, there are three types of core body temperatures by which the patient can be evaluated by: surface body temperature, how the patient says they feel, or how the patient feels to touch. However, the above three factors are not always the same and may differ according to the underlying disease process, which the medics should consider when taking the measurements. Additional factors are correctly calibrated and awareness of the difference in the core temperature between anatomical sites. (Siddiquee *et al.*,2018) noted significant differences in accuracy and consistency on devices used in temperature measurement e.g. oral disposable, oral electric or temporal artery. This qualifies the need for regular calibration and consistency to identify trends in patient's temperature measurement.

2.1.3 Pulse Rate

Pulse rate is the palpable rhythmic expansion of the artery resulting from increased blood volume being pushed into a vessel by contraction and relaxation of the heart (Yamin, A., 2020). Pulse is affected by factors such as medication (beta blockers), age, existing medical conditions (fever) and fluid status whether its hypervolemia or hypovolemia). It's worth noting that pulse rate is not always a true reflection of cardiac output or cardiac contractility; for resistant, where the client has aortic stenosis, the pulse rate may be weak despite forceful cardiac contractions (Elliott and Coventry, 2012 , Kyriacos *et*

al., 2015). The pulse rate shouldn't be considered heart rate, which is a measurable pulse characteristic. In case the pulse is palpated, characteristics other than pulse rate need to be assessed.

They include the pulse regularity's strength or amplitude and the pulses' peripheral equality. This is essential as it provides further insight into a patient's condition or response to treatment. Pulse should be assessed for 15 seconds or longer, but pulse rate is evaluated by counting pulse for 30 seconds or less, there exists a potentially problematic as an irregular pulse may not be easily be detected during this interval. Use of short periods, it has been noted that, there is increase in errors four to six-fold (Mu *et al.*, 2011 , Elliott and Coventry, 2012, Yamin, A., 2020). A client with atrial fibrillation may appear to have a regular pulse when he or she is assessed for 30 seconds or less. When the assessment is done for one minute, all the abnormalities not detected during short assessment intervals are easily noted. In addition, the use of pulse oximeter to determine patient's pulse rate, medic's knowledge and physical assessment skills to assess the pulse accurately are of great importance. For instance, the pulse oximeter may provide an inaccurate reading if the pulse rate is irregular or the client is cold or hypovolaemic. Use of technology wholly for taking observations may sometimes be detrimental to patient care, as some of key clues to the patient's condition can be easily be missed (Wheatley, 2006, Kramer *et al*., 2019).

2.1.4 Oxygen Saturation (SpO_2)

The fundamental assignment of cardiorespiratory organization is to deliver oxygen (O_2) into the cells and removal of carbon dioxide ($C0_2$), (along with other metabolic products) from them. Proper upholding of this task depends on undam elderly respiratory and cardiovascular systems, adequate hemoglobin level and number of red blood cells and supply of inspired gas containing sufficient oxygen concentration (O_2) (Saghiv, M.S. and Sagiv, M.S., 2020). Decreased O_2 accessibility to red blood cells results in an inhibition of the respiratory sequence and in turn, increased anaerobic glycolysis. This switch to anaerobic from aerobic metabolism, so called Pasteur effect reduces adenosine triphosphate (ATP) production. In case of severe hypoxia, ATP production is not enough to meet the energy requirements of ionic and osmotic equilibrium, cell membrane depolarization which leads to uncontrolled Ca^{2+} influx and activation of Ca^{2+}-dependent proteases and phospholipases. These actions in turn, results to cell swelling and eventually results to cell necrosis (Bonora *et al*.,2020) .

2.1.5 Respiratory rate

Respiratory rate is an important baseline observation and its accurate measurement is a fundamental part of patient assessment (Jevon, 2010). Respiratory rate measurement serves several purposes, such as being an early indicator of acidosis (Rolfe, 2019). It is also one of the most sensitive indicators of critical illness (Smith *et al.,* 2008). An increase from the patient's normal rate of three to five breaths per minute is an early and important sign of

respiratory distress and potential hypoxaemia (Rolfe, 2019). Despite this, research has found that the respiratory rate is often not recorded in clinical settings or is simply guessed (Mitchell and Van Leuvan, 2008). This is disturbing given that an abnormal respiratory rate is the best predictor of an impending adverse effect such as cardiac arrest (Cretikos *et al.*, 2007). The reason for this haphazard assessment is unclear. Perhaps it is because nurses assume that oxygen saturation provides a greater reflection of the patient's respiratory function or because there is no automated machine for measuring respiratory rate (Akel, 2021). In the acutely ill patient, respiratory rate should be counted for a full one minute, rather than 30 seconds and then doubled (Ringdal and Gullick, 2015). In measuring the respiratory rate, the pattern should also be assessed and classified as eupnoea, tachypnoea, bradypnoea or hypopnoea (Moore and Woodrow, 2009).

Labeling it as such encourages the medics to do more than simply count a number, and to consider why the respiratory rate may be fast or slow. Medics should also assess respiratory effort (depth of inspiration and use of accessory muscles) and equality of thoracic expansion (Perkins *et al*., 2021).

2.1.6 Blood pressure (BP)

Blood pressure (BP) is pressure that is exerted by blood against the arterial wall. This is influenced by a number of factors, including cardiac output, blood volume, peripheral vascular resistance, blood viscosity and vessel wall elasticity (Elliott and Coventry, 2012). Blood Pressure is an important vital sign as it reflects blood flow when the heart is contracting (systole) and

relaxing (diastole). It also indicates cellular oxygen delivery to all parts of the body. Changes in Blood Pressure may reflect the underlying body's pathophysiology or its attempts to maintain homeostasis. A drop in blood pressure has been recorded to be a common indicator in patients prior to cardiac arrest (Araujo-Moura *et al.*,2021). A change in Blood pressure alone, does not indicate that the patient will have a cardiac arrest, but it's a trigger to the medics to perform a more detailed assessment. BP measurement cannot be over-emphasized; but it is one of the most inaccurately measured vital signs (Pickering *et al.*, 2005). If the BP reading consistently underestimates the diastolic pressure by 5mmHg, this could result in two-thirds of hypertensive patients being denied preventative treatment (Sharman *et al.*, 2020). Heavy clinical workloads or ratios of patients and medics may result in using automated BP monitors to save time. Inadequate psychomotor skills, local culture or lack of confidence have contributed to their use. The use of automated BP monitors has significantly increased the risk for measurement error. A case study of 95 patients that was done to compare digital and aneroid monitors with a sphygmomanometer, out of 34% of systolic blood pressures measured with a digital device were within 5 mmHg of the sphygmomanometer (Araujo-Moura *et al*., 2021). Suppose one of these machines records a BP measurement that is outside normal range. In that case, it is easy for the medical personnel to perform another reading using the machine and keep doing so, until a value within normal range is obtained. This has been described as observer bias or prejudice, where the medic simply

‘adjusts’ the BP recording to what he or she thinks it should be or wants it to be. Blood pressure does vary substantially due to effects such as pain, emotions, eating, voiding, drugs, body position caffeine, nicotine, mental and physical activity. For if an elevated blood pressure reading has been obtained, the blood pressure level should be confirmed on a different day (Dadlani *et al*.,2019).

2.2.1 Body mass index (BMI)

Obesity is a pathological condition where excess adipose mass is deposited in body tissues which is often seen as an increase in body weight, this need not be the case. Lean but very muscular individuals may be overweight by numerical standards without increasing adiposity. Obesity is more effectively defined by assessing its linkage to morbidity or mortality. Adiposity is not measured directly, but obesity is widely gauged by body mass index (BMI), which is weight/height2 (in kg/m2) (Chatterjee *et al*.,2021). Other approaches that can be utilized to quantifying obesity are skin fold thickness (anthropometry), densitometry (underwater weighing), electrical impedance, computerized tomography (CT) and Magnetic resonance imaging (MRI). The obtained body mass index value is compared to already established standards values (Keys *et al.,* 2014). This is a key surrogate marker of adiposity. However, the limitation exists of considering differences in body composition and the contribution of body fat all over the body. In the last three decades, obesity has almost doubled globally (Fauc *et al.,* 2008; WHO 2013; Pasco *et al.*, 2014). Globally the estimates of body mass index (BMI) provide a guide to obesity levels. BMI

values greater than or equal to 30 are indicative of obesity. Use of BMI has clear shortcomings where it overestimates or underestimates adiposity in the muscular body builds for the elderly (Pasco *et al*., 2014). Generally, women have more body fat than men. Substantial data indicates that threshold of morbidity on based on obesity in both men and women are of BMI of 30 and above.

Epidemiologic studies suggest that all-cause, cancer, metabolic and cardiovascular morbidity begin to rise when BMIs are 25. BMI values of between 25 -30 should be considered as of medically importance and worthy of therapeutic intervention, especially where adiposity is an influential risk factor, such as glucose intolerance and hypertension (Huang *et al.*, 2021).

2.3. Biochemical assessment

The biochemical assessment indicates both tissue level of a certain nutrient or any abnormality of metabolism in the utilization of certain nutrients under study. These studies are determined from different body fluids such as serum, cerebral fluid and urine. Some of these studies may indicate recent intake or identify below-normal values of element under evaluation even when no clinical symptoms have been seen to indicate deficiency (Wishart,2019).

Lipids are fats that are found in blood and tissues. Lipids have four key roles, since they are mostly composed of mostly carbon- hydrogen (C-H) bonds; they are rich source of energy and an excellent way of storing excess calories in the body. Lipids have unique physical properties; thus, they form an integral part of cell membranes hence playing an important structural role in cells, aiding in

digestion and serves as a hormone. There are six lipids comprising cholesterol, diacylglycerols, prostaglandins, sphingolipids and fatty acids (Sunshine, H. and Iruela-Arispe, 2017). Triglycerides and cholesterol evaluation forms the basis of diagnosis and management of lipid associated disorders. Cholesterol is insoluble in body water but is attached to protein molecule enabling it to be transported within lipoproteins. In addition to the above role, Lipoproteins do have cell-targeting signals that direct lipids they carry to specific body tissues, thus there are several types of lipoproteins within blood that are identified depending on their density: chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).

Lipoprotein density is determined by the ratio between protein and cholesterol; the more protein is, the less cholesterol a lipoprotein has, hence the denser it is (Sacks,and Jensen,2018). Among these five types of lipoproteins, HDL and LDL are the most abundant and are of interest in clinical practice. The cholesterol within all the various lipoproteins is identical. Where the lipid levels are too high and unbalanced, they build up in walls of the artery walls forming plaque. These plagues result in obstruction of flow via the arteries and increase cardiovascular disease and stroke risks. Lipids include cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides.

The basic lipids blood test measures total cholesterol, triglyceride levels, HDL and LDL cholesterol. More extensive lipid profile testing also includes VLDL,

total cholesterol ratio to HDL and LDL to HDL ratio. Lipid profile testing is used to help determine the risk of heart diseases along with other factors such as age, cigarette smoking, family history, weight, diet, exercise, diabetes and blood pressure (Daly *et al.,* 2018)

Low-density lipoprotein (LDL), solely contains apoB-100 and is more cholesterol rich than other apo B–containing lipoproteins chylomicrons, VLDL, LDL and HDL. LDL is readily taken up by cells with help of LDL receptor in the liver and peripheral cells (Daly *et al*.,2018). LDL do infiltrate into the extracellular space of the vessel wall due to its size, which is smaller than chylomicrons and VLDL, which are oxidized and then taken up by macrophages. LDL subtractions, of late are of medical importance because small, dense LDL particles are pro-atherogenic as they are better marker for coronary heart disease risk, (Sethi et *al.,* 2010).

High-Density Lipoprotein (HDL) cholesterol comprises of 20-30 percent of total cholesterol. HDL molecule is the smallest and most dense particle that is synthesized by both intestine and the liver (Donato, and Meeusen, 2020). HDL is termed as “good cholesterol” as it helps remove excess cholesterol from peripheral cells (reverse cholesterol transport) which is the main mechanisms of anti-atherogenic property of HDL. There are two major types of spherical based on density. There are two HDL particles, HDL2 and HDL3. HDL2 are better in delivering lipids to the liver as they are larger in size and richer in lipid than HDL3, thus protecting the heart condition such as myocardial infarction and ischemic stroke (Daly *et al*.,2018).

Triglyceride is an organic compound made up of three fatty acids that esterify into glycerol. Most triglycerides from plant sources, for example corn and sunflower seeds are rich in polyunsaturated fatty acids and are oils. In contrast, those from animals contains saturated fatty acids and are solids at room temperature. Triglycerides are the major components of very-low-density lipoprotein (VLDL) and chylomicrons that play an important role in as energy metabolism and transporter of dietary fat. They have twice as much energy than what is derived from proteins and carbohydrates (Ebbeling,*et al.*, 2018). Triglycerides pass through cell membranes via special enzymes found on the cells of blood vessels called lipoprotein. Triglycerides are broken down by lipase into free fatty acids and glycerol. Fatty acids are then taken up by cells via the fatty acid transporter. High levels of triglycerides in the bloodstream are linked to hardening of arteries (atherosclerosis) which are associated with heart disease and stroke. When combined with other clinical data, population-specific reference intervals are important for clinical decision-making in managing patients considered to be at risk for atherosclerotic cardiovascular disease (CVD) (Donato, and Meeusen, 2020).

2.4. Haematological Assessment

Hematology is measurement of blood elements. It is important in early identification of disease or physical illness. Variations in the shape, size and number of blood cells do give early insight in the general functioning of blood and bone marrow where hematopoiesis of blood occurs and clinical factors that affect it. Hematology analyzers are used to determine between 5-24 blood

parameters simultaneously, the main being number of leukocytes, hematocrit and hemoglobin concentration , erythrocytes, distribution of red blood cells and platelet count. Since the advent of hematological measurement and analysis it has been shown that the cellular constituents of blood vary in number and are not stable constants. These elements are determined and influenced by several factors; age and sex, for instance, are very clear, definable influencing factors. Erythrocytes enlarge as people age, while significant differences have been noted between age 11 and 15 years, 21-50 years and above 60 years of the black American population (Rahmanitarini *et al.,* 2019).

Blood is a vital circulatory tissue which is composed of cells suspended in a fluid intercellular substance (plasma). The major function of blood is maintaining homeostasis (Isaac *et al.,* 2013). Hematological components, are composed of red blood cells (RBC), leucocytes or white blood cells (WBC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), platelets and mean corpuscular hemoglobin concentration (MCHC) investigate anemia, infections and pyrexia (fever) of unknown origin (PUO), hemoglobinopathies, monitoring of patients receiving antiretroviral therapy (ART) and bleeding disorder all which give specific guide in patient management (Shah *et al.,* 2021). Red blood cells (erythrocytes) serve as a carrier of hemoglobin. Red cells (erythrocytes) is the main cellular component of blood, which is about 45% of total blood volume in an adult, but the exact RBC number varying according to gender, age and state of health. Hemoglobin reacts with oxygen in the blood to form oxyhemoglobin during respiration

(Isaac *et al.*,2013) thus transporting oxygen and carbon dioxide in the body.(Soetan *et al.,* 2013 , Isaac *et al.,* 2013).

4.1 White blood cells

These are a combination of different blood cells involved in immune system responses. In an adult, the normal ranges are 4.0-11.0 x 10^9/litre of whole blood which is differentiated into neutrophils 1.5-7.5 x 10^9/l, lymphocytes 1.2-4.0 x 10^9/l, monocytes 0.2-1.0 x 10^9/l, eosinophils 0.02-0.6 x 10^9/l and basophils 0.01-0.1 x 10^9/l (Patil *et al.,* 2021).

2.4.2 Haemoglobin

Hemoglobin molecule contains four linked polypeptide chains (globin) where an adult consists of two alpha chains with 141 amino acids and two beta chains of 146 amino acids. When partial pressure of oxygen (pO_2) in the blood is high, hemoglobin takes oxygen and releases it when the pO_2 is low. When the blood is in the lungs therefore, it rapidly combines with hemoglobin, forming oxyhemoglobin. The blood becomes saturated with oxygen (97%) in the lungs and when it gets to the tissues, this reduces to about 30% (Dybas, 2020).

2.4.3 Neutrophil

Neutrophil has 3 (occasionally 4 or 5) clear lobes, separated by chromatin threads. The normal range is 45-70%. They have a short life-span, upon circulating in blood for 6-10 hours; they pass into the tissues as highly mobile phagocytes which are important in defending the body from infection. They mobilize and migrate to sites of infection or inflammation, chemically attracted by substances released by bacteria, complement components, damelderly

tissue, and other leukocytes (process called chemotaxis). Increased number of neutrophils above the normal ranges is referred to as neutrophilia, which usually indicates tissue damage or infection. The common causes include bone marrow failure, bacterial infection, viral infections, splenomegaly, drugs and megaloblastic anaemia. While reduction in neutrophils is neutropenia which is usually due to bone marrow failure or treatments with some drugs (Castellanos-Sinco *et al.*, 2015 ; Patil *et al.*, 2021).

2.4.4 Lymphocytes

Two main types of lymphocytes are produced in human bodies, with specific response being immune. They are produced as immune soilders; in an adult, there is about 10 % in circulation. Increased production in leucocytes (leucocytosis) is mostly due to bacterial infection, while a decreased which is known as leukopenia is mostly due to use of drug such as those used in cancer treatment (Monica, 2006 , Rydzewska *et al.*, 2018).

2.4.5 Monocytes

Monocytes are the largest type of WBCs; they share their function with neutrophils, but have a longer half-life. In an adult, the normal ranges are .0.2-1.0 x 10^9/l. From bone marrow monotypes passes to blood circulation, whereby within 2-3 days they get into body tissues where they develop into macrophages. Monocytosis is an increase in monocyte circulation due to protozoal parasitic infections, tuberculosis and other chronic bacterial infections (Monica, 2006 ,Yang *et al.*, 2014).

2.4.6 Eosinophils

Eosinophils originate, differentiate and mature in the bone marrow. They are slightly larger than a neutrophil, measuring 12-17 micrometer in diameter with two- three lobes. Their cytoplasm contains large and round orange-red granules. They circulate for about 8 hours and they are mainly found in gastrointestinal tract, skin and lungs where they are involved in hypersensitivity reactions, such as hay fever, asthma, and eczema; they contain substances that inactivate histamine and factors released during anaphylaxis. In parasitic immune responses, the IgG and IgE antibodies bind to mast cells at the site of parasitic infection (Monica, 2006, Ramirez *et al*., 2018).

2.4.7 Basophil

The nucleus of basophil is usually bi-lobed and obscured by large, irregular-in-size basophilic granules that are blue in color on a stained blood film. The normal basophil count is less than 0.1 x 10^9/l. They interact with macrophages and eosinophil in allergic reactions. Basophilia is often found in myeloproliferative disorder such as leukaemias and allergic disorders (Monica, 2006 , Uciechowski and Rink, 2014).

2.4.8 Platelets

In an adult the normal ranges are 100-400 platelets per liter of blood. They have rare practice, which are characterized by mucocutaneous bleeding such as nose bleeding, bcrucial role in hemostasis. Platelet dysfunctions are due to congenital and acquired etiologies. Congenital problems are common causes of bleeding encountered in clinicaruising, menorrhagia, and bleeding after

hemostatic stress after adenoidectomy and tonsillectomy, dental extraction and rarely, post-partum (Anjali, 2014, Ingram, 2021).

2.4.9 Packed Cell Volume

The packed cell volume (PCV), also known as hematocrit is used for anemia dehydration, burns, dengue hemorrhagic fever, and polycythaemia. PCV and hemoglobin values determine the mean cell hemoglobin concentration (MCHC). The normal ranges for PCV 0.36-0.54l/l for men and 0.36-.45l/l for women. PCV is reduced in people who are anemic and is elevated where there is loss of plasma. For example, in case of dehydration, severe burns and dengue hemorrhagic fever (Duncan, 2020).

The normal ranges of MCV in an adult are 80-100 fL. High values of MCV are indication of in pernicious anemia which can range up to 150 femtometers. Elevated levels of MCV can result from increased alcohol intake and folic acid / Vitamin B12 deficiency, which results in macrocytic anemia (Nagao and Hirokawa 2017). Where the MVC is low it's associated with microcytic anemia (iron deficiency anemia) which is due to iron deficiency; the MCV values are as low as between 60-70 fL which are mostly attributed to gastrointestinal blood loss, inadequate dietary intake or menstrual blood loss), sideroblastic anemia, thalassemia,or or chronic disease (Duncan, 2020).

2.4.11 Mean Cell Hemoglobin Concentration (MCHC)

MCHC is the hemoglobin concentration in g/l in a litre of packed red cells (PCV) and the normal range in a healthy adult is 315–360 g/l or (31.5-36.0 g/dl). In iron deficiency anemia, the MCHC values are low, while elevated

MCHC are found in patients with a rare condition spherocytosis (Nagao and Hirokawa 2017 , Duncan, 2020).

2.4.12 Mean Platelet Volume (MPV)

Mean platelet volume is a hematological parameter that is generated by fully automated blood count analyzers as a part of routine full hemogram (FH) / complete blood count (CBC) which estimates platelet function and activation. In an adult male, the normal values are 6.5-12 fL. (Doig and Zhang 2017).

2.4.13 Red blood cell distribution width (RDW)

This is the measurement of the variability of red blood cell size. Higher numbers indicate greater variation in size. The normal range for the red cell distribution width (RDW) is 11%–15%. The RDW is a standard part of the complete red blood cell count of automated cell counters that also includes the concentration of red blood cells (RBC), the mean corpuscular volume (MCV), the mean hemoglobin of a red blood cell (MCH), and mean hemoglobin concentration of a red blood cell (MCHC) (Doig and Zhang 2017, Nagao and Hirokawa 2017).

2.5.1 Liver and Liver Function Tests Profile

The liver is the largest organ in the human body, weighing between 1.2-1.5 kilograms; it is located at the upper right-hand portion of the abdominal cavity, above the diaphragm and above the stomach, right kidney and intestines. It is a dark reddish-brown organ with two main lobes with up to 8 segments. Each segment is made up of a thousand lobules. The liver has six broad critical biochemical roles: metabolism, digestion, immune response, synthetic,

excretory and detoxification. The liver is a unique organ because it is able to regenerate destroyed cells in a short-term after an injury or disease; also it's liable to frequent damages over a long period of time where it may suffer irreversible changes that permanently alter the normal and essential functions (Tsilimigras *et al.*, 2021). Throughout the world, liver diseases are the major causes of morbidity and mortality.

Most common liver ailment is due to alcohol abuse throughout the world, but the rise in obesity (lifestyle disease) rates is taking a toll in liver diseases. The incidence of non-alcoholic fatty liver disease (NAFLD) is on upward trend in the developed countries. This contrasts with developing worlds, where infections such as viral hepatitis viruses and parasitic infections are responsible for most chronic liver disease and hepatobiliary cancer (Chapman *et al.*, 2007). Hepatic disease (liver disease) is a term that describes any liver disorder which is often associated with icterus (jaundice). Clinical conditions that manifest liver disorders are jaundice, disorders in hemostasis, portal hypertension and release of enzymes in different body fluids (Kushwaha, 2020) Visible jaundice is noted when bilirubin concentration in the plasma rises to double its normal limit of 34 mol /l (2 mg. This is easily detected as white's part of the eyes; the skin and body fluids are all yellowish in colour due to breakdown of hemoglobin from dead blood cells (Monica, 2009).

2.5.2 Types of jaundice

Hemolytic (prehepatic) jaundice is observed when there is severe breakdown of red blood cells (hemolysis) producing more bilirubin than the liver can be able

metabolize, (breakdown of red cells). The excess produced bilirubin builds up in the plasma and is mostly the unconjugated type thus not found in the urine. In hepatocellular / hepatic jaundice, the amount of bilirubin builds up in the plasma as is not transported, conjugated, or excreted due to liver cells damage caused by infections such as viral hepatitis. In this type of jaundice, there is both unconjugated and conjugated bilirubin in blood and urine. In obstructive jaundice, which is also known as post-hepatic jaundice, there is buildup of bilirubin in the plasma as a result obstruction of small bile channels or in the main bile duct. Obstruction is caused by gallstones or a tumour obstructing or closing the biliary tract. In this type of jaundice, bilirubin is in the form of the conjugated type is found in the urine (Monica,2009, Tsilimigras *et al.*,2021).

Portal hypertension is a liver disorder that occurs when portal flow is obstructed in the spleen, gastrointestinal, gallbladder or pancreas. Depending on the site of obstruction, there are three types of portal hypertension: presinusoidal, sinusoidal and postsinusoidal. Schitosomiasis (Portal vein thrombosis) is the common cause of presinusoidal portal hypertension. Congestive heart failure and hepatic vein occlusion are causes of postsinusoidal hypertension, while cirrhosis is the common disorder in sinusoidal hypertension (Simonetto, 2019).

In Liver cirrhosis, scar tissue replaces the normal tissues, blocking blood flow through the organs, thereby preventing normal liver functioning. Liver cirrhosis rarely causes signs and symptoms in early stages. Still, as the liver function deteriorates, nausea, fatigue, unintended weight loss, bleeding from

the gastrointestinal tract, jaundice, intense itching, legs swelling and abdomen appears. Liver cirrhosis has poor prognosis, but patients have a prolonged survival rate (Simonetto, 2019). Liver cirrhosis is not easily reversed, but its progression can be delayed with treatment which is determined by its cause and patient's complications. For example, alcohol cirrhosis is treated by abstaining from alcohol. In contrast, hepatitis-related cirrhosis involves treatment of the different types of hepatitis with for example interferon for viral hepatitis and corticosteroids for autoimmune hepatitis.

Reye Syndrome describes a group of disorders caused by metabolic disorders, infections or drug induced or toxic disease found mostly in children and rarely in adults (Schmeltzer 2019). Reye syndrome's real cause is unknown, but viral syndromes such as gastroenteritis, varicella, or upper respiratory tract infections are thought to be the cause (Friedman and McKenna, 2008). Reye syndrome is characterized by fatty degeneration and non-inflammatory encephalopathy of the liver which is accompanied with profuse vomiting with varying degrees of neurologic impairment, such as deterioration in consciousness. There is also liver degeneration which characterized by mild hyper-bilirubinemia, increases in ammonia and aminotransferases ALT and AST. Rapid clinical deterioration occurs when delayed treatment leads to death (Schmeltzer 2019).

2.5.3 Drug- and Alcohol-Related Disorders

Drug-induced liver disease is a major problem that results in acute liver failure. This is attributed to the facts that liver is a major and primary organ that plays a

central role in drug metabolism. Many drugs are toxic and cause liver damage; liver damage ranges from very mild transient forms to fulminant liver failure via an immune-mediated injury to the hepatocytes. An adverse immune response initiated by the drugs directly affects the liver thus resulting to hepatic and cholestatic disease (Lucena *et al.*, 2020). In hepatic toxicity, ethanol is the most destructive ingested substance. When ingested in very small amounts, the damage caused ranges from very mild, transient, and unnoticed injury but with heavier and prolonged consumption, it leads to cirrhosis (alcoholic). Long-term and excessive consumption of alcohol results in liver abnormalities that ranges from alcoholic fatty liver with inflammation (steatohepatitis) to scar tissue formation (hepatic fibrosis) to the destruction of normal liver structure seen in hepatic cirrhosis. This results in three stages of liver damage (1) alcoholic fatty (2) liver alcoholic hepatitis (3) alcoholic cirrhosis. In alcoholic fatty liver only mild changes occur in the liver function with slight elevations in ALT, AST, and GGT (Adeli *et al.*, 2017).

In second stage, there is more evidence of liver damage with moderate elevation in AST, GGT ALT, ALP and total bilirubin is elevated up to 30 mg/dl. Other parameters of importance such as serum albumin are decreased while prothrombin time is prolonged. Alcoholic cirrhosis is the last stage and is fatal. This condition is common in males with nonspecific symptoms and includes; weight loss, general weakness, fever, hepatomegaly, ascites, splenomegaly, jaundice, malnutrition, and edema. Measurable laboratory abnormalities are elevated liver function tests (ALT, AST, ALP, GGT, and

total bilirubin), prolonged prothrombin time and decreased albumin (Lucena *et al*., 2020).

2.5.3.1 Enzyme release from diseased liver conditions

In most patients, the liver enzymes continue to function normally. So, to determine the damage in the liver, measurement of cytosolic membrane-associated and mitochondrial enzyme are elevated thus becomes essential diagnostic and differential parameters in liver disease evaluation. Commonly measured enzymes in medical laboratory medicine include ALT, AST, GGT, ALP and LDH (Lucena *et al.*, 2020). The use of these enzymes is determined by pattern of release, clearance from plasma, subcellular distribution, relative degree of enzyme activity in liver and plasma and tissue specificity (Dufour, 2008).

2.5.4 Liver panel tests

Liver function/panel tests: These are used to the functional capacity of the liver and any cellular damage that have occurred the liver cells. This done through via;1. Synthetic ability entails measuring the various plasma proteins (albumin, prothrombin and lipids) synthesized by the liver. 2. Liver secretory or excretory abilities where serum bilirubin levels are estimated (Mendoza-Elias, 2018).

Bilirubin is an end product of haem metabolism derived from the haem part of hemoglobin molecule which is yellow in colour. Upon breakdown of haem in the liver giving unconjugated bilirubin which is insoluble in water, is transferred from the spleen where it is broken down into the liver binding with

albumin (conjugation) with aid of glucuronyl transferase enzyme. The resulting bilirubin is water soluble and is excreted into bile. In laboratory setting, bilirubin is evaluated as total and direct bilirubin (conjugated bilirubin). Indirect bilirubin is calculated by subtracting direct bilirubin from total bilirubin (Mendoza-Elias, 2018).

Liver enzymes originate from the hepatic cells and leak into the circulation after cytolysis or necrosis of hepatic cells. They are evaluated to determine the cellular insult of the liver. Different liver enzymes are increased in conditions such as liver cirrhosis, viral and toxic hepatitis among others. In routine analysis, (1) Transaminases: ALT (SGPT), AST (SGOT) these are enzymes that do transfer an amino group from an amino acid to α keto acid. (b) Transpeptidases GGT Gamma glutamyltranspeptidase is an enzyme that is specific to biliary tree. Elevation indicates cholestasis and biliary tree damage even in minute damages (Monicah 2009 , Mendoza-Elias, 2018).

Alkaline Phosphatase is a hydrolase enzyme that removes phosphates from molecules such as proteins and nucleotides. Abundantly, it is found in biliary cells lining, hence a rise is indicative of biliary tree damage due to cholestasis. Other cause includes blockage of large ducts by stones, intrahepatic obstruction or biliary channel inflammation. Alkaline phosphatase is found in placenta and bones also. Increased levels are found in adults with Paget's disease and in growing children whose bones are undergoing remodeling (Bover *et al*., 2018).

Proteins are macromolecules that are built of one or more unbranched chains of amino acids. Typical, most protein contains 200–300 amino acids, but some

have smaller peptides and others are larger with their molecular mass ranging from 6000 for insulin to several million for some structural proteins (Tymchak, 2010). Most proteins in circulation are synthesized in the liver. The encoded information in genes specified by the nucleotide sequence provides each protein with its unique amino acid sequence. The amino acid sequence is determined by a corresponding sequence of bases (guanine, cytosine, adenine, and thymine) in the DNA contained in the specific gene. In genetic coding sets of three nucleotides, whose combination stands for a specific amino acid. Total protein measurements reflect nutritional status, kidney and liver diseases, hypothyroidism and redistribution by hemodilution. Conditions that are associated with protein are; hypoproteinemia and hyperproteinemia, (Tymchak, 2010). Protein daily in-takes are essential for proper nitrogen balance which have core value in anabolic influences on bone and muscle through an anabolic activity of those amino acids (Bover *et al*., 2018_ENREF_191).

Hyperproteinemia (increase in total plasma proteins) is as a result of dehydration. Upon loss of excess water from the vascular system, the proteins remain within the blood vessels. Hypoproteinemia results when levels of total protein is less than the reference interval (negative nitrogen balance exists), due to excessive loss in renal disease, leakage into the gastrointestinal tract in inflammation of the digestive system, and loss of blood in open wounds, internal bleeding, or extensive burns. Hypoproteinemia can also be also due malnutrition or intestinal malabsorption (Mutanei 2018).

2.6.1 Kidney and kidney disorders

The Kidney is a bean-shaped paired organ located retroperitoneally on either side of the spinal column with multiple functions. In an adult male, each kidney weighs about 150 grams while in a female weigh about 135 grams with a nephron being the functional unit. There are about one million nephrons in each kidney. The central role of the kidney is homeostatic mechanism in a human body. Each nephron is composed into glomerulus, proximal tubule, loop of Henle, distal tubule and collecting ducts which aid in maintaining the normal homeostatic body condition (Kurts *et al.*, 2020). The main roles of the kidney are to maintain the internal milieu, urine formation, endocrine, hemostatic regulation and excretion processes.

Diverse kidney diseases/conditions affect individuals with characteristic clinical features. The common clinical conditions that affect the kidney are renal cysts, nephritic and nephritic syndromes, diabetic nephropathy, toxic nephropathy, renal calculi, chronic kidney disease, Prostaglandins and non-steroidal anti-inflammatory drugs (NSAID) disease, nephrolithiasis urinary tract infection and obstruction. Renal cell carcinoma is the most common renal cancer disease affecting the adults (Kumar *et al*., 2018).

Diagnosis and screening of kidney disease occurs when a client presents to a clinician with a sign or symptom, an abnormality detected during a routine urinalysis or biochemical blood screening or a client with a systemic disease that affects the renal system e.g., diabetes mellitus. Nephrotic syndrome is caused by different diseases and conditions that result in injury and increased

permeability of the glomerular basement membrane. This results in an abnormality in which protein greater than 3.5 g/day is noted and serves as a confirmatory test. Another subsequent condition includes decrease in plasma oncotic pressure that causes generalized edema as body fluids moves out of vascular and into interstitial space as condition progresses, hyperlipidemia and lipiduria are also magnificent. The exact cause of Nephrotic syndrome is unknown, and it affects both children and the adults. In children the age that is frequently affected is between one and half to five years with boys being the most affected (Saleem, 2019.). Acute Renal failure (ARF) This is a sudden and sharp decline in renal function over hours to days due to an acute toxic or hypoxic insult to the kidneys, where the GFR is reduced to less than 10 ml/minute. This syndrome has three stages which also determine the type of ARF depending on where the defect is located.

Pre-renal failure: There is a blood supply defect before it gets to the kidney. The main causes are cardiovascular system failure and consequent hypovolemia. Primary renal failure involves the kidney, common causes of acute tubular necrosis, vascular obstruction (inflammation) and glomerulonephritis. Postrenal failure: Acute renal failure occurs due to lower urinary tract obstruction or rupture of the urinary bladder. Toxic insults that are severe enough to initiate (ARF) include; hemolytic transfusion reactions, heavy metal or solvent poisoning, myoglobinuria due to rhabdomyolysis, antifreeze ingestion, and aminoglycoside and analgesic toxicities that have direct damage the renal tubules. Hypoxic insults are conditions that severely compromise

renal blood flow, e.g, burns, septic or hemorrhagic shock, and cardiac failure. In ARF oliguria and anuria (400 ml/day) are commonly observed symptoms. As a result of diminished ability to excrete water and electrolytes results in a significant increase in extracellular fluid volume, which leads to peripheral edema, congestive heart failure and hypertension. However, this condition is potentially reversible, with serum creatinine measurement being a monitoring parameter to follow the changes in glomerular filtration rate (GFR) (Saleem, 2019).

Uremic syndrome (US): This is a group of physical signs, symptoms and abnormal findings on diagnostic evaluations that results from the kidney performing its core functions, that is, excretory, regulatory and endocrine functions (den Bakker *et al.,* 2018). US is considered as the terminal manifestation of kidney failure. The classical features of uremia (azotemia) are progressive weakness, easy fatigability, wasting, nausea and vomiting, loss of appetite, abnormal mental function, tremors, frequent and shallow respirations and metabolic acidosis. When a patient develops this condition, he eventually ends up in stupor, coma and then to ultimately death unless intervention is provided by either dialysis or a successful kidney transplant (Delaney *et al.,* 2008).

Chronic renal failure (CRF) or chronic kidney disease (CKD) encompasses a number of pathophysiologic processes that are associated with abnormal kidney function that progressive result to decline in glomerular filtration rate (GFR). Its prudence to monitor the risk factor of CKF even in individuals with

normal GFR. Risk factors are autoimmune disease, hypertension, diabetes mellitus, older age, family history with renal problem, African ancestry, previous episode of acute renal failure, presence of proteinuria, and structural abnormalities of the urinary tract and abnormal urinary sediment (De Candia, 2019). There is an increasing incidence of CKD due to increased diabetes, obesity, aging population, and metabolic syndrome. Patients who have been diagnosed with type one diabetes, will progressively have their kidney function deteriorate (diabetic nephropathy) within 15-20 years after diagnosis. Diabetes affects kidneys by causing them to become glycosuric, nocturic and polyuric. These are caused by the heavy demands made on the kidneys to diurese hyperosmotic urine (den Bakker *et al.*, 2018). Sometimes this may be accompanied by mild proteinuria (micro-albuminuria) which develops between 10 and 15 years after the original diagnosis. This is followed by hypertension. Early treatment of diabetes that focuses on tight control of blood glucose and prevention of high blood pressure may prolong the onset of chronic renal failure. In addition to cardiovascular disease, there is an increase in blood creatinine due to failure in GFR and decreased excretion of waste product by the kidney (De Candia, 2019).

2.6.2 Prevention of End -stage renal disease (ESRD)

KD progression to ESRD, the following interventions are essential to stabilize or slow renal function decline. 1) Protein restriction, 2) Reduction of intraglomerular hypertension, 3) Slowing of progress of diabetic renal disease, 4) Patient education. This is based on clinical evidence that protein-mediated

hyperfiltration contributes to the decline in renal function in different condition of renal disease. A patient in stage 5 CKD, protein intake tends to decrease, which may result in the patient getting into a state of protein-energy malnutrition. To check on this, a protein intake of up to 0.90 g/kg per day is recommended, emphasizing proteins of high biologic value to prevent protein-calorie malnutrition. Increased intraglomerular filtration pressures and glomerular hypertrophy that develops due to nephron number loss from different kidney pathological conditions. Control of systemic and glomerular hypertension is not essential in reducing cardiovascular disease risks but also slows nephron injury progression by reducing intra-glomerular hypertension.

Patient education is essential as it prepares patients with an intensive educational program that help explain the right time of initiation of renal replacement therapy and other available therapy forms. The more the client is knowledgeable about hemodialysis (both in-center and home-based), peritoneal dialysis, and kidney transplantation, the easier and more appropriate will be their decisions. This kind of approach becomes beneficial to the society as home-based therapy is less expensive and is associated with improved quality of life (Verberne, *et al.,* 2021).

2.6.3 Renal panel test

Clinical chemistry plays a magnificent role in investigating and managing kidney pathological disorders. The commonly Laboratory diagnostic profile tests that forms part of kidney diagnosis include: - electrolytes (sodium, potassium and chloride), blood urea / blood urea nitrogen, creatinine and

creatinine clearance. Sodium is the most abundant extracellular cation and, is the primary determinant of serum osmolarity. As serum sodium increases, osmolality increases; and vice versa. Determination of sodium levels reflects the amount of sodium in relative to volume of water in the body which is mediated by ADH, thirst and the kidneys. Elevated levels are hypernatremia and converse are hyponatremia which are pathological states which are indication of water imbalance. Excess water loss results to hypernatremia, while impairment of ADH and renal water excretion will result to hyponatremia (Morais and Biondo, 2012).

Potassium is an essential in muscle and nerve activities and is a major intracellular cation with 95% being found in the cells and 5% is found in blood. The pathological conditions that are associated with potassium are either hyperkalemia (elevated levels) or reduced levels (hypokalemia). These pathological conditions are caused by changes in potassium intake, altered excretion or transcellular shifts. The use of diuretic and gastrointestinal losses is the common causes of hypokalemia, while medication, kidney disease and hyperglycemia are common causes of hyperkalemia. In severe potassium pathological disorders do lead to life-threatening cardiac conduction and neuromuscular dysfunction (Viera and Wouk, 2015).

Urea is generated by urea cycle enzymes found in the liver but also ubiquitously expressed at low levels in other tissues. Metabolic process of urea is altered by factors such as hormones, diets and diseases. Elimination of urea is through fluids, major being urine. For decades, blood urea nitrogen (BUN)

has been used to evaluate renal function, but this have been extended to other systems such as circulation, digestive, respiratory, and nervous systems, which have suggested clinical significance of urea (Dewitte *et al*., 2012) . Urea is the pathway for nitrogen excretion, this occurs when 60% of glomerular must be destroyed to have a medically significant rise. There are conditions such as intravenous infusion, pregnancy, and inappropriate antidiuretic hormone secretion (ADH), all of which are due to increased glomerular filtrate rate (GFR). Low BUN levels are found in children due to use of amino acids in protein anabolism during growth, severe liver pathological conditions, low protein intake and inborn errors of urea cycle (Song *et al.,* 2019) .

Chloride is the most abundant anion in the extracellular fluid (ECF). Chloride determination is essential in differential diagnosis of acid-base disturbance in calculating the anion gap. Chloride is involved in maintenance of osmotic pressure, water distribution and anion-cation balance in extracellular fluid compartment. Elevated levels of chloride (hypochloremia) are associated diabetic ketoacidosis and renal kidney failure, while reduced levels (hyperchloremia) is metabolic acidosis accompanied by prolonged diarrhea, dehydration, acute renal failure loss of sodium bicarbonate and renal tubule acidosis (Kiutts and Scotts, 2008 , Wong *et al.,*2018). Bicarbonate (HCO3) is important anion in maintaining acid-base homeostasis, and is second most abundant in ECF. The regulation of HCO3 is happens in is reabsorbed by the proximal tubules at 85% and the remaining percentage at the distal tubules where it's reabsorbed as CO_2. This is facilitated when HCO_3 combines with H

to form carbonic acid that dissociates into H_2O and CO_2. The formed CO_2 then diffuses back to ECF. In alkalosis, an increase in HCO_3 compared to CO_2, and then the kidneys will initiate increase HCO_3 excretion in the urine, thereby carrying along with sodium. This is essential in maintaining the body PH. When the H excretion is increased, then the body internal environment becomes acidic (acidosis). The common causes of metabolic alkalosis are hypokalemia severe vomiting and excessive alkali consumption (Signorelli *et al.,* 2020).

2.7.1 Pancreas and related disorders

Pancreas is a large gland involved in digestive process, but located outside of the GI system and composed of both endocrine and exocrine tissue. The endocrine purpose is production of glucagon and insulin hormones involved in carbohydrate metabolism while exocrine produces enzymes used in digestive processes (Miller, 2020). Diseases that affect the pancreas are; 1) Cystic fibrosis (fibrocystic disease of the pancreas and mucoviscidosis) a disorder characterized by dysfunction of mucous and exocrine glands throughout the body, 2) Pancreatitis (inflammation of the pancreas) which is caused by auto digestion of the pancreas by reflux of bile or duodenal contents in the pancreatic duct, resulting to acute edema, 3) Pancreatic carcinoma- cancer that affects the head of the pancreas. Signs of pancreatic carcinoma are jaundice, weight loss and nausea (Walsh, 2008) . Depending on etiology and clinical picture, amylase and lipase are enzyme are elevated with pancreas disorders, thus are used in evaluating pancreatic functions (Hoenerhoff and Pandiri 2019).

2.7.2 Glucose metabolism

Glucose is the main source of energy for life processes and is the main end product of carbohydrate digestion by oxidation process via tri-carboxylic acid and glycolytic pathways. When the body does not need the energy immediately, glycogenesis takes place. This is where the glucose is converted to glycogen for storage in muscles and the liver and muscles. When the body is in dire need of glucose, stored glycogen is converted back to glucose (glycogenolysis). Glycogen in muscle, provides the glucose for muscular activity, the excess glucose is oxidized to fatty acids and stored as fat in the tissues. Fats and protein can also be source of glucose in case of need (gluconeogenesis). When the body has an increased breakdown of fats to provide energy it also results in increased ketones production (Hoenerhoff and Pandiri 2019). Regulation of carbohydrate metabolism (glucose metabolism), liver, pancreas among other endocrine glands is involved in regulating of blood glucose levels within a narrow range. In case of fasting, glucose supplied to ECF from liver is through glycogenolysis but if the fasting period prolongs to more than a day gluconeogenesis takes place. Blood glucose Concentration is controlled by two major hormones: glucagon and insulin produced by the pancreas. Insulin and glucagon hormones work by opposing action of each other (Petersen *et al.*, 2017)

Disorders of glucose metabolism are evaluated by plasma or blood glucose measurement. In management of blood glucose levels in diabetic patients helps to prevent or delay the development of complications which may lead to

kidney failure, stroke, coronary thrombosis, blindness, bacterial infections (particularly mycobacterial and anaerobic infections), fungal infections, premature disability and eventually death (Monica, 2009 , Pak *et al.,* 2021). The important concern on glucose metabolism disorders is diabetes. There four types of diabetes mellitus that differ in clinical course and treatment, with key classification being; Type 1 diabetes (insulin-dependent diabetes mellitus), Type 2 diabetes (non-insulin dependent diabetes mellitus), Gestational diabetes mellitus and Diabetes mellitus associated with other conditions or syndromes (Association, 2010).

2.7.3 Diabetes mellitus

Diabetes mellitus is a group of metabolic disorders of carbohydrates metabolism in which glucose is underutilized resulting to hyperglycemic condition which may be life threatening such as hyperosmolar coma or ketoacidosis. With prolonged disorder of diabetes, some individual may end up with neuropathy (nerve damage) that causes cardiovascular disorders, sexual dysfunction, gastrointestinal, genitourinary symptoms and retinopathy that may end up with loss of vision (Sacks, 2008 , Okur *et al.*, 2017). Deficiency of insulin action or insulin secretion due to pathological processes do result to development of diabetes due to destruction of β- cells of the pancreas (De León *et al.,* 2006). This causes elevation of blood glucose levels, decreased glucose metabolism in body tissues, increased glycogenolysis, and stimulation of gluconeogenesis. The pathological symptoms associated with this condition are excessive thirst, pruritus, polyuria, and unexplained weight loss. The disease

usually is detected from complications that are associated with untreated diabetes or may also be discovered accidentally during random blood glucose evaluation or may be detected from the complications associated with untreated diabetes (Monica, 2009 , Okur *et al*., 2017).

Genetic predisposition is attributed to the major factor of diabetes disease, along with diet, environmental factors, diet, geographical location and lifestyle (inactivity) (Cruickshank *et al.*, 2001). Diabetes is termed a "disease of opulence" as it's more prevalent among the powerful and the wealthy and is more pronounced in urban areas as the population there is physically less active, eating junk foods, diet rich in saturated fats and refined sugars. Obesity is the most significant contributors to increased prevalence of diabetes, thus "diabesity (International Diabetes Federation (IDF) (Khalil *et al.*, 2018), in rural and urban areas. Urban setting also presents an increased prevalence of obesity compared to rural settings (Kamadjeu *et al.*, 2006 , Hossain *et al.*, 2007). In Africa, World Health Organization (WHO) estimates over a third of women are obese compared to one-fourth of the men, with the poor being as vulnerable as the rich (Hasan 2017).

2.7.4 Classification of diabetes mellitus and other categories glucose of intolerance

Kumar and Kumar (2020) have categorized hyperglycemic disorder into seven sub-types; they include, Juvenile (insulin dependent diabetes mellitus (IDDM), non-insulin dependent diabetes (adult-onset diabetes), gestational diabetes, impaired fasting glucose, impaired glucose tolerance and potential abnormality of glucose tolerance. Type 1 diabetes is characterized by inappropriate

hyperglycemia primarily due to pancreatic islet -cell destruction. This is estimated to be 5-10% of all diabetes cases. It's characterized by insulinopenia (deficiency of insulin). The peak incidence of this type of diabetes being in children and adolescents with 75% of those affected before their 30th birthday (Barbara and Maschak-Carey, 2015, Freeman, 2010). Insulin dependent diabetes is found in people with certain HLA (human leukocyte antigen) types which are a cluster of genes responsible for transplantation antigens and other immune processes.

IDDM signs and symptoms include polyphagia (increased food intake), polydipsia (excessive thirst), polyphagia, polyuria (excessive urine production), hyperventilation, mental confusion and coma due to increased glucose in the brain). Idiopathic type 1 diabetes is a form of diabetes whose etiological cause is unknown and does not have β-cell autoimmunity but is genetically inherited. Individuals with type 1 idiopathic condition are manelderly by episodic insulin replacement. Type 2 diabetes also known as non-insulin dependent diabetes mellitus (NIDDM) is characterized by impaired insulin secretion and insulin resistance (Kumar and Kumar, 2020).

Insulin resistance is where there is decreased tissue sensitivity to insulin. This pathological condition comprises of 90% of all diabetic cases. NIDDM patient have minimal symptoms, not prone to ketosis and not insulin dependent. Type 2 diabetes goes undiagnosed for a couple of years. It's generally genetically predisposed, with increased risk in obesity, advanced age, and lack of physical exercise. Type 2 DM disease is milder than type 1 with seldom ketoacidotic

conditions but prone to hyperosmolar coma and have increased risk of developing microvascular and macrovascular complications (Banday *et al.,* 2020).

Gestational diabetes mellitus (GDM) results from glucose intolerance with its onset or first detection being during pregnancy. GDM develops during pregnancy due to secretion of placental hormone that causes resistance to insulin activity but patient who suffers from this condition, always return to normal in postpartum. Women with GDM are at significant risk of developing type 2 diabetes later in life. Infants born to mothers with diabetes are at increased risk for respiratory distress syndrome, hyper-bilirubinemia and hypocalcemia are all conditions that are associated with infants born to mothers with GDM. Fetal insulin secretion is stimulation of fetal insulin secretion is noted in neonate of mothers with gestational diabetes, but upon delivery, and the umbilical cord is severed, the infant's oversupply of glucose is abruptly terminated, causing severe hypoglycemia (Barbara and Maschak-Carey, 2015).

Impaired glucose tolerance (IGT) is a condition that is diagnosed in people whose fasting blood glucose levels are less than those required for diagnosis of diabetes mellitus but in plasma this is confirmed when OGTT is performed, the blood glucose is between normal and diabetic state. Impaired fasting glucose (IFG) this condition is similar to IGT and is diagnosed by determining fasting glucose concentration and the values are above the normal but below values that are indicative of diabetic levels (Chai *et al.,*2017).

2.7.5 Clinical manifestationss

Three Ps clinical manifestations is common in all diabetic condition. They include polyphagia, polyuria and polydipsia. Polydipsia (increased thirst) and Polyuria (increased urination) occur due to excess loss of fluid associated with osmotic dieresis. Polyphagia Increased appetite (polyphagia) occurs due to a catabolic state induced by insulin deficiency and the breakdown of fats and proteins. In addition to the above common symptoms at varying degrees are sudden vision changes, fatigue, skin lesions numbness of hands and feet, weakness, dry skin, wounds that take long to heal and recurrent infections (Barbara and Maschak-Carey, 2015).

2.7.6 Hypoglycemia

Hypoglycemia (abnormally low plasma glucose level) involves decreased blood glucose to less than 50-60 mg/dl (2.7-3.3 mmol/l). Hypoglycemia is normally due to high insulin levels, oral hypoglycemic agents, excessive physical activity, or too little food. Low blood glucose concentration occurs before meals, delayed meals or snacks are omitted. Mid-morning hypoglycemia occurs when the morning regular insulin is peaking, whereas afternoon hypoglycemia coincides with the morning NPH or Lente insulin peak. Middle-of-the-night hypoglycemia may occur because of peaking evening or pre-dinner NPH or Lente insulin in patients who have not taken bedtime snack. Symptoms that are associated with hypoglycemia are nausea

and sweating, vomiting increased hunger, nausea, dizziness, nervousness and shaking, mental confusion, blurring of speech and sight (Chai *et al., 2017*).

2.7.7 Glucose diagnostic profile

Laboratory diagnostic profiles that are used to evaluate hypoglycemic and hyperglycemic are random blood sugar (RBS), fasting blood glucose (FBG), oral glucose tolerance test (OGTT) and oral glucose screening test (OGST) (Mathew and Tadi 2020). In each of the above diagnostics to successfully evaluate plasma or whole blood sugar levels, certain conditions must be met. To be successful in fasting blood sugar (FBS), patients must abstain from taking food for at least eight hours except water. It's easier and cheap to perform FBS after overnight fasting as it confirms fasting duration. FBS is not affected by activities surrounding the patient or age. Where there is need to verify the results, repeat test is prompt. Blood glucose exceeding 126 mg/dl (7mmol/l) on more than one occasion are diagnostic of being diabetic (Freeman, 2010 , Mathew and Tadi 2020).

OGTT (Oral Glucose Tolerance Test) is superior to fasting glucose test. It's performed in the morning at around 9am to avoid glucose tolerance exhibiting a diurnal rhythm with a remarkable decrease in the afternoon (Mathew and Tadi 2020). OGTT procedure is inconvenient to patients. It's recommended that the patient be ambulatory and on a normal-to high carbohydrate intake for 3 days before test and should have fasted between 10-16 hours. During the test, the patients are advised to refrain from eating, exercising, drinking (except plain water), and smoking.

Oral glucose tolerance results are affected by medications (large doses of oral contraceptives, salicylates, corticosteroids, diuretics, anticonvulsants. gastrointestinal problems, (malabsorption problems and gastrointestinal surgery) endocrine dysfunctions and vomiting. American diabetic association recommends that only fasting and a serial measurement of plasma glucose after the patient is given the recommended glucose orally. A 2-hour sample should be measured, except when the client is pregnant. Adults are given a glucose solution (glucola) is 75 g; while children receive 1.75 g/kg of glucose to a maximum dose of 75 g (Freeman, 2010 , Chai *et al.*,2017). Two occasional tests should be performed for OGTT results to be conclusive, unless initial results are grossly abnormal (Freeman, 2010).

Glycosylated hemoglobin (GHb)/hemoglobin A1c ($HbA1_c$) this is a method used to monitor blood sugar when an amino group of hemoglobin (a protein) reacts with glucose (a reducing sugar). Glucose molecule attaches non-enzymatically to a hemoglobin molecule to form a ketoamine whose formation rate is directly proportional to glucose concentrations. Glycosylated hemoglobin level reflects the average plasma glucose concentration of previous 2 to 3 months, as the average lifespan for red blood cells is roughly 120 days. Therefore, measuring the glycosylated hemoglobin provides the clinician with a time-aver elderly picture of the patient's blood glucose concentration over the past 3 months. GHb is useful in evaluating management of diabetes which is to maintain the blood glucose levels within normal or near the non-diabetic

reference range with a minimal number of fluctuations (Barbara and Maschak-Carey, 2015, Chai *et al.,* 2017).

2.8 Uric acid metabolism and related disorders

In man uric acid is produced as the end product of endogenous and exogenous pool of purine that involves complex processes that regulate hepatic production, renal and gut excretion. Exogenous pool varies with diet and animal proteins contribute to amount of purine pool (Chaudhary *et al*., 2013 , Mandal and Mount, 2015).

Uric acid is a $C_5H_4N_4O_3$ (7, 9-dihydro-1H-purine-2, 6, 8(3H)-trione) heterocyclic organic compound with a molecular weight of 168 Da. Uric acid is filtered by glomerulus, secreted by distal tubules into urine, reabsorbed at the proximal tubules, and reused. Due to it characteristic of uric acid being insoluble in plasma when in high concentrations, it is deposited in tissues and joints causing painful inflammation. Uric acid is used to assess and diagnose gout, inherited disorders of purine metabolism, renal calculi, prevent uric acid nephropathy during chemotherapeutic treatment, and detect kidney dysfunction (Ahmed *et al*.,2018).

2.9 Thyroid hormones and associated disorders

Thyroid hormones have several biological effects major being; control of basal metabolic rate, promote of sexual maturation, stimulation of neural and normal growth, increase vitamin requirement, stimulate protein synthesis and carbohydrates metabolism, increase synthesis and degradation of cholesterol and triglycerides, stimulation of adrenergic activity with increased heart rate

and myocardial contractility, increase in calcium and phosphorous metabolism in addition to enhancing the sensitivity of adrenergic receptors to catecholamine (Grotzke, 2010 , Joan, 2010, Biondi *et al.*,2019).

These effects are evident when the thyroid activity is either reduced (hypothyroidism) or overactive (hyperthyroidism) (Jang *et al.,* 2018). Thyroid hormone circulates as free T3 and T4 in the bloodstream and can move across the cell membrane. T4 is deiodinated into T3 in the cytoplasm which is the active form of thyroid hormone. Production of proteins that are triggered by the combination of T3 with nuclear receptors on thyroid hormone that are responsive genes which influences metabolism and development. Clinically, patients who have thyrotoxicosis (excess thyroid hormone) will present with increased metabolism such as tremor and tachycardia, while those individuals with hypothyroidism will have lowered metabolism like constipation and edema (Grotzke, 2010). Clinical Manifestations in infants appear normal at birth, but less than 10% are diagnosed based on clinical features which may include prolonged jaundice, hypotonia (abnormal loss of muscle tone), feeding problems, enlarged tongue, umbilical hernia and delayed bone maturation. Where treatment is delayed, permanent neurologic damage is prominent. Cardiac malformation is four times common in congenital hypothyroidism (Jang *et al.,* 2018).

Hypothyroidism results due to suboptimal levels of thyroid hormone. Thyroid deficiency leads from mild, subclinical forms to advanced form (myxedema), Hashimoto’s (autoimmune thyroiditis) in adults is the prominent medical

condition where the immune system attacks the thyroid gland. Hyperthyroidism is generally followed by hypothyroidism and myxedema especially in patients who have been on medication with antithyroid or radioiodine medications or those who had surgical interventions who have had surgery and is mostly found in older women (Joan, 2010). Thyroid cancer is common in feminine in their third to fifth decades of the life. Mostly found at the neck region and is rarely painful fine needle aspirate in addition to radiological studies are used to diagnose thyroid cancer (Sokoll and Chan 2021).

2.9.1 Thyroid stimulating hormone (TSH)

TSH regulates thyroid gland function through the TSH-R, a seven-trans-membrane G protein–coupled receptor (GPCR). The TSH-R is coupled to the subunit of stimulatory G protein (G_s), which activates adenylyl cyclase, leading to increased cyclic AMP production. TSH-R functional role is affected by the consequences of naturally occurring mutations. Recessive loss-of-function mutations cause hypoplasia and congenital hypothyroidism, while dominant gain-of-function mutations results to familial or sporadic hyperthyroidism which is characterized by thyroid cell hyperplasia, goiter and autonomous function (Grotzke, 2010 , Muthamizhveena, 2017).

Physiological changes in pregnant women alter Thyroid Function. These factors include; estrogen raises thyroxine binding globulin (TBG) in the first three month of pregnancy alteration of immune system, thus resulting to autoimmune thyroid diseases, increased metabolism of thyroid hormone by the

placenta and increased iodide excretion via urine which results to reduce thyroid hormone production especially in areas with iodine insufficiency. This is common in women and results to goiter in pregnancy. Iodine supplementation is used as a remedy to fetal and maternal hypothyroidism and reduces the development of neonatal goiter (Jang *et al.,* 2018).

TSH falls in the first trimester as circulating hCG levels rises and this persists in the middle of pregnancy. This is due to weak binding of hCG, whose levels are higher than TSH-R. Changes that are hCG-induced to thyroid function result in transient gestational hyperthyroidism or hyperemesis gravidarum, a condition characterized by severe vomiting and nausea that puts one at risk of volume depletion. This is corrected with parenteral fluid replacement till the condition resolved. In 2-3% of women of childbearing age experience maternal hypothyroidism and this is associated with increased risk of delayed development in offspring. TSH screening becomes a curtail diagnostic tool for hypothyroidism that is common in early pregnancy, especially for women who are planning pregnancy, particularly those with a strong family history of autoimmune thyroid disease or goiter (Jang *et al.*, 2018).

2.9.2 Triiodothyronine (T3)

Triiodothyronine test is a diagnostic and monitoring parameter to evaluate hyperthyroidism or in addition to determine its severity. The majority of Patients suffering from hyperthyroid have elevated T3 levels but some have a low TSH, and normal FT4, and the only elevated parameter is T3. As far as hypothyroidism is concerned; T3 evaluation is rarely helpful as it remains

normal for a long time. For Patients can have a severe hypothyroidism with a high TSH, low FT4, but with normal T3 levels, hence a full thyroid profile is essential in the evaluation of thyroidism (Laurberg *et al.,* 1998). T3 values are age specific. Lower levels are observed in patients over seventy years of age, compared to the younger population. This is due to reduced peripheral conversion of T4 to T3 with advance with age (Dayan and Panicker 2018).

2.9.3 Tetraiodothyronine or Thyroxine or (T4)

In blood thyroxine circulates in two forms as T4 bound to proteins and free T4. Free T4 fraction is the most crucial in determining thyroid functions and free T4 measurement is used. FT4 is elevated in Individuals with hyperthyroidism while those with hypothyroidism will have low FT4 levels. For accurate thyroid function testing, combining of the FT4 and TSH tests is essential. Elevated TSH with a low FT4 level indicates primary hypothyroidism due to diseased thyroid gland, while low FT4 and low TSH indicate hypothyroidism due to pituitary gland problems. Elevated FT4 with A low TSH is common phenomena with patient suffering from hyperthyroidism (Dayan and Panicker 2018). Graves' disease is the most common hyperthyroidism due to excessive production of thyroid hormones that is triggered by abnormal stimulation of thyroid gland by circulating immunoglobulin. Graves's disease affects women at 80% than men, with its onset between second and fourth decades (Tierney *et al.,* 2001 , McPhee *et al.,* 2010). Elevated levels may appear after stress, infection, emotional shock or stress. Other causes of hyperthyroidism are

excessive ingestion of artificial thyroid hormone and thyroiditis (Spencer, 2017).

2.10 Causes of Cancer

Cancer is the term applied to a group of diseases in which cells no longer respond to normal restraints on growth. Normal cells in the body respond to signals, such as contact inhibition, that direct them to stop proliferating. Cancer cells do not require growth stimulatory signals and they are resistant to growth inhibitory signals. They are also resistant to apoptosis, the programmed death process whereby unwanted or irreparably dam elderly cells self-destruct. They have an infinite proliferative capacity and do not become senescent (i.e., they are immortalized) (Chan *et al.,* 2008, Vineis and Wild, 2014 , Abbas and Rehman 2018). Furthermore, they can grow independently of structural support, such as the extracellular matrix (loss of anchorage dependence). A single cell that divides abnormally eventually forms a mass called a tumor. A tumor can be benign and harmless; the common wart is a benign tumor formed from a slowly expanding mass of cells. In contrast, a malignant neoplasm (malignant tumor) is a proliferation of rapidly growing cells that progressively infiltrate, invade, and destroy surrounding tissue (Rokita and Sindy, 2010). Most cancers are also preventable (Golemis *et al*., 2018).

Tumors develop angiogenic potential, which is the capacity to form new blood vessels and capillaries. Thus, tumors can generate blood supply to bring oxygen and nutrients. Cancer cells also can metastasize, separating from the growing mass of the tumor and traveling through the blood or lymph to

unrelated organs, where they establish new growths of cancer cells (Fauci *et al.,* 2008). Causes of cancer are divided into two, depending on cause, (1) genetically which causes about 5-10% (Mattiuzzi and Lippi, 2019) (2) Others are environmental factors, e.g. radiation, diet, lack of physical exercises, tobacco and environmental pollutants (Gorlova *et al.,* 2007). Symptoms are the first indicators or suspect of presence of cancer. However, a definite diagnosis is done with examination of a biopsy microscopically. Healing of cancer depends on stage of the cancer and the type of cancer. The commonly used method in cancer management is surgery, chemotherapy or radiotherapy. In medicine different cancers have been reported, but the common ones include leukemia, breast cancer, prostate, lung cancer, ovary and pancreas (Yagawa *et al.,* 2017).

2.10.1 Cancer Markers

Tumor markers are produced either directly by the tumor or as an effect of the tumor on healthy tissue thus they are used for screening, prognosis diagnosis, therapy monitoring, and eventually detecting recurrence. A variety of enzymes are elevated nonspecifically in tumors due to proliferative cells. Although cancer may affect any age group, mostly population above 65 years of age are at a higher risk, also the incidence of cancer is higher in men than women and higher in industrialized nations (Rokita and Stern, 2010). In ascending order, Lung, colorectal cancer and prostate in men and lung, breast and colorectal cancer in women are the leading causes of death in America (Jemal *et al.*, 2010). Tumor markers are constituents of healthy cells, but they are produced

in abundance in case of malignancy. In clinical practice, cancer antigen 19-9 (CA 19-9), total prostate specific antigen (TPSA), cancer antigen 125 (CA125), Carcinoembryonic antigen (CEA) and Cancer antigen 15-3(CA 15-3) are mostly used in diagnosis and evaluating the prognosis of the disease (Nair *et al* 2018).

2.10.2 Breast cancer (CA 15-3)

Breast cancer is a malignant proliferation of epithelial cells lining the lobules or ducts of the breast. Potentially, breasts do provide common sites of fatal malignancy in women but also at lower grades in men. Breast physical examination is essential to both men and women as it gives clues of underlying systemic disorders (Chen *et al.,* 2018). CA 15-3 is a protein expressed in different adenocarcinomas, and in higher levels in breast cancer (Kesisis *et al*., 2010 , Rau *et al.,* 2012).

FDA recommends CA15-3 be used only in determining breast cancer relapses before the appearance of symptoms and in monitoring response to therapy. C15-3 has also been indicated in literature that it is elevated in non-cancerous conditions e.g. hepatitis, endometriosis, benign breast disease, and pelvic inflammatory disease . Worldwide, breast disorder is the most prevalent with over one million new cases diagnosed per year (Chen *et al.,* 2018).

2.10.3 Ovarian cancer (CA 125)

CA 125 is an antigenic glycoprotein recognized by a monoclonal antibody (OC 125). CA 125 antigenic determinant is expressed in more than 80% of non-mucinous epithelial ovarian carcinomas and most carcinomas of Mullerian

origin, such as lungs, cervical, primary serous peritoneal carcinoma, endometrial and fallopian tube.CA 125 is the marker that has been clinically accepted for diagnosis of ovarian cancer (Macuks *et al.*, 2011 , Chen *et al.*, 2018).

2.10.4 Colon cancer (CEA)

Carcinoembryonic antigen (CEA) is a glycoprotein produced by goblet and columnar cells in normal colon cells and colonic cancer cells and has a half-life of 3-11 days (Al-Shuneigat *et al.*, 2011). CEA is produced during stages of development (fetal) but it stops just before birth. Colorectal carcinoma (CRC) is one of the deadly cancers in women and men worldwide (Chan *et al.*, 2010). In United States, CRC is ranked third (Siegel *et al.*, 2015). CRC is curable if detected during the initial stage, as with other cancers (Levin *et al.*, 2011). Thus, early diagnosis is of utmost importance for better prognosis and management of CRC (Chan *et al.*, 2010 , Zeller *et al.*, 2014 , Van Schaeybroeck 2014, Zhang *et al.*, 2015).

2.10.5 Pancreas, Lungs, Ovary Cancer Marker (CA 19-9)

CA 19-9 is a glycosylated protein that is produced by adenocarcinomas of the ovary, pancreas, colon, stomach, gall-bladder, and lung. CA 19-9 levels are also high with patients with hepatocellular and bile duct cancer. Literature do indicate that CA 19-9 levels are associated with advance of the disease. This also correlates well with the stage of disease and the ability for successful surgical intervention. CA 19-9 levels may also be detected in elevated patients with benign pancreatic or liver disease (Dong *et al.*, 2014 , Zhang *et al.*, 2015).

Other conditions that are Non-cancerous and have been associated with elevated CA 19-9 levels are cholecystitis, gallstones, cirrhosis of the liver and pancreatitis (Chen *et al.,* 2018).

2 10.6 Prostate Cancer (PSA)

Prostate specific antigen (PSA) is a glycoprotein that produced by prostate cells is found in small levels in semen as a normal component. In abnormal prostate events, e.g., overproduction of PSA, prostate cancer, or overabundance of prostate cells, PSA leaks into the bloodstream, resulting in measurable levels of PSA in the blood, which is considered to be most effective method of prostate cancer detection. The rising PSA levels can be due to localized or metastatic cancer of the prostate Chen *et al.,* 2018). Prostate cancer is a malignant tumor that affects the urinary system. In recent years, there is increased incidence and mortality due to prostate malignancy (Chang *et al.,* 2015 ,Koo *et al.,* 2015). Prostate cancer affects mostly men of over fifty years of age. In most cases, this type of cancer is slow growing and symptom free. Due to the advanced age of the patients, most die of other conditions related to ageing and not from prostate cancer (Munteanu *et al.,*2020).

2.11 Quality control

Analytical stages cover the principle of the test method, reagents, standard control material and equipment used. Quality control procedures are used to detect and minimize errors in the performance of tests. This in turn, ensures the results are reliable, precise and accurate (Monica, 2009). Control is evaluated using a quality control chart (Levy Jennings control chart) prepared daily.

Control values within ± 2 SD are a good sign; the results produced are reliable and, therefore can be reported confidently (Katayev and Fleming, 2020).

Developing countries have been found to have problems in issuing accurate results. (Gitimu et al., 2016) found the variation of the results in Kenya. This was also recorded in Canada, where quality control is performed and the results have been wanting. This has necessitated the building of reliable quality control systems in order to report excellent results assured of quality and proper documentation of tests carried out on quality control material.

Reliability and reproductivity of laboratory results are affected by the environment, laboratory materials, specimen handling, personnel, test methods, equipment, reading and reporting. To check on the above, internal and external quality procedures are employed. External quality control is an objective system of assessing the laboratory ability to produce reliable results. Participation of external quality assessments should always be regarded as additional to internal quality control (Monica, 2009 , Braga *et al.,* 2021).

Quality control involves all the procedures that are followed from collection of specimen, analysis, reporting, and dispatch of the results. This is essential to countercheck the quality of test performed. This in return assists in detecting and ratifying different procedures responsible for the errors. External quality control is performed once in a while where the control material is from outside the laboratory setting from where the values are performed and compared with what is received from the laboratory after using the same sample material, which is usually lyophilized. In this study, quality control was followed to the

latter; i.e proper collection of blood samples, to avoid hemolysis, transportation from the field to the laboratory in a cooled ice box, separation of the serum, refrigeration and the standard operating procedure (SOP) on analysis. For proper management of patients, it is of paramount importance to build reliable quality control mechanism to be able to give excellent results, and proper documentation in addition to assured quality of all procedures carried on quality control samples (Chesebrough, 2009 , Kim *et al.*, 2017).

2.12 Reference intervals Terms

Reference ranges are useful as a quality control tool in medical laboratory science (He *et al.*,2018).These are crucial to clinicians in aiding making informed decisions on subsequent steps in patient management. To achieve these harmonization and allow for universality, the International Federation of Clinical Chemistry and Laboratory Medicine has adopted definitions of standard terms when establishing normal reference interval ranges. The terms have also been certified by World Health Organization (Ozarda, 2018). The terms adopted include; Reference individual:- this is a person selected for testing on the basis of well-defined inclusion and exclusion criteria; Reference population:- A group consisting of all the reference individuals; Reference sample group:- An adequate number of persons selected to represent the reference population; Reference value:- The value (test result) obtained by the observation or measurement of a particular type of quantity on a reference individual; Reference distribution:- The distribution of reference values; Reference limit:- A value derived from the reference distribution and used for

descriptive purposes; Reference interval:- The interval between, and including, two reference limits; Observed value:- The value of a particular type of quantity, obtained by observation or measurement of a test subject (patient), to be compared with reference interval values (Ozarda *et al.*, 2018).

2.12.1 Guidelines for Establishing Reference Intervals

Clinical and Laboratory Standards Institute has published recommended steps to be followed for establishing or verification of reference intervals (CLSI, 2008 , Bishop *et al*., 2013, Ozarda *et al*., 2018). They are:

I. Compile an appropriate list of biological variations and analytical interferences from medical and scientific literature

II. Generate a selection (or exclusion) and partition principles and a suitable questionnaire designed to reveal these principles in the potential reference individuals.

III. Prepare an appropriate written consent form for participation in the reference interval study and have the reference individual complete the questionnaire.

IV. Classify the potential reference individuals based on the questionnaire findings and results of other appropriate health assessments.

V. Exclude individuals from the reference sample group based on the exclusion criteria or other assessments indicating a lack of good health.

VI. Establish an appropriate number of reference individuals in consideration of desired confidence limits.

VII. Prepare, properly and consistently, the selected persons for specimen collection to measure a given analyte consistent with the routine practice for patients.

VIII. Collect and handle the biological specimens properly and consistently with the routine practice for patient specimens.

IX. Derive the reference values by analyzing the specimens according to the respective analytical methodology under well-defined conditions and consistent with the routine practice for patient specimens.

X. Inspect the reference value data and prepare a histogram to evaluate the data distribution.

XI. Identify possible data errors and outliers.

XII. Analyze the reference values by selecting a method of estimation and estimate reference limits and the reference interval.

XIII. Document all the previously mentioned steps and procedures.

2.13 Selection of Reference Interval Study Individuals

Reference ranges can be derived from healthy blood donors direct from selected from a healthy population. Developed reference ranges help diagnose pathological conditions (Akinrinmade and Akinrinde, 2013). They may also be determined for observing a physiological state or therapeutic drugs from the relevant reference sample cluster (Bhuvanendranath, 2012 , Bishop *et al*., 2013).

In order to determine the best individuals for participation in reference intervals establishment, a researcher has to develop comprehensive inclusion

and exclusion criteria. Inclusion consists of all those factors that prequalify a study subject while in exclusion standard is made of all the factors that disqualify a study subject. To qualify as a reference interval establishment participant, an individual must meet the set inclusion parameters (Bishop *et al*., 2013, Humphries *et al.*, 2018). The principles that are engaged the elderly in any reference range establishment should be defined and put down for interested study subjects to determine the health wellbeing of the reference sample group (CLSI, 2008, Borai *et al.*, 2016). Sometimes the investigator must contemplate on partitioning criterion also it is exceptional factors among the selected reference subject which permit subdivision of the reference sample into subclasses (Bishop *et al*., 2013 , Humphries *et al.*, 2018,). While selecting reference range participants, it is required that the designate subjects bear a resemblance to the target patient population undertaking medical assessment ((Humphries *et al.*, 2018). The sampling method commonly used to determine reference sample groups may be priori or posteriori. A priori sampling method ensures pre-establishment of elaborate exclusion and partitioning criteria before selecting the individuals. In posteriori sampling method, the individuals are excluded and partitioned after analyte sampling and testing have been completed (CLSI, 2008 , Humphries *et al.*, 2018)

CHAPTER THREE

3 Materials and methods

3.1 Study site

The study was conducted in Taita Taveta county-Kenya. It lies approximately 200 km northwest Mombasa and 360 km southeast of Nairobi. It has a population of 250,000, with population densities ranging from 3 persons per km^2 to more than 800 persons per km^2. This is due to the varied rainfall and terrain, with the lower zones receiving an average of 440 mm of rain per annum and the highland areas receiving up to 1900 mm of rain. The range rises in altitude from 500 m above sea level to almost 2,300 above sea level at Vuria peak, which is the highest. The county covers an area of 17,083.9 km^2 of which a bulk 62% or 11,100 km^2 is within Tsavo East and Tsavo West National Parks. The remaining 5,876 km^2 is occupied by ranches, sisal estates, water bodies such as Lakes Chala and Jipe in Taveta and Mzima springs, and the hilltop forests, which occupy less than 100 km^2 or approximately 10 km^2 out of 587.5 km^2.

The lowland areas of the district that do not belong to national parks are divided to ranches, estates and wild life sanctuaries. The district has approximately 25 ranches. The main land use in ranch is cattle grazing. The three operating sisal estates of the district are the Teita Sisal Estate, Voi Sisal Estate and Taveta Sisal Estate. Many ranches also utilize wildlife tourism and conservation. The Taita Hills and Saltlick Lodges sanctuary is located in the district.

Sampling was done in four sub-counties of Taita Taveta: Mwatate, Taveta, Wundanyi and Voi. The main analytical centre was the Department of Clinical Chemistry, Moi Subcounty Hospital, Voi, Kenya.

3.2 Study population

The target subjects were divided into five categories. The first category that was involved in the study was recruited from the four sub-counties was those of ages between. 1-17 years (infants, children and adolescents (129 males and 132 females), the second category of pregnant mothers (296), Category three the elderly (above 55 years of age (153 males and 177 females) and fourth being of a group comprising ages 18-93 years of age (191 males and 317 females). The first category was recruited to establish reference ranges for full blood count and routine biochemical parameters. The second category (pregnant mothers) recruited from second and third trimesters to establish reference ranges for full haemogram and routine biochemical parameters. The third category comprising the geriatric population was recruited for the establishment of special and routine chemistry and full blood count parameters. The fourth category was for those under special instruction to establish normal ranges for fasting lipid profile. The fifth category evaluated normal ranges for vital signs, including temperatures, oxygen concentration, pulse rate, blood pressure and body mass index. Sensitization was done prior to the recruitment of study population. Sensitization exercise was done through medical talks in various institutions that included chief barazas, churches, blood donation centers, Taita Taveta University and hospitals maternal health clinics. All those

subjects who fulfilled the inclusion criteria and consented were recruited in the study.

3.2.1 Inclusion criteria

The study population that was recruited for the study was from Kenyan citizens living in Taita Taveta County between 1-100 years of age who were categorized into five groups and were all subjected to age and special requirements as required by the parameters under study. A questionnaire was administered to consenting study subjects to gather some socio-demographic data (Appendix I and II).

3.2.2 Exclusion criteria

Subjects whose blood specimens tested positive results for VDRL, HIV, and HBs-Ag were excluded from the study. The three conditions were screened because they could be easily be missed during the history taking and one can have the disease without having any visible signs and symptoms. Also, the diseases greatly impact the biochemical and hematological effects. Also excluded from the study were those who were on medication or those involved in excessive exercises.

3.3 Study design

This was a random cross-sectional prospective study involving 2390 healthy male and female subjects who were divided into 320 geriatric (males and females), radom blood sugar 306 participants, 261 of age 1-17 years, 272 for lipid profile, 504 for vital signs, 252 participants were recruited for body mass

indexs and 296 pregnant mothers. The study was undertaken between May 2015 and December 2017.

3.4 Ethical approval

This study was approved by Kenyatta University Ethical Committee Ref Number I84/31987/15/ NACOSTI Ref number 16/22096/14531, Taita-Taveta county medical director (Appendix III, IV and VI).

3.5 Taking of vital parameters

A Mindray automatic vital monitor (Avante Health Solution) took vital signs. Three tubes are used. Blood pressure; the patient was allowed to sit comfortably with a bare arm at heart level. The brachial artery was palpated just above the antecubital fossa and BP cuff was wrapped around the upper arm about 2.5 cm above the brachial artery, the results are given as diastolic and systolic and respiration rates. Pulse oximeter sensor was attached to a patient's finger to measure the light absorption of hemoglobin that gave arterial SpO_2; the second tube is clipped on index finger while the temperature metallic attached tube is placed at the axillary. The machine is then switched on. Upon determining the readings, the results are displayed on the LCD monitor, the results displayed are oxygen concentration in tissues (SpO_2), Temperatures in degree centigrade (^oC), pulse rate and blood pressure giving both systolic and diastolic in millimeter of mercury (mmHg). All the results were recorded in addition to age and gender.

3.6 Body mass index

Body weight was measured to ± 0.1 kg using electronic scales, standing height was measured to ± 0.001 m using a wall-mounted stadiometer (Unicef) and BMI was calculated as weight/height^2 (kg/m^2). Based on WHO criteria, underweight was identified as BMI < 18.5 kg/m^2, overweight as BMI 25.0-29.9 kg/m^2, and obese as BMI ≥ 30.0 kg/m^2

3.7.1 Collection for blood specimens for fasting lipid profile

In this study, 246 study subjects were drawn from the four sub-county of Taita Taveta. This was done in churches, chief barazas and door to door visits. Instructions were given to the study subject to fast after super. Using a five-milliliters syringe, 5 milliliter of blood was drawn from auxiliary vein of the study subject who met the inclusion criteria and was put in a plain vacutainer tube (Revital Healthcare Ltd, Kenya) for lipid profile analysis. The specimen tubes were labeled correctly with the study number and subject's name. The samples were allowed to clot then placed in a cool ice box ready to be transported to main analyzing laboratory. In the analyzing laboratory the collected specimens were then centrifuged at 3000 g for five minutes. The serum was separated with a Pasteur pipette for each specimen and transferred into two (duplicate) vials on which a bar cord was used for identification purposes. Then the samples were run using Intergra 400 auto analyzer chemistry machine (Cobras Integra 400 Roche Diagnostics Ltd, CH-6343 Rotkreuz Switzerland). Cholesterol high performance reagent which comprises of (Total cholesterol, HDL, LDL, triggycylides) was used to estimate the

cholesterol levels. The duplicate samples were then stored at -20^{o}C in case of need to rerun the any test.

3.7.2 Collection of blood for haematological parameters

In this study, 2335 study subjects were drawn from the four sub-county of Taita Taveta (Mwatate, Wundanyi, Voi and Taveta). This was done in churches, chief barazas and door to door visits. Instructions were given to the study subject to fast after super Using a five milliliters syringe, 5 milliliter of blood was drawn from venous vein of the study subject who met the inclusion criteria and was put in EDTA vacutainer tubes (Revital Healthcare Ltd, Kenya). Gentle swirling of the sample in labeled vacutainer tube was done for half a minute to mix the blood in the EDTA to prevent the sample from clotting for hematological analysis. The specimen tube was labeled correctly with the study number and subject's name and was placed in ice box ready for transport to Moi County Laboratory for analysis.

3.7.3 Collection of blood samples for routisne biochemistry and special chemistry

In this study 752 and 244 study subjects were involved for routine and special chemistry respectively were drawn from the four sub-counties of Taita Taveta. This was done in churches, chief barazas and door to door visits. Those who meet the criteria of the study they were sampled. Using a five milliliters syringe, 5 milliliter of blood was drawn from venous vein of the study subject who met the inclusion criteria and was put in a plain vacutainer tube for lipid profile analysis. The specimen tubes were labeled correctly with the study number and subject's name. The samples were allowed to clot then placed in

an ice cool box ready to be transported to main analyzing laboratory. In the analyzing laboratory the collected specimens were then centrifuged at 3000 g for five minutes. The serum was separated with a Pasteur pipette for each specimen and transferred into two (duplicate) vials on which a bar cord was used for identification purposes. Routine biochemistry tests were run using Intergra 400 (Cobras Integra 400 Roche Diagnostics Ltd.CH-6343 Rotkreuz Switzerland) auto analyzer chemistry machine, while separated samples for special chemistry were then stored at -20^{o}C awaiting analysi

3.7.4 Collection of blood samples for random blood glucose

In this study, 306 study subjects involved were drawn from the four sub-counties of Taita Taveta. This was done in churches, chief barazas and door to door visits. The procedure for Random glucose test involved testing blood glucose using capillary blood obtained by a finger prick.

3.8 Specimen analysis

3.8.1 Screening for Hepatitis B Surface Antigen (HBsAg)

The HBs-Ag one step hepatitis B Surface Antigen Test Strip (HBs-Ag, Beijing, China) was used to screen HBs-Ag. This was a qualitative lateral flow immunoassay test. The test strip was immersed in a tube containing the serum for screening for 10 to 15 minutes. It was then removed and placed on a non-absorbent flat surface and the results read within 15 minutes. Positive result was indicated by the appearance of two distinct red bars, one on the control region and the other on the test region. Negative results were indicated by the appearance of only one red bar at the control window.

3.8.2 Random blood glucose analysis

In the study 306 samples were analysed with an automated auto care machine (on call plus- Germany) and Acon glucostrips (America) were used. The results were recorded in excel spread sheet as per age and gender.

3.8.3 Routine biochemical parameters

The collected blood-separated serum was analyzed for alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, potassium, γ-glutamyl transaminase, chloride, albumin, total bilirubin, direct bilirubin, creatinine, sodium, blood urea nitrogen, total protein, phosphorus and amylase These were analysed with biochemical reagents from Span Chemic (India). The serum was used for the screening for syphilis, hepatitis and HIV.

3.8.4 Analysis of special chemistry parameters

The collected samples were analyzed for fasting triglycerides, total fasting cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total prostatic specific antigen (TPSA), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), cancer antigen15-3 (CA 15-3), carcinoembryonic antigen (CEA) and Thyroid profile Tri-iodothyronine (T3), Tetraiodothyronine (T4) and Thyroid stimulating hormone (TSH). The thyroid profile was determined with reagents from Span chemic laboratories (India) while anti-(TSH, T4, T3, TPSA, CA125, CEA (Pishtaz Teb Diagnostics /Germany), CA 15-3, and CA 19-9 Cortez Diagnostic California USA).

3.9 Equipment that was used in the analysis

The machine used for the specimen analysis was Clinical Chemistry Autoanalyzer (Cobras Integra 400 Roche Diagnostics Ltd CH-6343 Rotkreuz Switzerland)). The Integra 400 is a discrete, Random Access Clinical Chemistry analyzer capable of performing a wide range of chemical tests in a single run. Chemwell (United States of America**)** was used to analyze tumor makers, while stat fax 4200 was used as a backup in case of breakdown of Chemwell Auto-analyzer. A glucometer (On Call Plus glucose meter from Roche Diagnostics GmbH, Mannheim, Germany) was used Random glucose analysis. For hematological analysis, Mindray (Coulter Ac T differential, Beckman Coulter, Miami, FL mindray BC 5200) five-part Auto Analyzer was used.

3.10 Reagents preparation

All reagents that were used with Integra 400 auto analyzer, chemwell for clinical chemistry and B C 5200 Mindray hematological auto machine were commercially prepared to fit the required concentrations and volumes. Reagents used in Integra 400 chemistry auto analyzer machine were in specific reagent containers referred to as “reagent cartridges”. The reagent cartridges were bar-coded for identification by the chemistry machine. User-defined chemistry programme was used for those reagents, which were not bar-corded. Biosensor strips (on call plus Germany) were used with a glucometer to analyze glucose.

For hematology auto analyzer, (BC 5200) uses ready to use. This is done by commanding the machine using the operational window to acctualise the machine and upload a new set of reagents (lyse, diieunt and cleaner). Before running any sample, the machine was primed and calibrated using control sample and the result was recorded (quality control).

3.11 Machine calibration

To ensure accurate and precise results that are recovered from analysed patient sample, it's of paramount importance that the machine calibration is performed before any procedure is undertaken to analysis of different parameters. The purpose of the calibration procedure was to determine the relationship between measured absorbance and known concentration of these same analytes contained in calibrator solutions. After achieving the calibration, the factors were installed and the required tests were analyzed. These were done for all the machines (Intergra 400, Chemwell and BC 5200 mindray hematological auto-analyzer).

3.12 Quality control (QC)

Normal multi-sera were used for the quality control for the analytical work during the study period. The QC multi-sera were either ready to use or in lyophilized form. Those that were lyophilised were reconstituted as per the manufacturer's instructions. CBctrol was used with intrgra 400 for clinical chemistry while Bc-30 hematology control was used wit BC 5200 auto analyzer. The prepared quality control multi-sera was used to perform internal quality control assessment or any other time the study procedures were

undertaken. HIV, VDRL and Hepatitis where test strips that had their control incorporated into the test strip (Kim *et al.,* 2017).

3.13 Analytical methods for haematological parameters

Automated equipment: The system measures those parameters plus conductivity cell volume, packed cell volume ratio, platelets count, and leucocyte differential count based on the cytochemical staining reaction of different white cells. Hematocrit is measured by automatic centrifugation. The counter is designed that employ a mercury manometer, a specific volume containing particles of an electrolyte (0.9% sodium chloride) is forced through an aperture of specific dimension. An aperture tube and another outside the aperture. As a particle passes through the aperture, it lowers the electrolytic conductivity between the two electrodes, producing an impulse whose magnitude is directly proportional to the particle's volume. The voltage pulses are fed into a threshold circuit that discriminates between pulses of different sizes, generating impulses for those particles that exceed the threshold level alone. The results are displayed visually and then digitally printed.

3.13.1 Hemoglobin

Whole blood was diluted 1 in 201 in a modified drabkin’s solution which contains potassium ferricyanide and potassium cyanide. The red cells are Haemolysed, and the ferricyanide oxidizes the hemoglobin to methaemoglobin. The cyanide converts this to stable hemiglobincyanide (HiCN). Absorbance of the HiCN solution is read in a spectrophotometer at wavelength 540 nm. The absorbance obtained is compared with that of a reference HiCN standard

solution. Hemoglobin values are obtained from tables prepared from a calibration graph using a direct readout hemoglobin meter and the results are digitally displayed.

3.14. Analytical methods for biochemical parameters

3.14.1 Glucose (GLU)

Glucose oxidase method

Glucose reagent was used to measure glucose concentration by a timed end-point reaction method. In the reaction, glucose oxidase (GoD) catalyzed the oxidation of glucose to gluconic acid with the formation of hydrogen peroxide (H_2O_2). The H_2O_2 reacted with phenol and 4-aminophenazone under the catalysis of peroxidase (POD) to form a red–violet quinoneimine which was directly proportional to the glucose concentration in the sample. 2µl of the sample was reacted with 300 µl of reagent and the change in absorbance was monitored at 500 nm. This change was directly proportional to the concentration of GLU in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37^{o}C for three and half minutes.

Principle of the reaction

$$\text{Glucose} + O_2 + H_2O \xrightarrow{GoD} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{POD} \text{quinoneimine} + 4H_2O.$$

3.15.1 Triglycerides (TG)

This was based on a series of coupled enzymatic reactions. Triglyceride in the sample was hydrolyzed by lipase to give glycerol and fatty acids. ATP

phosphorylated the glycerol in the presence of glycerol kinase (GK) and Mg^{2+} to produce glycerol-3-phosphate. Glycerol-3-phosphate was oxidized in the presence of glycerol phosphate oxidase (GPO) to produce hydrogen peroxide (H_2O_2) and dihydroxyacetone phosphate. The hydrogen peroxide formed reacts with 4-aminoantipyrine (4-AAP) (aminophenazone) and 3, 5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) in the presence of peroxidase to form a red quinoneimine dye. 2 µl of sample was reacted with 200 µl of reagent and the change in absorbance was monitored at 660 nm. This change was directly proportional to the concentration of TG in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37^{o}C for two minutes.

Principle of the reaction

$$TG + 3H_2O \xrightarrow{LIPASE} \text{Glycerol 3 fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{GKMg^{2+}} \text{Glycerol-3-phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{GPO} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{DHBS} \xrightarrow{POD} \text{Quinoneimine (red) dye} + HCl + 2H_2O$$

3.15.2 Total cholesterol (TC)

Cholesterol esters in the sample were hydrolyzed in the presence of cholesterol esterase to form cholesterol and fatty acids. The cholesterol was then oxidized in the presence of cholesterol oxidase to cholestene-3-one and hydrogen peroxide. Hydrogen peroxide formed reacted with 4-aminoantipyrine and phenol in the presence of peroxidase to form a red dye quinoneimine and water. 2.5 µl of sample was reacted with 250 µl of reagent and the change in

absorbance was monitored at 540 nm. This change was directly proportional to the concentration of TC in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37^{o}C for two minutes.

Principle of the reaction

Cholesterol ester + $H_2O \xrightarrow{CHE}$ Cholesterol + Fatty acids

Cholesterol + $O_2 \xrightarrow{CHO}$ Cholestene-3-one + H_2O_2

$2H_2O_2$+ 4-AAP + Phenol $\xrightarrow{POD}$ Quinoneimine (red) dye + $4H_2O$

3.15.3 HDL-Cholesterol (HDL-C)

Antihuman-β-lipoprotein antibody binds to lipoproteins other than HDL (LDL, VLDL. and chylomicrons). The antigen antibody complexes formed block enzyme reactions. The presence of an enzyme chromogen system quantified HDLC. 2.5 µl of sample was reacted with 250 µl of reagent and the change in absorbance was monitored at 540 nm. This change was directly proportional to the concentration of HDL in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37^{o}C for two minutes.

Principle of the reaction

LDL, VLDL and chylomicrons $\xrightarrow{ANTIHUMAN-\beta-LIPOPROTEINANTIBODY}$ Antigen-antibody complexes

HDL-C + H_2O + $O_2 \xrightarrow{CHO/CHE}$ Cholestene-3-one + Fatty acid + H_2O_2

$2H_2O_2$+ 4-AAP + Phenol $\xrightarrow{POD}$ Quinoneimine (red) dye + $4H_2O$.

3.15.4 LDL-Cholesterol (LDL-C)

A protecting agent protected LDLC from enzymatic reactions. All non-LDLC lipoproteins (HDL, VLDL, CM) were broken down by reaction with

cholesterol esterase (CHE) and cholesterol oxidase (CHO). H_2O_2 produced by this reaction was decomposed by catalase in the first step of the reaction. In the second step of the reaction, the protecting agent was released from the LDL-C and the catalase inactivated by sodium azide. LDL-C was quantified by the CHO/POD system. 2.5 µl of sample was reacted with 250 µl of reagent and the change in absorbance was monitored at 540 nm. This change was directly proportional to the concentration of LDLC in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37ºC for two minutes.

Principle.of the reaction

$$2\ \text{LDLC} + 2\ H_2O + 2\ O_2 \xrightarrow{CHO/CHE} 2\ \text{Cholestene-3-one} + 2\ \text{Fatty acid} + 2H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{Phenol} \xrightarrow{POD} \text{Quinoneimine (red) dye} + 4H_2O$$

3.16 Electrolytes (Sodium, Potassium and Chloride)

Ion selective electrode method

The Intergra 400 system determined sodium, potassium and chloride serum levels by measuring electrolytes ion activity in solution. A specific ion-selective electrode made the measurement. 3µl of sample was reacted with 60 µl of buffer solution (sodium bicarbonate) in a ration of 1:20 to establish a constant activity coefficient for the electrodes. With constant activity established, the electrodes system was calibrated to concentration values. The mixture was transported to the flow cell which houses the electrodes. The

sodium, chloride and potassium determination were made by measuring potentials developed at the face of specific ion-selective electrodes.

Principle of the reaction

For the three electrolytes, the change in potential voltage developed at each specific electrode's face was calculated using the Nernst equation.

Sodium E = constant + (slope) (log [Na^+]

Potassium E = Constant + (slope) (log [K^+])

Chloride E = constant + (slope) (log [Na^+]

3.17 Liver function panel test

3.17.1 Total protein (TP)

Total protein reagent was used to measure total protein concentration by a timed endpoint biuret method. In the reaction, the peptide bonds in the protein sample bind to cupric ions in an alkaline medium to form a peptide/copper complex. 6µl of the sample was reacted with 300 µl of reagent, and the change in absorbance was monitored at 560 nm. This change was directly proportional to the concentration of TP in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37ºC for four minutes.

Principle of the reaction

Protein + $Cu^{2+} \xrightarrow{OH^-}$ Protein-Copper complex

3.17.2 Albumin (ALB)

An albumin reagent was used to measure albumin concentration by a timed endpoint method. Albumin combined with bromocresol green to form a coloured product. 3 µl of the sample was reacted with 300 µl of reagent, and

the change in absorbance was monitored at 600 nm. This change was directly proportional to the concentration of ALB in the sample and was used to calculate and express concentration in g/l. The reaction took place at 37^{o}C for one and a half minutes.

Principle of the reaction

Albumin +BCG $\longrightarrow$ Albumin / BCG complex

3.17.3 Alkaline phosphatase (ALP)

An alkaline phosphatase reagent was used to measure alkaline phosphatase activity by a kinetic UV method using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction, alkaline phosphatase catalyzed the hydrolysis of the colorless organic phosphate ester substrate, p-nitrophenyl phosphate, to the yellow-colored product, p-nitrophenol and phosphate. The reaction occurred at an alkaline pH of 10.3. 5µl of the sample was reacted with 250 µl of the reagent. The change in absorbance was monitored at 410 nm and was directly proportional to the activity of ALP. The activity was calculated and expressed in U/L. The reaction took place at 37^{o}C for three minutes.

Principle of the reaction

ρ-Nitrophenyl Phosphate + H_2O $\xrightarrow{pH10.3 Mg^{2+}}$ ρ-Nitrophenol + Phosphate

(colorless) (yellow)

3.17.4 Alanine aminotransferase (ALT)

An enzymatic kinetic UV rate method estimates Alanine aminotransferase in the sample. In the assay reaction, the ALT catalyzed the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamine.

The pyruvate then reduces to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of β-Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to β-Nicotinamide Adenine Dinucleotide (NAD). Pyridoxal-5-phosphate was required in this reaction as a cofactor for transaminase activity by binding to the enzyme using Schiff-base linkage. 10µl of the sample was reacted with 110µl of the reagent. The change in absorbance was monitored at 340 nm and was directly proportional to the ALT activity. The activity was calculated and expressed in U/L. The reaction took place at 37^{o}C for three minutes.

Principle of the reaction

$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{ALT} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{LDH} \text{Lactate} + NAD^{+}$$

3.17.5 Aspartate aminotransferase (AST)

The AST reagent was used to measure aspartate amino transferase activity using an enzymatic kinetic UV rate method. In the reaction, aspartate aminotransferase catalyzed the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in malate dehydrogenase (MDH) presence with the concurrent oxidation of reduced β-nicotinamide adenine dinucleotide (NAD). 10µl of the sample was reacted with 110µl of the reagent. The change in absorbance was monitored at 340 nm and was directly proportional to the activity of AST. The activity was calculated and expressed in U/L. The reaction took place at 37^{o}C for three minutes.

Principle.of the reaction

L-Aspartate + α-Ketoglutarate $\xrightarrow{AST}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ Malate + NAD^+

3.17.6 Gamma glutamyltransferase (GGT)

GGT reagent was used to measure γ-glutamyl transferase activity by an enzymatic kinetic UV rate method. In the reaction, γ-glutamyl transferase catalyzed the transfer of the glutamyl group from the substrate to glycylglycine forming glutamylglycylglycine and 5-amino-2-nitrobenzoate. 5µl of the sample was reacted with 200µl of the reagent. The rate of formation of 5-amino-2-nitrobenzoate was proportional to the activity of GGT present in the sample and was measured kinetically at 405 nm. The activity was calculated and expressed in U/L. The reaction took place at 37^{o}C for three minutes.

Principle of the reaction

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{GGT}$ L-γ-Glutamylglycylglycine + 5-Amino-2-nitrobenzoate

3.17.7 Total bilirubin (T-BIL)

A stabilized diazonium salt (3, 5-dichlorophenyl diazonium tetrafluoroborate (DPD), reacted with conjugated bilirubin directly and with unconjugated bilirubin in the presence of an accelerator (caffeine) to form azobilirubin (purple). 8 µl of sample was reacted with 280 µl of reagent and the change in absorbance was monitored at 578 nm. This change was directly proportional to the concentration of T BILI in the sample and was used to calculate and express concentration in µmol/L. The reaction took place at 37^{o}C for two

minutes. A separate sample blank was performed (set) to reduce endogenous serum interference.

Principle.of the reaction

Bilirubin + DPD $\xrightarrow{CAFFEINE}$ Azobilirubin

3.17.8 Direct bilirubin (D-BIL)

A stabilized diazonium salt (3, 5-dichlorophenyl diazonium tetrafluoroborate (DPD), reacts with conjugated bilirubin directly in an acidic medium to form azobilirubin (purple). 5 µl of sample was reacted with 160 µl of reagent and the change in absorbance was monitored at 546 nm. This change was directly proportional to the concentration of D BILI in the sample and was used to calculate and express concentration in µmol/L. The reaction took place at 37^{o}C for two minutes.

Principle of the reaction

Bilirubin + DPD $\xrightarrow{H^+}$ Azobilirubin (purple colour)

3.18 Kidney function test

3.18.1 Uric acid (UA)

Uric acid reagent was used to measure the concentrate on of the uric acid concentration by a timed endpoint method. Uric acid was oxidized by uricase to produce allantoin and hydrogen peroxide. The hydrogen peroxide reacted with 4-aminoantipyrine (4-AAP) and 3, 5-dichloro-2-hydroxybenzene sulphonate (DCHBS) in a reaction catalysed by peroxidase to produce a coloured product. A of sample 4µl was reacted with 100 µl of reagent and the change in absorbance was monitored at 520 nm. This change was directly

proportional to the concentration of UA in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37ºC for three and half minutes.

Principle of the reaction

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{UREASE} \text{Allantoin} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{4-AAP} + \text{DCHBS} \xrightarrow{PEROXIDASE} \text{Quinoneimine} + H_2O.$$

3.18.2 Creatinine (CREAT)

The creatinine reagent was used to measure the creatinine concentration by a modified rate Jaffe method. In the reaction, creatinine is combined with picrate in an alkaline solution to form a creatinine - picrate complex. A sample of 20 µl was reacted with 220 µl of reagent and the change in absorbance was monitored at 512 nm. This change was directly proportional to the concentration of CREAT in the sample and was used to calculate and express concentration in µmol/L. The reaction took place at 37ºC for two minutes.

Principle of the reaction

$$\text{Creatinine} + \text{Picric Acid} \xrightarrow{ALKALINESOLUTION} \text{Creatinine / picrate (Red colour complexone).}$$

3.18.3 Blood Urea Nitrogen (BUN)

BUN can be analyzed using two methods: (i) Berthelot Reaction and (ii) Glutamate Dehydrogenase (GLDH).

Berthelot Reaction

In the first reaction step, urea is hydrolyzed with urease to form ammonia and carbon dioxide. In the second step of the reaction, ammonia is reacted with

phenol and hypochlorite in alkaline medium to form a green indophenol complex which is directly proportional to the concentration of urea in the sample. 2 µl of sample was reacted with 200 µl of reagent and the change in absorbance was monitored at 600 nm. This change was directly proportional to the concentration of BUN in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37^{o}C for one minute.

Principle of the reaction

$$\text{Urea} + H_2O \xrightarrow{UREASE} 2NH_3 + CO_2$$

$$NH_4^+ + 5\ NaOCl + 2\ \text{Phenol} \xrightarrow{OH^-} \text{Indophenol} + 5\ NaCl + 5H_2.$$

3.19 Analytical Methods for Special biochemical parameters (Tumour Markers)

Tumour markers were analyzed using Chemwell Auto-Analyzer machine. Every analyte had a specific master lot data entry card which contained all the specifications: analyte identification, factory master calibration curve, control data, and lot number of the reagent.

The strip consisted of 96 wells covered with a labeled foil seal. The label comprised a bar code that mainly indicated the assay code, kit lot number and expiration date. The foil of the first well was perforated to facilitate sample Introduction. The last well of each strip was a cuvette in which the fluorimetric reading was performed. The wells in the centre section contained the various reagents required for the assay.

Solid Phase Receptacle (SPR): Interior of the SPR was coated during production with specific monoclonal anti-(TSH, T4, T3, TPSA, CA 19-9,

CA125, CEA and CA 15-3) immunoglobulins (mouse). Each SPR was identified by specific analyte code.

Analytical Principle: The assay combines an enzyme immunoassay sandwich method with a final fluorescent detection (enzyme-linked fluorescent assay (ELFA)).

Analytical method: The SPR served as the assay's solid phase and pipetting device. Reagents for the assay were ready for use and pre-dispensed in sealed reagent strips. All the assay steps were performed automatically by the machine. The reaction medium was cycled in and out of the SPR several times. The sample was taken and transferred into the well-containing alkaline-phosphatase labeled anti-(TSH, T4, T3, TPSA, CA125, CEA (Pishtaz Teb Diagnostics /Germany), CA 15-3, and CA 19-9 Cortez Diagnostic California USA) immunoglobulins (conjugate). The sample/conjugate mixture was cycled in and out of SPR several times to increase reaction speed. The antigens bind with the antibodies coated on the SPR and to the conjugate forming a "sandwich". Unbound components were eliminated during the washing steps of the assay.

In the final detection step, the substrate (4-methylumbelliferyl phosphate) was cycled in and out of the SPR. The conjugate enzyme catalyzed the hydrolysis of the substrate into a fluorescent product (4-methylumbelliferone). The fluorescence intensity was directly proportional to the antigen present in the sample. At the end of the assay, the machine automatically calculated results in relation to the calibration curve stored in memory and then printed out.

3.20 Screening for Human Immunodeficiency Virus (HIV)

Immunochromatographic reagent strip (Determine HIV-1/2, Tokyo, Japan) was used to screen HIV 1 and 2. A sample of 50 µL was applied to the sample pad. After 1 minute chase buffer was applied to the sample pad and the test results read within 15 minutes. Two red bars each indicated positive results on the control window and the patient window. Negative results were indicated by the appearance of only one red bar at the control window.

3.21 Screening for Venereal Disease Research Laboratory (VDRL)

The syphilis ultra-rapid test, a qualitative membrane strip-based immunoassay (Treponema pallidum Strip, Beijing, China), was used to screen Treponema pallidum, which is the causative agent of the venereal disease, syphilis. A sample of 50 µl was placed on the sample pad followed by 1 drop of buffer. The result was read after 10 minutes. A positive result was indicated by the appearance of two red lines, one on the control region and the other on the test region. A negative result was indicated by the appearance of one red line on the control region.

3.22.1 Statistical Methods used in the Establishment of Reference Ranges

To produce unbiased national reference ranges for the adult Kenyan population, the data from 2335 study subjects were statistically treated using the following steps; (i) Partitioning of reference values, (ii) Inspection of data distribution, (iii) Detection and handling of outliers, (iv)Determination of reference limits (v) Selection of statistical method

3.22.2 Partitioning of Reference Values

This was done according to sex and age. The study subjects were divided into males and females. The data from each group was used to produce reference ranges for the nineteen biochemical analytes and twenty-three hematological parameters. The reference ranges produced were compared with the adult reference ranges for other populations as given in the literature. The data was also used to categorize the study subjects in three categories: (1) young adult and children (2), pregnant mothers that were divided into two groups; second and third trimester, and (3) the elderly those above 55 years of age. By categorizing it was possible to get the effect of sex and age on the reference ranges.

3.22.3 Inspection of Data Distribution

The use of computer prepared histograms and box plot for the analytes. The visual examination of the histograms (testing fit to Gaussian distribution) was to safeguard against the misapplication or misinterpretation of statistical methods and it also gave some valuable information about the data. The following characteristic of the data distribution was expressed:

(a) Outliers (highly deviating values) were easily detected, representing abnormal values in the collected data, which could affect the production of reference ranges.

(b) The histograms and box plot were used to examine the shape of data distribution.

3.23.4. Identification and handling of abnormal values

Identifying abnormal values (outliers) was done by visually inspecting the histograms. According to (Solberg, 2004), there is no other statistical test for identifying outliers, which is more sensitive or more reliable than the simple visual inspection of a histogram. The data that remained after removing the values (outliers) of both tails of the Gaussian curve (representing 95 % normal reference population) was used to construct the reference ranges.

3.23.5 Determination of reference limits

By definition, reference limit is descriptive of reference distribution that tells us something about the observed variation of values in the selected set of reference individuals. In this study, each analyte's lower and upper reference limits were obtained by the formula: mean ± 1.96 multiplied by the standard deviation (x ±1.96SD). All the values in between, including the two reference limits, give the reference range (interval) of different parameters. This reference interval is also defined as the central 95% interval bounded by 2.5 and 97.5 percentiles, that is, 2.5 % of the values cut off in both tails of the reference distribution. The confidence interval of percentiles which showed that they were within the true percentiles, were located with a specified degree of confidence for each analyte or determined parameter.

3.23.6 Selection of statistical method

The cleaned data from the randomly selected individuals was subjected to normality distribution testing using the Kolmogorov-Smirnov Test. The data was found to be normally distributed and computed using parametric approach

methods, whereby the lower and upper limits of the reference intervals were obtained using the following formula:

$X \pm 1.960SD$, where X = Mean and SD = standard deviation.

The collected analytical data was entered into the Excel spread sheet, cleaned and then exported to the Statistical Package for Social Sciences (SPSS) for analysis. T-test was used for means comparison, while ANOVA and post-Anova-tests were used for multiple comparisons of means. The tests were conducted at a 95% confidence interval and a significance level of 5%; p less than or equal to 0.05 was considered statistically significant. The performance of Analytical instruments and methods to analyse the levels of the selected analytes were achieved by using the paired t-test.

CHAPTER FOUR

4. RESULTS

4.1 Study population

The study subjects that were recruited for this research were 2390, of which triage parameters were taken, which were six vital signs (systolic blood pressure, diastolic blood pressure, oxygen concentration, pulse rate and temperature) and body mass index ((BMI)) (weight in kg/[height in m]^2). For vital signs, 508 participants were studied (191 [36.9%] were males, and 317 [63.1%] were females, where those who were evaluated for BMI were 252, with 125 [49.6%] males and 127 [50.4] females.

One percent (25) of the recruited participants were not used for analyses of hematological and biochemical parameters as they were excluded from the study as explained in the inclusion and exclusion criteria: 7 were positive for syphilis, 5 for hepatitis, 13 for HIV and 6 samples were hemolysed. The remaining subjects were used in the study. They were distributed as tabulated below: For routine biochemical and hematological parameters, 1404 subjects were evaluated, including 261 under 17 years (129 males) [49.4%] and 132 females [50.6%], 296 pregnant mothers, 320 for the elderly (153 males [47.8%] and 177 females [52.2%]). The biochemical parameters that were analysed included albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), chloride (CL), alkaline phosphatase (ALP), random glucose (GLU), γ-glutamyl transaminase (GGT), total bilirubin (T-BIL), direct bilirubin (D-BIL), creatinine (CREAT), sodium (SOD), potassium (POT),

blood urea nitrogen (BUN), uric acid (UA), total protein (TP) and calcium (CA). Two hundred and seventy-two participants were included in the development of reference ranges for special chemistry, including fasting lipid profile consisting of 123 [45.2%] males and 149 [54.8%] females, CEA where a total of 249 subjects were used including 123 [49.2%] were males, and 126 [51.8%] were females. The development of a reference range for thyroid profile parameters used 244 subjects including 124 [50.8%] males and 120 [49.2%] females), CA-15-3 used 244 participants including 120 [49.8%] males and 124 [50.8%] females), CA-19-9 used 244 participants including 120 [49.8%] males and 124 [50.8%] females), CA-12-5 used 126 subjects, and TPSA used 130 subjects as tumor markers for females and males, respectively.

The hematological parameters evaluated in this study included the red blood cells (RBC), hemoglobin level (HB), hematocrit (HCT), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), red cell distribution width by volume coefficient of variation (RDW-CV), red cell distribution width by volume standard variation (RDW-SD), platelets (PLT), platelet distribution width (PDW) and mean platelet volume (MPV), total white cell count (WBC), absolute and percentage of differential counts for white blood cell including neutrophil (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS) and basophil (BAS)

Table 4.1: Parameters that were evaluate and the population that was under study in each profile

	Vital signs	Hematological	Biochemical	Cancer marker	Fasted lipid profile	Thyroid profile
Category	Age 4-93	✓ 2-17 years ✓ Gravid mother ✓ Geriatric	✓ 2-17 years ✓ Gravid mother ✓ Geriatric	Geriatric	22-55 years	Geriatric
1	Temperature	RBC ($x10^{12}$/L)	RBS (mM)	CA-19-9 (U/L)	TOTAL CHOL (mmol/L)	TSH (µ/mL)
2	Systolic	HB (g/dL)	ALB (U/L)	CEA (U/L)	HDL (mmol/L)	T3 (nmol/l)
3	Diastolic	PCV (%)	ALP (U/L)	CA 15-3 (U/L)	TG (mmol/L)	T4 (nmol/l)
4	Oxygen (SpO_2)	MCH (pg)	ALT (U/L)	CA 12-5 (U/L	TOTAL CHOL: HDL	-
5	Pulse rate	MCHC (g/dL)	AST (U/L)	PSA (U/L)	NON-HDL (mmol/L)	-
6	BMI	MCV (fL)	D-BIL (µM)	-	-	-
7	-	RDW-CV (%)	T-BIL (µM)	-	-	-
8	-	RDW-SD (%)	CL (mM)	-	-	-
9	-	WBC ($x10^9$/L)	CREAT (µM)	-	-	-
10	-	NEU ($x10^9$/L)	GGT (U/L)	-	-	-
11	-	LYM ($x10^9$/L)	K (mM)	-	-	-
12	-	MON ($x10^9$/L)	NA (mM)	-	-	-
13	-	MON ($x10^9$/L)	TP (g/L)	-	-	-
14	-	EOS ($x10^9$/L)	BUN (mM)	-	-	-
15	-	BAS ($x10^9$/L)	UA (µM)	-	-	-
16	-	NEU (%)	CA (mM)	-	-	-
17	-	LYM (%,)	-	-	-	-
18	-	MON (%)	-	-	-	-
19	-	EOS (%)	-	-	-	-
20	-	BAS (%)	-	-	-	-
21	-	PLT ($x10^9$/µL)	-	-	-	-
22	-	PCT (%)	-	-	-	-
23	-	PWD (fL)	-	-	-	-
24	-	MPV (fL)	-	-	-	-

Table 4.2: Subjects studied for triage parameters by gender

	TEST	MALE	FEMALE	TOTAL
1	VITAL	191	317	508
2	BMI	125	127	252

Table 4.3: Tests performed on the participants recruited for hematological and biochemical parameters

	Test	Male	Female	Total
1	Under 17 years	129	132	261
2	Pregnant mothers	0	296	296
3	Lipid profile	123	149	272
4	TPSA	130	0	130
5	CEA	123	126	249
6	Thyroid profile	124	120	244
7	CA-15-3	120	124	244
8	CA 19 -9	120	124	244
9	CA 12-5	0	126	126
10	RBS	131	175	306

4.2 Internal quality control for parameters under study

Internal quality control material for all parameters used in this study was included in each analytical cycle. The analytical phase included 61 for routine biochemical parameters, 50 for hematology parameters, 12 for thyroid hormones, 15 for tumor makers, 12 for TPSA, 45 for lipid profile and 70 for full blood count. Quality control results for the analyzed parameters were within the specific assigned QC range (Table 4.4) of the target value ± 2 standard deviations (SD). Quality control was observed throughout the study, and this made sure that all the obtained results were acceptable because the methods were operating within the recommended ranges of reporting; this was achieved by the use of normal and pathological (PNU and PPU) predetermined values, respectively (Table 4.4 and Table 4.5).

Table 4.4: Internal quality control for qualitative parameters under study

Analyte (unit)	QC Type	Assigned QC Report			Study QC Report		
		Mean	SD	% CV	Mean	SD	% CV
TP (g/L)	PPU	46	2.00	4.3	46.6	2.3	4.9
	PNU	68	3.40	5.07	67.5	3.4	5.03
ALB (g/L)	PPU	29.6	2.0	6.07	46.0	2.3	5.00
	PNU	48.8	2.0	4.1	102	5.0	4.90
ALP (U/L)	PPU	259	13.0	5.0	226.8	5.1	2.23
	PNU	102	5.0	4.9	83.8	2.6	3.16
ALT (U/L)	PPU	139	7.0	5.04	143.1	2.5	1.75
	PNU	51	3.0	5.88	49.3	2.0	4.09
AST (U/L)	PPU	122	6.0	4.92	145.5	1.9	1.32
	PNU	38	2.0	5.26	44.8	1.3	2.99
D-BIL (µM)	PPU	33.7	2.5	7.42	36.32	0.75	2.06
	PNU	12.7	1.9	14.96	8.52	0.29	3.35
T-BIL (µM)	PPU	66.3	4.9	7.39	93.5	3.1	3.27
	PNU	17.1	1.0	5.85	21.7	0.9	4.26
BUN (mM)	PPU	26.3	1.3	4.94	22.5	1.9	8.31
	PNU	7.4	0.4	5.4	7.0	0.3	4.83
CREAT(mM)	PPU	398	20	5.03	422	20	4.65
	PNU	92	5	5.43	93	5	5.56
Na (mM)	PPU	144	4.0	2.8	144	2.5	1.7
	PNU	124.3	2.1	1.7	129	5	3.87
K(mM)	PPU	5.2	0.6	3.0	6.5	0.1	2.0
	PNU	5.2	0.6	3.0	4	0.6	1.5
Cl (mM)	PPU	116	3.0	2.6	115.8	2.9	2.5
	PNU	85.3	2.6	3.1	105	5	4.76
UA (µM)	PPU	628	28	4.46	605	18	3.4
	PNU	276	12	4.35	265	15	5.77
CA (mM)	PPU	3.26	0.12	3.77	0.0	0.0	0.0

	PNU	2.12	0.08	3.9	0.0	0.0	0.0
CK (U/L)	PPU	531	27	5.08	554	25.9	4.67
	PNU	151	8	5.30	152	7.5	4.93
GGT (U/L)	PPU	259	13	5.02	264	10	3.77
	PNU	53	3.0	5.66	53.0	3.0	6.30
LDH (U/L)	PPU	277	14	5.05	293	16.6	3.02
	PNU	161	8	4.96	154.4	13.7	8.9
TC (mM)	PPU	4.4	2	7.3	4.2	4	4
	PNU	3.7	0.3	7.6	3.9	0.2	7.9
HDLC(mM)	PPU	2	0.5	11.8	1.8	0.22	7.8
	PNU	1.3	0.14	12	2	0.17	13
LDLC (mM)	PPU	3	0.15	12.2	2.8	0.19	8.1
	PNU	1.7	0.17	7.7	1.9	0.19	7.9
TC (mM)	PPU	1.7	0.19	7.5	1.9	0.2	8
	PNU	0.94	0.07	7.2	0.98	0.08	7.6
TSH(µ/ml)	PPU	5	0.08	7	4.8	0.08	8
	PNU	3.6	0.1	3.1	4	0.1	4
T3 (nM)	PPU	3.3	0.11	3.2	3	0.11	3.8
	PNU	2.2	0.2	2.8	2.1	0.1	2.7
T4 (nM)	PPU	105	0,4	3.1	103	0.15	3
	PNU	84	1.3	1.6	85	1.4	1.8
CA-125 (U/L)	PPU	22.3	1.5	1.8	21	1.72	2
	PNU	18.8	0.7	3.9	19	0.9	4.2
CA-19-9(U/L)	PPU	25.5	0.8	4	24.3	1	4.5
	PNU	20.6	0.9	4.4	22	1.2	4.5
CA-15-3 (U/L)	PPU	40	1.2	4.7	38	1.4	3.9
	PNU	30.8	2	6.2	31.2	2.3	6.6
CEA (U/L)	PPU	39.6	2.3	7	38.6	2.5	6.5
	PNU	31.3	1.9	6.1	32.1	2.1	6.5
TPSA(ng/ml)	PPU	4.5	2.1	6.8	4.3	2	6.3
	PNU	3.3	0.2	6	3.5	0.4	6.2
RBS (mM)	PPU	7	0.33	4	3.6	0.35	3
	PNU	3.0	0.22	3.1	3.2	0.24	3.2

Table 4.5: Results of the commercial quality control material for hematological parameters

Parameter	Assigned QC report			Study QC report		
(unit)	Upper Limit	Target	Lower limit	Mean	SD	CV (%)
HB (g/dL)	23.50	15.50	7.50	15.48	0.14	0.90
RBC ($x10^{12}$/L)	5.13	4.83	4.53	4.875	0.069	1.42
PCV (%)	0.75	0.51	0.27	0.51	0.008	1.57
MCH (pg)	34.6	32.1	29.6	31.75	0.36	1.13
MCHC (g/dL)	33.4	30.4	27.4	29.87	0.37	1.24
MCV (fL)	110.5	105.5	100.5	106.33	0.87	0.82
RDW-SD (%)	67.0	59.0	51.0	57.86	0.99	1.71
RDW-CV (%)	16.5	13.5	10.5	13.10	0.21	1.60
WBC ($x10^9$/L)	19.90	17.40	14.90	17.99	0.45	2.50
NEU ($x10^9$/L)	12.97	11.57	10.17	12.36	0.18	1.46
NEU (%)	74.5	66.50	58.5	68.72	1.24	1.80
LYM ($x10^9$/L)	4.53	3.13	1.73	3.38	0.11	3.25
LYM (%)	26.0	18.00	10.00	18.78	0.34	1.81
MON ($x10^9$/L)	1.48	0.78	0.08	0.58	0.11	18.97
MON (%)	8.5	4.5	0.5	3.18	0.54	16.98
EOS ($x10^9$/L)	3.30	1.91	0.52	1.69	0.16	9.47
EOS (%)	19.0	11.0	3.0	9.33	0.70	7.50
BAS ($x10^9$/L)	15.36	13.62	11.88	13.84	0.38	2.75
BAS (%)	86.3	78.3	68.3	76.88	0.58	0.75
PLT ($x10^9$/L)	514	454	394	445.6	12.9	2.89

PCT (%)	0.61	0.41	0.21	0.38	0.01	2.63
PDW (%)	19.5	16.5	13.5	16.91	0.11	0.65
MPV (fL)	11.9	8.9	5.9	8.46	0.08	0.95

4.3. Reference interval limits for body mass index and vital signs for infants, children, adolescents, adults and geriatric population of Taita-Taveta county, Kenya

4.3.1 Established reference interval limits for BMI and selected vital signs for male and female infants, children, adults, and the geriatric population of Taita-Taveta County, Kenya

The established reference intervals for body mass index and the selected vital signs for male infants, children, adolescents, adults, and the geriatric population of Taita-Taveta County, Kenya for peripheral oxygen saturation (SpO_2) and temperature (Temp) were similar to those of the female infants, children, adolescents, adults, and geriatric population of Taita-Taveta County, Kenya ($\rho > 0.05$). Therefore, combined reference interval limits for peripheral oxygen saturation (SpO_2) and temperature (Temp) were established for this population. The established combined reference interval limits for SpO_2 are 99 (86.8-100) %, and Temp is 34 (32-35.9) ^oC. Further, the established reference interval limits for BMI, Systolic and Diastolic, and Pulse rates for the male infants, children, adolescents, adults, and the geriatric population of Taita-Taveta County, Kenya, significantly differed from that of the female infants, children, adolescents, adults, and the geriatric population of the same County ($\rho < 0.05$). The established reference interval limits for infants, children, adolescents, adults, and the geriatric population of Taita-Taveta County, Kenya

for BMI is 19.7 (14.48-31.84) kg/m^2 for males and 22.0 (14.14-31.84) kg/m^2 for females; systolic is 119 (88-157) mmHg for males and 133 (84-178.2) mmHg for females; diastolic is 68 (46-100.8) mmHg for males and 74 (49-108) mmHg for females and pulse rate is 84 (62-117.6) for males and 87 (60-129) for females (Table 4.6).

Table 4.6: Established reference interval limits for BMI and selected vital signs for male and female infants, children, adults, and the geriatric population of Taita-Taveta County, Kenya

Vital Sign (unit)	Sex	N	Median	Percentile		Reference Interval	IV	Difference between M&F	
				2.5th	97.5th			z-value	Sig
BMI (kg/m^2)	M&F	252	20.3	14.3	38.0	14.3-38	23.7	3.411	**ρ = 0.0006**
	F	**127**	**22.0**	**14.1**	**39.9**	**14.1-39.9**	**25.8**		
	M	**125**	**19.7**	**14.5**	**31.8**	**14.5-31.8**	**17.3**		
Systolic BP (mmHg)	M&F	508	116	84.7	174	84.7-174	89.3	3.135	**ρ = 0.0017**
	F	**317**	**119**	**84**	**178.2**	**84-178.2**	**94.2**		
	M	**191**	**113**	**88**	**157**	**88-157**	**69**		
Diastolic BP (mmHg)	M&F	508	71	49	107	49-107	58	3.246	**ρ = 0.0012**
	F	**317**	**74**	**49**	**108**	**49-108**	**59**		
	M	**191**	**68**	**46**	**100.8**	**46-100.8**	**54.8**		
Pulse rate (beats per minute)	M&F	508	86	60.7	125	61-125	64	2.575	**ρ = 0.01**
	F	**317**	**87**	**60**	**129**	**60-129**	**69**		
	M	**191**	**84**	**62**	**117.6**	**62-117.6**	**55.6**		
Oxygen saturation ($S\rho O_2$) (%)	**M&F**	**508**	**99**	**86.8**	**100**	**86.8-100**	**13.2**	1.031	ρ = 0.3027
	F	317	99	92.9	100	92.9-100	7.1		
	M	191	99	73.9	100	73.9-100	26.1		
Temperature (0^oC)	**M&F**	**508**	**34**	**32**	**35.9**	**32-35.9**	**3.9**	1.557	ρ = 0.1195
	F	317	33.9	31.6	35.7	31.6-35.7	4.1		
	M	191	34.1	32	36.14	32-36.1	4.1		

Results are expressed as Median and range for the number of referent participants in the column labeled N. Statistical comparisons of the median values between male and female referent participants were carried out using Mann-Whitney U test. Differences were considered statistically significant at $\rho < 0.05$.

4.3.2 Effects of age on the developed reference interval limits for BMI and the selected vital signs for the male and female population of Taita-Taveta County, Kenya

The effects of age on the developed reference interval limits for BMI and the selected vital signs for infants and children, adolescents, adults, and geriatric male and female population of Taita-Taveta County, Kenya, are presented in Table 4.7. The different age groups were categorized as follows: (a) age category 1 (1-12 years), (b) age category 2 (13-17 years), (c) age category 3 (18-55 years), and (d) age category 4 (56-95 years). The reference interval limits for BMI and the selected vital signs differences across age categories were compared for both males and females using ANOVA and post-ANOVA test where ρ-values less than 0.05 was considered statistically significant (Table 4.7).

Results indicate that infants, children, and adolescents had similar reference interval limits for BMI, after which the BMI reference interval limits significantly increased in adults and then significantly decreased in geriatrics. Further, results indicate that reference interval limits for systolic blood pressure for infants and children were significantly lower than those of adolescents who were significantly lower than those of adults who were significantly lower than those of geriatrics. In addition, results indicate that reference interval limits for diastolic blood pressure for infants and children were significantly lower than those of adolescents, which were significantly lower than those of adults, similar to those of geriatrics. The pulse rate reference interval limits for infants

and children were significantly lower than those of adolescents, who were significantly lower than those of adults who were similar to those of geriatrics. The reference interval limits for temperature and peripheral oxygen saturation were statistically similar for infants, children, adolescents, adults and geriatrics. In summary, there was a statistically significant increase in BMI, systolic and diastolic blood pressure, and pulse rate with advancement of age. Temperature and peripheral oxygen saturation was not significantly altered with advancement of age.

Table 4.7: Effects of age on the developed reference interval limits for BMI and the selected vital signs for the male and female population of Taita-Taveta County, Kenya

Parameter (units)	Sex	Changes on the value of body mass index and the measured vital signs with age (years)							
		N	1-12 years	N	13-17 years	N	18-55 years	N	56-95 years
BMI (kg/m^2)	M	55	18.00±4.31	54	19.00±2.41	10	**23.50±5.30^{ab}**	31	**22.00±5.43^{abc}**
	F	55	18.00±3.17	18	20.00±2.01	26	**29.00±6.48^{ab}**	29	**27.00±5.55^{abc}**
Systolic BP (mmHg)	M	59	102.27±10.01	64	**109.88±12.26^{a}**	43	**126.40±18.82^{ab}**	25	**138.0±15.11^{abc}**
	F	92	100.82±13.28	53	**112.38±11.31^{a}**	116	**132.68±22.48^{ab}**	56	**142.64±23.71^{abc}**
Diastolic BP (mmHg)	M	59	61.68±13.28	64	**67.13±9.89^{a}**	43	**77.58±17.30^{ab}**	25	**81.20±12.23^{ab}**
	F	92	61.72±8.80	53	**68.51±10.14^{a}**	116	**80.89±13.34^{ab}**	56	**86.18±22.13^{ab}**
Temperature (^0C)	M	59	33.96±0.86	64	34.93±5.07	43	33.89±0.97	25	33.88±0.70
	F	92	33.72±1.22	53	34.25±0.80	116	34.30±6.78	56	34.28±2.78
Pulse rate (beats per minute)	M	59	79.31±10.69	64	**83.45±10.34^{a}**	43	**88.70±14.00^{ab}**	25	**93.32±18.23^{ab}**
	F	92	77.11±11.59	53	**83.08±10.44^{a}**	116	**96.14±15.98^{ab}**	56	**98.71±19.40^{ab}**
Oxygen saturation ($S\rho O_2$) (%)	M	59	98.19±4.47	64	**98.18±4.74^{a}**	**43**	**95.44±8.41^{ab}**	**25**	**98.40±1.05^{ab}**
	F	92	98.60±1.82	53	97.94±4.30	116	98.25±2.00	56	96.76±9.00

Results are expressed as Mean ± Standard deviation of the number of subjects indicated in the column labeled N. $^{*}\rho < 0.05$ when male reference interval limits are compared to female reference interval limits per each age category; $^{a}\rho < 0.05$ when reference interval limits in age range 1-12 years is significantly different when compared to reference interval limits in age range 13-17 years; $^{b}\rho < 0.05$ when reference interval limits in age range 1-12 years is significantly different when compared to reference interval limits in age range 18-55 years; $^{c}\rho < 0.05$ when reference interval limits in age range 1-12 years is significantly different when compared to reference interval limits in age range 56-90 years; $^{d}\rho < 0.05$ when reference interval limits in age range 13-17 years is compared to reference interval limits in age range 18-55 years; $^{e}\rho < 0.05$ when reference interval limits in age range 13-17 years is significantly different when compared to reference interval limits in age range 56-95 years; $^{f}\rho < 0.05$ when reference interval limits in age range 18-55 years is significantly different when compared to reference interval limits in age range 56-95 year.

4.3.3 Effects of the hand-arm side on the reference interval limits for vital signs for the male and female population of Taita Taveta County, Kenya

The effects of the hand-arm side on the reference interval limits for vital signs for the male and female population of Taita Taveta County, Kenya, are presented in Table 4.8. The established reference interval limits for the selected vital signs for the left-hand and right-hand arm side diastolic blood pressure and pulse rate for the male population of Taita-Taveta County, Kenya were similar to the right-hand arm side of the female population of the same county ($\rho > 0.05$). Therefore, combined reference interval limits of these parameters for this population were established. The established reference interval limits for the combined left-hand-arm side diastolic blood pressure (46.8-97.4 mmHg) for male and female population of Taita-Taveta County, Kenya were similar to the combined right-hand-arm side diastolic blood pressure (45.4-100.7 mmHg) and the combined left hand arm side pulse rate of 58-111.2 mmHg was similar to the combined right hand arm side pulse rate of 58.0-113.8 mmHg. The established reference interval limits for the left-hand arm side systolic blood pressure for the male population of Taita-Taveta County< Kenya was significantly lower compared to the male population right-hand arm side systolic blood pressure of the same County ($\rho < 0.05$). The established reference interval for the left-hand arm side systolic blood pressure for the male population (92.2-149.8 mmHg) of Taita-Taveta County, Kenya was significantly higher than that of the female population (85.6-144.4 mmHg) of the same County; the right-hand arm side systolic blood pressure of the male

population (91-165 mmHg) of Taita-Taveta county, Kenya was significantly higher than that of the female population (90.8-145.2 mmHg) of the same County. The left-hand arm side systolic blood pressure for the male population (92.2-149.8 mmHg) of Taita-Taveta County, Kenya was significantly lower than the male right hand arm side systolic blood pressure (91-165 mmHg) (Table 4.8).

Table 4.8: Effects of the hand-arm side on the reference interval limits for vital signs for the male and female population of Taita Taveta County, Kenya

Vital Sign (unit)	Sex	N	Mean±SD	Percentiles (2.5% and 97.5%)		Reference Interval (RI)	IV	Difference between M&F	
				X-1.96SD	X+1.96SD			t-value	Sig
Left systolic BP (mmHg)	M&F	65	119.1±15.2	89.3	148.9	89.3-148.9	59.6	1.962	ρ = **0.0498**
	F	32	**115.0**±15.0*	**85.6**	**144.4**	**85.6-144.4**	**58.8**		
	M	33	**121.0**±14.7	**92.2**	**149.8**	**92.2-149.8**	**57.6**		
Right systolic BP (mmHg)	M&F	65	123.0±18.4	95.2	167.0	92.0-167.0	**75**	6.821	ρ = **0.0017**
	F	32	**118.0**±13.9*	**90.8**	**145.2**	**90.8-145.2**	**55.5**		
	M	33	**128.0**±18.9[a]	**91.0**	**165.0**	**91.0-165.0**	**73.9**		
Left diastolic BP (mmHg)	M&F	**65**	**72.0±12.9**	**46.8**	**97.4**	**46.8-97.4**	**50.6**	0.0788	ρ = 0.9372
	F	32	71.5±13.9	44.3	98.7	44.3-98.5	54.2		
	M	33	70.0±12.0	46.5	93.5	46.5-93.5	47		
Right diastolic BP (mmHg)	**M&F**	64	**73.0±14.1**	**45.4**	**100.7**	**45.4-100.7**	**54.3**	-0.806	ρ =0.4265
	F	32	71.9±14.3	44.0	99.8	44.0-100.0	56		
	M	33	74.0±14.1	46.3	101.7	46.3-101.7	55.4		
Left pulse rate (beats per minute)	M&F	65	**79.9±12.0**	**58.0**	**113.8**	**58.0-113.8**	55.8	0.525	ρ = 0.5995
	F	32	80.0±13.0	67.0	105.4	67.0-105.4	38.4		
	M	33	79.8±11.1	58.0	101.5	58.0-101.5	43.5		
Right pulse rate (beats per minute)	M&F	65	**79.9±11.4**	**58.0**	**111.2**	**58.0-111.2**	53.2	0.643	ρ = 0.5201
	F	32	80.4±10.1	60.6	100.2	60.6-100.2	39.6		
	M	33	79.3±12.8	54.4	104.3	54.3-104.3	50		

Results are expressed as Mean ± standard deviation (SD) for the number of subjects shown in the column labeled N. M = for male, F = female, M&F = combined male and female values, RI = reference interval limits; $\rho < 0.05$ is considered statistically significant by 2-tailed t-test.

4.4.4 Comparison of the developed reference interval limits for BMI and vital signs for infants, children, adolescents, adults and geriatric population of Taita-Taveta County, Kenya, with those reported in the medical literature

A comparison of this study's reference interval limits for BMI and the selected vital signs with those reported in the medical literature is presented in Table 4.9. This comparison indicates that: this study's lower reference interval limit for BMI is lower than that of the separate male and female American population, and the upper limit is higher and lower for males and females, respectively. Further, this study's lower reference interval limit is lower than that reported by WHO, while the upper reference interval limit is higher. This study's lower reference interval limit for systolic blood pressure is higher for both gender in the American population, while the upper limits are similar and lower for males and females, respectively; **d**iastolic blood pressure lower reference interval limit are lower than that of the American population, while the upper limits are lower (Foppa *et al.,* 2016). This study's lower reference interval limits for systolic blood pressure is lower and higher than that of the male and female **C**hinese population, respectively, while the upper limit is higher; diastolic lower reference interval limits is lower than that of the Chinese population, and the upper limits are lower. For pulse rate, this study's lower reference interval limits are higher than those of the Americans and Chinese, while the upper reference interval limits are lower (King *et al.,* 2006, Foppa *et al.,* 2016). For peripheral oxygen saturation ($S\rho O_2$), this study's

combined lower reference interval limits are lower than that reported by WHO, while the upper limits are similar. For axillary body temperature (^{0}C), this study's lower and upper reference interval limits are lower than the combined reference interval limits reported by Geneva *et al.* (2019) of 35.01-36.93^{o}C, Marui *et al.* (2017) of 35.54-37.40^{o}C, Gunes and Zaybak (2008) of 34.50-36.50^{o}C, and Thomas *et al.* (2004) of 34.62-37.40^{o}C and 33.11-35.67^{o}C for females of 21-36 years and 39-59 years, respectively, for other populations.

Table 4.9: Comparison of the developed reference interval limits for BMI and vital signs for infants, children, adolescents, adults and geriatric population of Taita-Taveta County, Kenya with those reported in literature

Parameter	Gender	This study RI	WHO	Foppa et al., 2016	King et al., 2006
BMI (kg/m^2)	M	14.5-31.8	18.5-24.9	20.5-36.5	
	F	14.1-39.9		16.7-37.9	
Systolic BP (mmHg)	M	88.0-157.0		94.6-157.4	91.5-138.2
	F	84.0-178.2		88.7-159.3	80.7-116.0
Diastolic BP (mmHg)	M	46.0-100.8		58.4-93.6	55.5-83.4
	F	49.0-108.0		54.4-89.6	53.0-74.6
Pulse Rate (heart beats per minute)	M	62.0-117.6		39.5-86.5	48.2-88.6
	F	60.0-129.0		46.4-85.6	52.6-90.6
Oxygen saturation (SpO_2) (%)	M	86.8-100	95-100		
	F	86.8-100			
Temperature (0^oC)	M	32.0-35.9			
	F	32.0-35.9			

The Chinese population studied by King et al., (2006) and the American population was studied by Foppa et al., (2016).

4.5 Established reference intervals for hematological and biochemical parameters for children and adolescents of Taita-Taveta county, Kenya

4.5.1Established reference intervals for hematological parameters for children and adolescents of Taita-Taveta County, Kenya

The established reference intervals for hematological parameters for male children and adolescents of Taita-Taveta, County, Kenya for RBC ($x10^{12}$/L), MCH (pg), MCHC (g/dL), RDW-SD (%), RDW-CV (%), WBC ($x10^{9}$/L), NEU (%), LYM ($x10^{9}$/L), LYM (%), MON ($x10^{9}$/L), MON (%), BAS (%), PDW (%) and MPV (fL) were similar to that of the female population of the same county ($\rho > 0.05$). Therefore, combined reference interval limits were established for these parameters for this population. The established reference interval limits were 5 (3-8) $x10^{12}$/L for RBC, 27 (20-33.9) pg for MCH, 33 (25-36) g/dL for MCHC, 46 (39-68.6) % for RDW-SD, 13 (11-19) % for RDW-CV, 8 (3-9) $x10^{9}$/L for WBC, 47 (24-58) % for NEU, 3 (0-4) $x10^{9}$/L for LYM, 44.5 (8-52) % for LYM, 0 (0-1) $x10^{9}$/L for MON, 4 (0-7) % for MON, 0 (0-1) % for BAS, 16 (8-17) % for PDW, and 9 (7-11) fL for MPV.

The established reference intervals for male children and adolescents of Taita-Taveta County, Kenya, for HB (g/dL), MCV (fL), NEU ($x10^{9}$/L), EOS ($x10^{9}$/L), EOS (%), and PLT ($x10^{9}$/L) significantly differed from that of the female population of the same County ($\rho < 0.05$). The established reference intervals for this population for HB is 12 (6-19.9) g/dL for males with mean Srank of 137.95 and 12 (8-17) g/dL for females with mean rank of 117.84 ($U = 6860$, $z = -2.226$, $\rho = 0.026$, $r = 0.1397$), MCV is 83 (56-103) fL for males with

mean rank of 116.5 and 85 (67-104.7) fL for females with mean rank of 137.66 (U = 6710.5, z = -2.296, ρ = 0.026, r = 0.1441), NEU is 3 (1-5) $x10^9$/L for males with mean rank of 112.72 and 4 (2-7) $x10^9$/L for females with mean rank of 140.3 (U = 6248.5, z = -3.048, ρ = 002, r = 0.1912), EOS is 0 (0-2) $x10^9$/L for males with mean rank of 137.27 and 0 (0-1) for females with mean rank of 118.47, EOS is 3 (0-6) % for males with mean rank of 148.09 and 1 (0-6) % for females with mean rank of 107.36 (U = 5418, z = -4.559, ρ = 0.000, r = 0.2861), and PLT is 301 (22-886) $x10^9$/L for males with mean rank of 139.22 and 273 (50-584) $x10^9$/L for females with mean rank of 116.67 (U = 6622.5, z = -2.444, ρ = 0.015, r = 0.1534) (Table 4.10).

Table 4.10: Established reference intervals for hematological parameters for children and adolescents of Taita Taveta County, Kenya

Analyte (Unit)	Sex	N	Median	Percentiles		Reference Interval	IV	Difference between M&F	
				2.5th	97.5th			z-value	Sig
RBC (x10^{12}/L)	**M&F**	254	4.65±1.05						
			5	**3**	**8**	**3-8**	**5**		
	F	132	4.57±0.81					-1.323	ρ = 0.186
			4.5	**3**	**6**	**3-6**	**3**		
	M	122	4.74±1.25						
			5	**3**	**8.93**	**3-8.9**	**5.9**		
HB (g/dL)	**M&F**	**254**	12.96±6.53						
			12	**8**	**18.25**	**8-18.3**	**10.3**		
	F	**132**	12.19±1.98					-2.226	ρ = 0.026
			12	**8**	**17**	**8-17**	**9**		
	M	**122**	13.79±9.15						
			12	**6**	**19.93**	**6-19.9*↑**	**13.9**		
MCH (pg)	**M&F**	**254**	29.22±25.66						
			27	**20**	**33.88**	**20-33.9**	**13.9**		
	F	**132**	26.89±3.29					-0.904	ρ = 0.366
			27	**20**	**34**	**20-34**	**14**		
	M	**122**	31.75±15.87						
			27.5	**18**	**45.88**	**18-45.9**	**27.9**		
MCHC (g/dL)	**M&F**	**254**	31.82±4.48						
			33	**25**	**36**	**25-36**	**11**		
	F	**132**	31.61±2.59					-1.764	ρ = 0.078
			32	**27**	**35**	**27-35**	**8**		
	M	**122**	32.04±5.88						
			33	**25**	**36**	**25-36**	**11**		
MCV (fL)	M&F	**254**	83.71±10.51						
			84	**64.38**	**103.63**	**64.4-103.6**	**39.2**	-2.296	ρ = 0.022
	F	**132**	85.28±±8.30						

			85	**67**	**104.68**	**67-104.7*↑**	**37.7**		
	M	**122**	82.01±12.29						
			83	**56**	**103**	**56-103**	**47**		
RDW-SD (%)	**M&F**	**254**	49.51±8.70						
			46	**39**	**68.63**	**39-68.6**	**29.6**		
	F	**132**	49.08±8.43					-0.995	ρ = 0.320
			46	**38.33**	**68**	**38.3-68**	**29.7**		
	M	**122**	49.98±8.99						
			46	**41**	**78**	**41-78**	**37**		
RDW-CV (%)	**M&F**	**254**	13.83±2.51						
			13	**11**	**19**	**11-19**	**8**		
	F	**132**	13.55±1.67					-1.340	ρ = 0.180
			13	**11**	**18**	**11-18**	**7**		
	M	**122**	14.15±3.15						
			14	**12**	**20**	**12-20**	**8**		
WBC ($x10^9$/L)	**M&F**	**254**	8.07±3.48						
			8	**3**	**9**	**3-9**	**6**		
	F	**132**	8.02±3.89					-0.614	ρ = 0.539
			7	**3**	**9**	**3-9**	**6**		
	M	**122**	8.12±3.07						
			8	**3.33**	**15.68**	**3.3-15.7**	**12.4**		
NEU ($x10^9$/L)	M&F	**254**	4.55±3.75						
			3	**1.35**	**5.09**	**1.4-5.1**	**3.7**		
	F	**132**	4.85±3.56					-3.048	ρ = 0.002
			4	**2**	**7.01**	**2-7*↑**	**5**		
	M	**122**	4.22±3.93						
			3	**1**	**5.03**	**1-5**	**4**		
NEU (%)	**M&F**	**254**	50.22±15.72						
			47	**24**	**58**	**24-58**	**34**		
	F	**132**	51.52±14.86					-1.602	ρ = 0.109
			50	**29**	**69**	**29-69**	**40**		
	M	**122**	48.80±16.55						
			45	**24**	**58.25**	**24-58.3**	**34.3**		
LYM ($x10^9$/L)	**M&F**	**254**	3.19±1.70						
			3	**0**	**4**	**0-4**	**4**		
	F	**132**	3.19±1.57					-0.808	ρ = 0.419
			3	**0**	**5.58**	**0-5.6**	**5.6**		
	M	**122**	3.18±1.83						

			3	**0.08**	**4**	**0.1-4**	**3.9**		
LYM (%)	**M&F**	**254**	40.92±14.76					-1.143	ρ = 0.253
			44.5	**8**	**52**	**8-52**	**44**		
	F	**132**	40.12±14.24						
			43.5	**6.65**	**56.7**	**6.7-56.7**	**50**		
	M	**122**	41.79±15.30						
			46	**9.15**	**54**	**9.2-54**	**44.8**		
MON ($x10^9$/L)	**M&F**	**254**	0.41±0.70					-1.478	ρ = 0.139
			0	**0**	**1**	**0-1**	**1**		
	F	**132**	0.48±0.84						
			0	**0**	**1.67**	**0-1.7**	**1.7**		
	M	**122**	0.34±0.57						
			0	**0**	**1**	**0-1**	**1**		
MON (%)	**M&F**	**254**	5.07±3.30					-1.588	ρ = 0.112
			4	**0**	**7**	**0-7**	**7**		
	F	**132**	5.34±3.18						
			4.5	**0**	**9.7**	**0-9.7**	**9.7**		
	M	**122**	4.77±3.41						
			4	**0**	**6**	**0-6**	**6**		
EOS ($x10^9$/L)	**M&F**	**254**	0.19±0.57					-3.331	ρ = 0.001
			0	**0**	**1**	**0-1**	**1**		
	F	**132**	0.08±0.27						
			0	**0**	**1**	**0-1**	**1**		
	M	**122**	0.30±0.76						
			0	**0**	**2**	**0-2*↑**	**2**		
EOS (%)	M&F	**254**	3.15±2.96					-4.559	ρ = 0.000
			2	**0**	**4**	**0-4**	**4**		
	F	**132**	2.35±2.32						
			1	**0**	**6**	**0-6**	**6**		
	M	**122**	4.01±3.33						
			3	**0**	**6**	**0-6*↑**	**6**		
BAS (%)	**M&F**	**254**	0.48±0.83					-0.705	ρ = 0.481
			0	**0**	**1**	**0-1**	**1**		
	F	**132**	0.39±0.49						
			0	**0**	**1**	**0-1**	**1**		
	M	**122**	0.58±1.07						
			0	**0**	**1**	**0-1**	**1**		
PLT	M&F	**254**	295.43±137.75					-2.444	ρ = 0.015

($x10^9$/L)			**288**	**22**	**610**	**22-610**	**588**		
	F	**132**	275.92±113.91						
			273	**50**	**584.7**	**50-584.7**	**534.7**		
	M	**122**	316.53±157.36						
			301	**22**	**886.2**	**22-886.2*↑**	**864.2**		
PDW (%)	**M&F**	**254**	14.60±2.90						
			16	**8**	**17**	**8-17**	**9**		
	F	**132**	14.14±3.26						
			16	**7.3**	**17**	**7.3-17**	**9.7**	-1.246	ρ = 0.213
	M	**122**	15.11±2.36						
			16	**8**	**17**	**8-17**	**9**		
MPV (fL)	**M&F**	**254**	8.91±0.94						
			9	**7**	**11**	**7-11**	**4**		
	F	**132**	8.89±1.04						
			9	**7**	**11**	**7-11**	**4**	-1.005	ρ = 0.315
	M	**122**	8.93±0.83						
			9	**7.1**	**10**	**7.1-10**	**2.9**		

Results are expressed as Median and range for the number of referent participants in the column labeled N. Statistical comparisons of the median values between male and female referent participants were carried out using the Mann-Whitney U test. Differences were considered statistically significant at $\rho < 0.05$.

4.5.1 Effects of age on the reference intervals for hematological parameters for children and adolescents of Taita-Taveta County, Kenya

The effects of age on the reference interval limits of the measured hematological parameters for children and adolescents of Taita-Taveta County, Kenya, are presented in Table 6.4. The different age groups were categorized as follows: (a) category 1 (>1-5 years), (b) category 2 (> 5-10 years), (c) category 3 (>10-15 years), and (d) category 4 (>15-18 years). Reference interval differences between males and females were estimated within each age category using the Mann-Whitney U test, where a ρ-value of less than 0.05 was considered statistically significant. Estimating the differences within and between the four age categories for separate males and females or combined males and females was carried out using the Kruskal-Wallis H test followed by the Mann-Whitney U test with an adjusted statistically significant ρ-value of less than 0.0083. The mean ± standard deviation (SD), median and 95 % range, sex and age categories are presented in Table 4.11

Results of the Kruskal-Wallis H test indicate that the reference interval limits for percent monocytes (MON %), absolute (EOS $x10^9$/L) and percent (EOS %) eosinophils, mean cell hemoglobin (MCH pg), mean cell volume (MCV fL), platelets (PLT $x10^9$/L), platelet distribution width (PDW %), and mean platelet volume (MPV fL) for combined male and female children and adolescents of Taita-Taveta County, Kenya were not statistically significantly affected by age ($\rho > 0.05$). However, the reference interval limits for white blood cells (WBC 10^9/L) (χ^2 (3) = 28.54, $\rho = 0.000$), absolute (NEU 10^9/L) (χ^2 (3) = 14.526, ρ =

0.002) and percent (NEU %) (χ^2 (3) = 9.959, ρ = 0.019) neutrophils, absolute (LYM 10^9/L) (χ^2 (3) = 12.785, ρ = 0.005) and percent (LYM %) (χ^2 (3) = 12.260, ρ = 0.007) lymphocytes, absolute monocytes (MON 10^9/L) (χ^2 (3) = 18.905, ρ = 0.000), percent basophils (BAS %) (χ^2 (3) = 9.842, ρ = 0.020), red blood cells (RBC 10^{12}/L) (χ^2 (3) = 22.872, ρ = 0.000), hemoglobin (HB g/dL) (χ^2 (3) = 15.675, ρ = 0.001), mean cell hemoglobin concentration (MCHC g/dL) (χ^2 (3) = 15.636, ρ = 0.001), red cell distribution width-standard deviation (RDW-SD %) (χ^2 (3) = 9.366, ρ = 0.025), and red cell distribution width-coefficient of deviation (RDW-CV %) (χ^2 (3) = 9.942, ρ = 0.019) for combined male and female children and adolescents of Taita-Taveta County, Kenya were statistically significantly affected by age. Mann-Whitney U test was used as a follow-up test for pairwise comparisons to identify the significant age group pairs with adjusted significant ρ-value of less than 0.0083. Results indicate that the reference interval limits for red blood cells for combined male and female children and adolescents of age greater than 1-5 years (4 (3-5) x10^{12}/L) with a mean rank of 57.7 is significantly lower than that of age greater than 5-10 years (5 (4-5.28) x10^{12}/L) with a mean rank of 82 (U = 1541, z = -3.881, ρ = 0.000, r = 0.3304), for mean cell hemoglobin concentration for combined male and female children and adolescents of age greater than 1-5 years (33 (27.6-34) g/dL) with a mean rank of 80.3 is significantly higher than that of age greater than 5-10 years (32 (25-34) g/dL) with a mean rank of 58.05 (U = 1611.5, z = -3.304, ρ = 0.001, r = 0.2813), for red blood cell distribution with-coefficient of variation for combined male and

female children and adolescents in age 1-5 years (14 (12-15) %) with a mean rank of 80.02 is significantly higher than that of age greater than 5-10 years (13 (11-14) %) with a mean rank of 58.35 (U = 1631.5, z = -3.262, ρ = 0.001, r = 0.2777).

Further, the reference interval limits for red blood cells for combined male and female children and adolescents in age 1-5 years (4 (3-5) $x10^{12}$/L) with a mean rank of 55.02 is significantly lower than that of age greater than 10-15 years (5 (4-5.22) $x10^{12}$/L) with a mean rank of 80.72 (U = 1350.5, z = -4.169, ρ = 0.000, r = 0.3615), for hemoglobin for combined male and female children and adolescents of age greater than 1-5 years (12 (7.8-13) g/dL) with a mean rank of 57.26 are significantly lower than that of age greater than 10-15 years (12.5 (10-14) g/dL) with a mean rank of 78.15 (U = 1509.5, z = -3.170, ρ = 0.002, r = 0.2749), for mean cell hemoglobin concentration for combined male and female children and adolescents of age greater than 1-5 years (33 (27.6-34) g/dL) with a mean rank of 77.35 is significantly higher than that of age greater than 10-15 years (32 (26.15-33.25) g/dL) with a mean rank of 55.15 (U = 1466, z = -3.353, ρ = 0.001, r = 0.2907).

In addition, the reference interval limits for white blood cells (WBC $x10^{9}$/L) for combined male and female children and adolescents of age greater than 1-5 years (10 (3-13) $x10^{9}$/L) with mean rank of 78.35 is significantly higher than that of age greater than 5-10 years (8 (3.4-9) $x10^{9}$/L) with mean rank of 60.12 (U = 1750, z = -2.691, ρ = 0.007, r = 0.2291), for absolute neutrophils (NEU $x10^{9}$/L) for combined male and female children and adolescents of age greater

than 1-5 years (5 (1.80-7.36) $x10^9$/L) with mean rank of 78.04 is significantly higher than that of age greater than 5-10 years (3 (1.68-5.28) $x10^9$/L) with mean rank of 59.28 (U = 1701.5, z = -2.813, ρ = 0.005, r = 0.2395), and for percent basophils (BAS %) for combined male and female children and adolescents of age greater than 1-5 years (1 (0-1) %) with mean rank of 78.04 is significantly higher than that of age greater than 5-10 years (1 (0-1) %) with mean rank of 60.45 (U = 1772, z = -3.007, ρ = 0.003, r = 0.2560).

Further, the reference interval limits for white blood cells (WBC $x10^9$/L) for combined male and female children and adolescents in age greater than 1-5 years (10 (3-13) $x10^9$/L) with mean rank of 80.37 is significantly higher than that of age greater than 10-15 years (7 (4-8) $x10^9$/L) with mean rank of 51.69 (U = 1252, z = -4.302, ρ = 0.000, r = 0.3730), for absolute neutrophils (NEU $x10^9$/L) for combined male and female children and adolescents of age greater than 1-5 years (5 (1.80-7.36) $x10^9$/L) with mean rank of 77.71 is significantly higher than that of age greater than 10-15 years (3 (1-4.25) $x10^9$/L) with mean rank of 54.73 (U = 1440.5, z = -3.479, ρ = 0.001, r = 0.3017), and for absolute monocytes (MON $x10^9$/L) for combined male and female children and adolescents of age greater than 1-5 years (1 (0-1) $x10^9$/L) with mean rank of 77.96 is significantly higher than that of age greater than 10-15 years (0 (0-0.61) $x10^9$/L) with mean rank of 54.44 (U = 1440.5, z = -3.479, ρ = 0.001, r = 0.3017).

The reference interval limits for absolute eosinophils (EOS $x10^9$/L) for combined male and female children and adolescents in age greater than 1-5

years (0 (0-0.27) $x10^9$/L) with a mean rank of **62.73** is significantly lower than that of age greater than 15-18 years (0 (0-0.68) $x10^9$/L) with a mean rank of 63.36 (U = 1533.5, z = -3.190, ρ = 0.001, r = 0.2853), and for percent eosinophils (EOS %) for combined male and female children and adolescents of age greater than 1-5 years (1 (0-4) %) with a mean rank of **59.87** is significantly lower than that of age greater than 15-18 years (2 (0-4) %) with a mean rank of **67.11** (U = 1372.5, z = -2.960, ρ = 0.003, r = 0.2648).

Further, the reference interval limits for percent eosinophils (EOS %) for combined male and female children and adolescents in age greater than 6-10 years (3 (0-5) %) with a mean rank of **64.52** is significantly higher than that of age greater than 10-15 years (3 (1-4.21) %) with a mean rank of **64.48** (U = 1312.5, z = -3.575, $\rho < 0.001$, r = 0.3148).

Reference interval limits for red blood cell distribution width-standard deviation (RDW-SD %) for combined male and female children and adolescents of age greater than 6-10 years (45 (38-51) %) with a mean rank of **52.42** is significantly lower than that of age greater than 15-18 years (47.5 (39.13-64) %) with a mean rank of **71.65** (U = 1234.5, z = -3.008, $\rho < 0.001$, r = 0.2735). The reference interval limits for percent neutrophils (NEU %) for combined male and female children and adolescents of age greater than 6-10 years (43 (28.7-56) %) with mean rank of **53.01** is significantly lower than that of age greater than 15-18 years (50 (30.25-59.5) %) with mean rank of **70.91** (U = 1274, z = -2.791, ρ = 0.005, r = 0.2537), for absolute lymphocytes (LYM $x10^9$/L) for combined male and female children and adolescents of age greater

than 6-10 years (4 (1-4) $x10^9$/L) with mean rank of **71.40** is significantly higher than that of age greater than 15-18 years (2 (0-3) $x10^9$/L) with mean rank of **48.09** (U = 1112, z = -3.735, $\rho < 0.001$, r = 0.3395), and for percent lymphocytes (LYM %) for combined male and female children and adolescents of age greater than 6-10 years (47 (9-53) %) with mean rank of **69.66** is significantly higher than that of age greater than 15-18 years (40 (5.5-47) %) with mean rank of **50.26** (U = 1229, z = -3.027, $\rho = 0.002$, r = 0.2752).

Further, the reference interval limits for percent lymphocytes (LYM %) for combined male and female children and adolescents of age greater than 10-15 years (46 (11.75-53.25) %) with a mean rank of **69.66** is significantly higher than that of age greater than 15-18 years (40 (5.5-47) %) with a mean rank of **50.26** (U = 1229, z = -3.027, $\rho = 0.002$, r = 0.2752).

Results of the Kruskal-Wallis H test indicate that the reference interval limits for mean cell hemoglobin (MCH pg), mean cell volume (MCV fL), percent neutrophils (NEU %), absolute (LYM $x10^9$/L) and percent (LYM %) lymphocytes, absolute monocytes (MON $x10^9$/L), absolute (EOS $x10^9$/L) and percent (EOS %) eosinophils, platelets (PLT $x10^9$/L), and platelet distribution width (PDW %) for male children and adolescents of Taita-Taveta County, Kenya were not significantly affected by age ($\rho > 0.05$).

Interestingly, the reference interval limits for red blood cells (RBC $x10^{12}$/L) (χ^2 (3) = 20.865, $\rho = 0.000$), hemoglobin (HB) (χ^2 (3) = 13.139, $\rho = 0.004$), mean cell hemoglobin concentration (MCHC g/dL) (χ^2 (3) = 15.332, $\rho = 0.002$), red cell distribution width-standard deviation (RDW-SD %) (χ^2 (3) = 13.447, ρ =

0.004), red cell distribution width-coefficient of variation (RDW-CV) (χ^2 (3) = 10.637, ρ = 0.014), white blood cells (WBC x10^9/L) (χ^2 (3) = 25.542, ρ = 0.000), absolute neutrophils (NEU x10^9/L) (χ^2 (3) = 10.537, ρ = 0.015), percent monocytes (MON %) (χ^2 (3) = 10.720, ρ = 0.013), percent basophils (BAS %) (χ^2 (3) = 8.363, ρ = 0.039), and mean platelet volume (MPV %) (χ^2 (3) = 14.910, ρ = 0.002) for male children and adolescents of Taita-Taveta County, Kenya were significantly affected by age.

Results for the pairwise comparison using Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for red blood cells (RBC x10^{12}/L) for male children and adolescents of age greater than 1-5 years (4 (3-5) x10^{12}/L) with mean rank of **24.32** is significantly lower than that of age greater than 6-10 years (5 (4-5.72) x10^{12}/L) with mean rank of **41** (U = 232, z = -3.952, ρ < 0.001, r = 0.4979), for red blood cell distribution width-standard deviation (RDW-SD %) for male children and adolescents of age greater than 1-5 years (47 (41-53.5) %) with mean rank of **38.56** is significantly higher than that of age greater than 6-10 years (44 (39-52.76) %) with mean rank of **24.31** (U = 270, z = -3.090, ρ = 0.002, r = 0.3839), and for red blood cell distribution width-coefficient of variation (RDW-CV %) for male children and adolescents of age greater than 1-5 years (14 (12-15) %) with mean rank of **39.03** is significantly higher than that of age greater than 6-10 years (13 (11-14.01) %) with mean rank of **23.76** (U = 254, z = -3.386, ρ = 0.001, r = 0.4266). Reference interval limits for percent basophil count (BAS %) for male children and adolescents of age

greater than 1-5 years (1 (0-1) %) with a mean rank of **37.62** is significantly higher than that of age greater than 6-10 years (0 (0-0.39) %) with a mean rank of **25.41** (U = 302, z = -3.072, ρ = 0.002, r = 0.3870).

In addition, the reference interval limits for red blood cell count (RBC $x10^{12}$/L) for male children and adolescents of age greater than 1-5 years (4 (3-5) $x10^{12}$/L) with a mean rank of **25.96** is significantly lower than that of age greater than 10-15 years (5 (4-5.7) $x10^{12}$/L) with a mean rank of **40.73** (U = 287.5, z = -3.476, ρ = 0.001, r = 0.4311), and for mean cell hemoglobin concentration (MCHC g/dL) for male children and adolescents of age greater than 1-5 years (33 (31-35) g/dL) with a mean rank of **41.19** is significantly higher than that of age greater than 10-15 years (32 (27-33) g/dL) with a mean rank of **24.02** (U = 248.5, z = -3.735, ρ < 0.001, r = 0.4633).

Further, the reference interval limits for white blood cell count (WBC $x10^{9}$/L) for male children and adolescents of age greater than 1-5 years (9 (3-12.74) $x10^{9}$/L) with a mean rank of **41.71** is significantly higher than that of age greater than 10-15 years (7 (4-8) $x10^{9}$/L) with a mean rank of **23.45** (U = 231, z = -3.914, ρ < 0.001, r = 0.3962), and for absolute neutrophil count (NEU $x10^{9}$/L) for male children and adolescents of age greater than 1-5 years (5 (2-8.56) $x10^{9}$/L) with a mean rank of **39.40** is significantly higher than that of age greater than 10-15 years (3 (1-4) $x10^{9}$/L) with a mean rank of **25.98** (U = 309.5, z = -2.902, ρ = 0.004, r = 0.3599).

Reference interval limits for red blood cell count (RBC $x10^{12}$/L) for male children and adolescents of age greater than 1-5 years (4 (3-5) $x10^{12}$/L) with

mean rank of **24.82** is significantly lower than that of age greater than 15-18 years (5 (0-5.2) $x10^{12}$/L) with mean rank of **39.61** (U = 249, z = -3.514, $\rho < 0.001$, r = 0.4463), hemoglobin (g/dL) for male children and adolescents of age greater than 1-5 years (12 (9-12.67) g/dL) with mean rank of **25.75** is significantly lower than that of age greater than 15-18 years (14 (6-16) g/dL) with mean rank of **38.48** (U = 280.5, z = -2.809, $\rho = 0.005$, r = 0.3567), mean cell hemoglobin concentration (MCHC g/dL), and for male children and adolescents of age greater than 1-5 years (33 (31-35) g/dL) with mean rank of **37.47** is significantly higher than that of age greater than 15-18 years (32 (0-34) g/dL) with mean rank of **24.25** (U = 273, z = -2.919, $\rho = 0.001$, r = 0.3707), and for white blood cell count (WBC $x10^9$/L) for male children and adolescents of age greater than 1-5 years (9 (3-12.74) $x10^9$/L) with mean rank of **39.04** is significantly higher than that of age greater than 15-18 years (6 (5-8) $x10^9$/L) with mean rank of **22.34** (U = 219.5, z = -3.654, $\rho < 0.001$, r = 0.4641).

Reference interval limits for red cell distribution width-standard deviation (RDW-SD %) for male children and adolescents of age greater than 5-10 years (44 (39-52.76) %) with a mean rank of 25.97 is significantly higher than that of age greater than 10-15 years (47 (42-59) %) with a mean rank of 34.74 (U = 253.5, z = -2.910, $\rho = 0.004$, r = 0.3757), for white blood cell count (WBC $x10^9$/L) for male children and adolescents of age greater than 5-10 years (8 (4-9.5) $x10^9$/L) with a mean rank of 38.03 are significantly higher than that of age greater than 10-15 years (7 (4-8) $x10^9$/L) with a mean rank of 23.45 (U = 231, z = -3.277, $\rho = 0.001$, r = 0.4231), and for mean platelet volume (MPV fL) for

male children and adolescents of age greater than 5-10 years (9 (8-9) fL) with a mean rank of 25.03 is significantly lower than that of age greater than 10-15 years (9 (8-10) f/L) with a mean rank of 37.47 (U = 233.5, z = -3.413, ρ = 0.001, r = 0.4406).

Reference interval limits for red cell distribution width-standard deviation (RDW-SD %) for male children and adolescents of age greater than 5-10 years (44 (39-52.76) %) with a mean rank of 22.59 is significantly lower than that of age greater than 15-18 years (49 (42-60) %) with a mean rank of 35.64 (U = 220, z = -2.983, ρ = 0.003, r = 0.3951), and for white blood cell count (WBC $x10^9$/L) for male children and adolescents of age greater than 5-10 years (8 (4-9.5) $x10^9$/L) with a mean rank of 34.88 is significantly higher than that of age greater than 15-18 years (6 (5-8) $x10^9$/L) with a mean rank of 22.91 (U = 235.5, z = -2.755, ρ = 0.006, r = 0.3649).

Reference interval limits for percent monocytes (MON %) for male children and adolescents of age greater than 10-15 years (3 (0-6) %) with a mean rank of 23.85 is significantly lower than that of age greater than 15-18 years (6 (1-7.49) %) with a mean rank of 36.80 (U = 243.5, z = -2.916, ρ = 0.004, r = 0.3796), and for mean platelet volume (MPV fL) for male children and adolescents of age greater than 10-15 years (9 (8-10) fL) with a mean rank of 36.06 is significantly higher than that of age greater than 15-18 years (9 (7-9.03) fL) with a mean rank of 23.29 (U = 246, z = -3.096, ρ = 0.002, r = 0.4031).

Results of the Kruskal-Wallis H test indicate that the reference interval limits for red blood cell count (RBC $x10^{12}$/L), hemoglobin (HB g/dL), mean cell hemoglobin (MCH pg), mean cell volume (MCV fL), red cell distribution width-standard deviation (RDW-SD %), red blood cell distribution width-coefficient of variation (RDW-CV %), white blood cell count (WBC $x10^{12}$/L), absolute neutrophil count (NEU $x10^{9}$/L), absolute (EOS $x10^{9}$/L) and percent (EOS %) eosinophil count, percent basophil count (BAS %), platelet count (PLT $x10^{9}$/L), and platelet distribution width (PDW %) for female children and adolescents of Taita-Taveta County, Kenya were not significantly affected by age ($\rho > 0.05$).

However, the reference interval limits for mean cell hemoglobin concentration (MCHC g/dL) (χ^2 (3) = 9.174, $\rho = 0.027$), percent neutrophil count (NEU %) (χ^2 (3) = 9.638, $\rho = 0.022$), absolute (LYM $x10^{9}$/L) (χ^2 (3) = 8.749, $\rho = 0.033$) and percent (LYM %) (χ^2 (3) = 11.014, $\rho = 0.012$) lymphocytes, absolute (MON $x10^{9}$/L) (χ^2 (3) = 15.915, $\rho = 0.001$) and percent (MON %) (χ^2 (3) = 12.717, $\rho = 0.005$), and mean platelet volume (MPV %) (χ^2 (3) = 13.921, $\rho = 0.003$) for female children and adolescents of Taita-Taveta County, Kenya were significantly affected by age.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for mean cell hemoglobin concentration (MCHC g/dL) for female children and adolescents of age greater than 1-5 years (34 (26-34) g/dL) with a mean rank of **45.78** is significantly lower than that of age greater

than 6-10 years (30.5 (27-33) g/dL) with a mean rank of **30.42** (U = 415, z = -3.092, $\rho < 0.001$, r = 0.3670).

The reference interval limits for absolute monocyte count (MON $x10^9$/L) for female children and adolescents of age greater than 1-5 years (1 (0-1) $x10^9$/L) with a mean rank of **40.81** is significantly higher than that of age greater than 10-15 years (0 (0-1.11) $x10^9$/L) with a mean rank of **26.97** (U = 340, z = -3.295, $\rho = 0.001$, r = 0.3996), and for percent eosinophil count (EOS %) for female children and adolescents of age greater than 1-5 years (1 (0-2.44) $x10^9$/L) with a mean rank of **28.65** is significantly lower than that of age greater than 10-15 years (2 (1-4) $x10^9$/L) with a mean rank of **40.60** (U = 357, z = -2.638, $\rho = 0.008$, r = 0.3199).

Further, the reference interval limits for absolute monocyte count (MON $x10^9$/L) for female children and adolescents of age greater than 1-5 years (1 (0-1) $x10^9$/L) with a mean rank of **37.51** is significantly higher than that of age greater than 15-18 years (0 (0-0.43) $x10^9$/L) with a mean rank of **24.15** (U = 277, z = -3.269, $\rho = 0.001$, r = 0.4119), for percent monocyte count (MON %) for female children and adolescents of age greater than 1-5 years (7 (0-8) $x10^9$/L) with a mean rank of **37.12** is significantly lower than that of age greater than 15-18 years (3 (0-5.5) $x10^9$/L) with a mean rank of **24.71** (U = 291.5, z = -2.660, $\rho = 0.008$, r = 0.3351), and for mean platelet volume (MPV fL) for female children and adolescents of age greater than 1-5 years (9 (7-11) fL) with a mean rank of **40.10** is significantly lower than that of age greater

than 15-18 years (10 (7-11) fL) with a mean rank of **26.31** (U = 270.5, z = -3.049, ρ = 0.002, r = 0.3841).

In addition, the reference interval limits for percent neutrophil count (NEU $x10^9$/L) for female children and adolescents of age greater than 5-10 years (4 (1-6.5) $x10^9$/L) with mean rank of 26.87 is significantly lower than that of age greater than 15-18 years (5 (2-6) $x10^9$/L) with mean rank of 40.73 (U = 280, z = -2.927, ρ = 0.003, r = 0.3659), for absolute lymphocyte count (LYM $x10^9$/L) for female children and adolescents of age greater than 5-10 years (3 (1-4) $x10^9$/L) with mean rank of 37.93 is significantly higher than that of age greater than 15-18 years (2.5 (0-3) $x10^9$/L) with mean rank of 24.56 (U = 287.5, z = -2.908, ρ = 0.004, r = 0.3635), for percent lymphocyte count (LYM %) for female children and adolescents of age greater than 5-10 years (47.5 (9-53) %) with mean rank of 38.12 is significantly higher than that of age greater than 15-18 years (37.5 (4-43.25) %) with mean rank of 24.29 (U = 280.5, z = -2.921, ρ = 0.003, r = 0.3651), and for mean platelet volume (MPV fL) for female children and adolescents of age greater than 5-10 years (9 (7-9.18) fL) with mean rank of 27.33 is significantly lower than that of age greater than 15-18 years (10 (7-11) fL) with mean rank of 40.06 (U = 297.5, z = -2.789, ρ = 0.005, r = 0.3486).

The reference interval limits for percent lymphocyte count (LYM %) for female children and adolescents of age greater than 10-15 years (46 (6-49) %) with a mean rank of 35.03 is significantly higher than that of age greater than 15-18 years (37.5 (4-43.25) %) with a mean rank of 21.81 (U = 216, z = -3.00,

ρ = 0.003, r = 0.3974), and for mean platelet volume (MPV fL) for female children and adolescents of age greater than 10-15 years (9 (8-9) fL) with a mean rank of 22.60 is significantly lower than that of age greater than 15-18 years (10 (7-11) fL) with a mean rank of 36.63 (U = 204.5, z = -3.328, ρ = 0.001, r = 0.4408).

Results of the investigation on the effect of sex at each of the four age categories on the reference interval limits of the measured hematological parameters of children and adolescents of Taita-Taveta County, Kenya are also presented in Table 4. 6. Results of the age category of greater than 1-5 years indicate that the reference interval limits for percent monocytes (MON %) for males (3 (0-8) %) with a mean rank of 29.91 are significantly lower than those of females (7 (0-8) %) with a mean rank of 41.59 (U = 422, z = -2.397, ρ = 0.017, r = 0.2845), for absolute eosinophil count (EOS $x10^9$/L) for males (0 (0-1) $x10^9$/L) with a mean rank of 41.49 are significantly higher than those of females (0 (0-0.88) $x10^9$/L) with a mean rank of 30.96 (U = 442, z = -3.307, ρ = 0.001, r = 0.3925), and for percent eosinophil count for males (3.5 (0-7) %) with a mean rank of 42.59 are significantly higher than those of females (1 (0-2.44) %) with a mean rank of 29.95 (U = 405, z = -2.659, ρ = 0.008, r = 0.3156).

Results of the age category of greater than 5-10 years indicate that the reference interval limits for mean cell hemoglobin concentration (MCHC g/dL) for males (33 (25-36.62) g/dL) with a mean rank of 41.28 are significantly higher than those of females (30.5 (27-33) g/dL) with a mean rank of 28.45 (U

= 340, z = -2.694, ρ = 0.007, r = 0.3291), for mean cell volume (MCV fL) for males (82 (68-86.53) fL) with a mean rank of 28.10 are significantly lower than those of females (85.5 (67-89) fL) with a mean rank of 38.50 (U = 380, z = -2.172, ρ = 0.030, r = 0.2654), and for percent eosinophil count (EOS %) for males (4 (1-7) %) with a mean rank of 43.50 are significantly higher than those of females (1 (0-4) %) with a mean rank of 26.75 (U = 275.5, z = -3.592, ρ < 0.001, r = 0.4388).

Results of the age category of greater than 10-15 years indicate that the reference interval limits for mean platelet volume (MPV fL) for males (9 (8-10) fL) with a mean rank of 39.03 was significantly higher than that of the females (9 (8-9) fL) with a mean rank of 23.97 (U = 247, z = -3.515, ρ < 0.001, r = 0.4464).

Results of the age category of greater than 15-18 years indicate that the reference interval limits for red blood cell count, and hemoglobin with mean rank 31.39 and 32.50, respectively, for males (5 (0-5.2) $x10^{12}$/L; 14 (6-16) g/dL) was significantly higher than that of females (5 (4-5) $x10^{12}$/L; 12 (10-13.01) g/dL) with a mean rank of 23.31 (U = 255, z = -2.130, ρ = 0.033, r = 0.2899) and 22.12 (U = 224, z = -2.490, ρ = 0.013, r = 0.3388), respectively, while reference interval limits for mean cell volume for males (82.5 (13-86) fL) with a mean rank of 21.88 was significantly lower than that of females (89 (73-92) fL) with a mean rank of 33.56 (U = 206.5, z = -2.735, ρ = 0.006, r = 0.3722).

Results of the age category of greater than 15-18 years indicate that the reference interval limits for white blood cell count (WBC $x10^9$/L) for males (6 (5-8) $x10^9$/L) with mean rank of 22.64 are significantly lower than those of females (8 (6-9) $x10^9$/L) with mean rank of 32.73 ($U = 228$, $z = -2.401$, $\rho = 0.016$, $r = 0.3267$), for absolute neutrophil count (NEU $x10^9$/L) for males (3 (2-5.18) $x10^9$/L) with mean rank of 21.48 are significantly lower than those of females (5 (2-6) $x10^9$/L) with mean rank of 33.98 ($U = 195.5$, $z = -2.977$, $\rho = 0.003$, $r = 0.4051$), for percent neutrophil count (NEU %) for males (46 (28-59) %) with mean rank of 22.20 are significantly lower than those of females (55 (34-68.25) %) with mean rank of 33.21 ($U = 215.5$, $z = -2.574$, $\rho = 0.010$, $r = 0.3503$), for percent lymphocytes count (LYM %) for males (43 (24-48) %) with mean rank of 31.54 are significantly higher than those of females (37.5 (4-43.25) %) with mean rank of 23.15 ($U = 251$, $z = -1.959$, $\rho = 0.050$, $r = 0.2666$), and for percent monocyte count (MON %) for males (6 (1-7.49) %) with mean rank of 32.88 are significantly higher than those of females (3 (0-5.5) %) with mean rank of 21.71 ($U = 213.5$, $z = -2.622$, $\rho = 0.009$, $r = 0.3568$). Results of the age category of greater than 15-18 years indicate that the reference interval limits for platelet count (PLT $x10^9$/L) for males (291 (16-408.11) $x10^9$/L) with a mean rank of 32.29 are significantly higher than those of females (239 (72-289) $x10^9$/L) with a mean rank of 22.35 ($U = 230$, $z = -2.321$, $\rho = 0.020$, $r = 0.3158$), and for mean platelet volume (MPV fL) for males (9 (7-9.03) fL) with a mean rank of 21.64 are significantly lower than

those of females (10 (7-11) fL) with a mean rank of 33.81 ($U = 200$, $z = -2.986$, $\rho = 0.003$, $r = 0.4063$).

Table 4.11:Effect of age and sex on the reference intervals for hematological parameters for children and adolescents of Taita-Taveta County, Kenya

Analyte (Unit)	Gender	N	Median reference limits						
			≥ 1-5 years	N	≥ 5-10 years	N	≥ 10-15 years	N	≥ 15-18 years
RBC ($x10^{12}$/L)	M&F	71	4.28±0.87	67	4.87±1.00	62	4.95±1.15	54	4.52±1.06
			4 (3-5)		**5 (4-5.3)[a]↑**		**5 (4-5.2)[b]↑**		**5 (0-5)**
	F	37	4.38±1.06	38	4.63±0.**68**	31	4.81±0.**79**	26	4.46±0.51
			4 (3-5)		**5 (4-5)**		**5 (4-5)**		**5 (4-5)**
	M	34	4.18±0.58	29	5.17±1.26	31	5.10±1.42	28	4.57±1.40
			4 (3-5)		**5 (4-5.7)[a]↑**		**5 (4-5.7)[b]↑**		**5 (0-5.2)*↑[c]**
HB (g/dL)	M&F	71	11.85±2.45	67	12.46±1.72	62	12.81±1.76	54	15.20±13.47
			12 (7.8-13)		**12 (9.7-13)**		**12.5 (10-14)[b]↑**		**12 (6-19.4)**
	F	37	11.81±2.85	38	12.05±1.34	31	12.68±1.76	26	12.35±1.41
			11 (7-13.5)		**12 (9-13)**		**12 (10-14)**		**12 (10-13)**
	M	34	11.88±1.97	29	13.00±2.02	31	12.94±1.79	28	17.86±18.41
			12 (9-12.7)		**12 (11-14)**		**13 (11-14)**		**14 (6-16)*↑[c]**
MCH (pg)	M&F	71	27.41±3.75	67	26.85±3.27	62	26.90±2.83	54	37.22±54.94
			28 (19.8-29)		**27 (21-28)**		**28 (21-29)**		**27 (15-30)**
	F	37	27.16±4.20	38	26.18±2.25	31	26.84±3.24	26	27.62±3.13
			27 (19-29)		**26.5 (21-28)**		**26 (21-30)**		**27.5 (20-30)**
	M	34	27.68±3.24	29	27.72±4.13	31	26.97±2.40	28	46.14±75.79
			28 (21-30)		**27 (24-29.5)**		**28 (21-28)**		**27 (15-33.2)**
MCHC (g/dL)	M&F	71	33.00±2.17	67	31.93±5.21	62	31.47±2.67	54	30.54±6.62
			33 (27.6-34)		**32 (25-34)[a]↓**		**32 (26.2-33.3)[b]↓**		**33 (0-34)**
	F	37	32.16±2.49	38	30.79±2.23	31	31.48±2.92	26	**31.88±2.55[c]**
			34 (26-34)		**30.5 (27-33)[a]↓**		**32 (25-34)**		**33 (27-34)**
	M	34	33.68±1.53	29	33.41±7.30	31	31.45±2.45	28	29.29±8.76
			33 (31-35)		**33 (25-36.6)*↑**		**32 (27-33)[b]↓**		**32 (0-34)[c]↓**
MCV (fL)	M&F	71	83.31±9.55	67	84.07±7.01	62	85.95±7.80	54	81.20±16.23
			82 (63-90)		**83 (67-88)**		**86 (69.7-88.4)**		**86 (13-90)**
	F	37	84.14±10.75	38	84.76±6.51	31	85.58±7.97	26	87.31±6.98
			83 (59-88.3)		**85.5 (67-89)*↑**		**87 (68-88)**		**89 (73-91)*↑**
	M	34	82.41±8.11	29	83.17±7.64	31	86.32±7.73	28	75.54±20.07

			82 (64-90)		**82 (68-86.5)[a]↓**		**85 (75-90)[d]↑**		**82.5 (13-86)**
RDW-SD (%)	M&F	71	49.75±7.80	67	47.76±9.09	62	49.89±8.70	54	50.94±9.22
			46 (39-56)		**45 (38-51)**		**47 (39.6-59)**		**47.5 (39.1-64)[e]↑**
	F	37	49.97±9.53	38	47.82±6.85	31	49.19±9.71	26	49.50±7.43
			46 (39-59)		**45 (38-53.3)**		**46 (39-56)**		**47 (38-54)**
	M	34	49.50±5.47	29	47.69±11.52	31	50.58±7.64	28	52.29±10.58
			47 (41-53.5)		**44 (39-52.8)[a]↓**		**47 (42-59)[d]↑**		**49 (42-60)[e]↑**
RDW-CV (%)	M&F	71	14.00±1.64	67	13.19±1.57	62	13.61±1.61	54	14.67±4.37
			14 (12-15)		**13 (11-14)[a]↓**		**14 (11-15)**		**14 (12-16)**
	F	37	13.84±1.82	38	13.21±1.28	31	13.52±1.79	26	13.65±1.83[c]
			14 (12-15)		**13 (11-14)**		**14 (11-15)**		**13 (12-15.3)**
	M	34	14.18±1.42	29	13.17±1.91	31	13.71±1.44	28	15.61±5.69[c]
			14 (12-15)		**13 (11-14)[a]↓**		**14 (12-15)**		**14 (12-18.2)**
WBC ($x10^9$/L)	M&F	71	9.86±5.01	67	7.97±2.45	62	6.87±2.47	54	**7.22**±1.88
			10 (3-13)		**8 (3.4-9)[a]↓**		**7 (4-8)[b]↓**		**7 (5-11.6)**
	F	37	9.43±3.94	38	7.79±2.65[a]	31	7.26±2.84[b]	26	**7.77±1.75[c]**
			10 (3-13)		**8 (2-9)**		**7 (4-8.6)**		**8 (6-9)*↑**
	M	34	10.32±5.99	29	8.21±2.18[a]	31	6.48±1.99[b]	28	6.71±1.88[c]
			9 (3-12.7)		**8 (4-9.5)**		**7 (4-8)[b]↓[d]**		**6 (5-8)[c]↓[e]**
NEU ($x\ 10^9$/L)	M&F	71	5.96±5.15	67	4.23±3.74	62	3.58±2.03	54	4.19±2.43
			5 (1.8-7.4)		**3 (1.7-5.3)[a]↓**		**3 (1-4.3)[b]↓**		**4 (2-5)**
	F	37	5.81±4.24	38	4.76±4.52	31	4.03±2.26	26	4.58±1.50
			5 (1-7.4)		**4 (1-6.5)**		**4 (2-5.1)**		**5 (2-6)*↑**
	M	34	6.12±6.05	29	3.55±2.29	31	3.13±1.69	28	3.82±3.03
			5 (2-8.6)		**3 (2-4.6)**		**3 (1-4)[b]↓**		**3 (2-5.2)**
NEU (%)	M&F	71	53.32±18.86	67	47.58±15.28	62	47.10±12.97	54	52.98±13.60
			52 (24-66)		**43 (28.7-56)**		**46 (25.2-55)**		**50 (30.3-59.5)[e]↑**
	F	37	53.41±16.80	38	47.63±13.51	**31**	48.81±11.04	**26**	57.77±16.01
			52 (28-64)		**44 (28-53)**		**50 (30-53.6)**		**55 (34-68.3)*↑[e]**
	M	34	53.24±21.14	29	47.52±17.57	**31**	45.39±14.64	**28**	48.54±9.12
			46 (24-71)		**41 (30-56)**		**42 (24-55)**		**46 (28-59)**
LYM ($x\ 10^9$/L)	M&F	71	3.59±2.34	67	3.42±1.29	62	3.05±1.18	54	2.52±1.45
			3 (0-4.2)		**4 (1-4)**		**3 (1-4.2)**		**2 (0-3)[e]↓**
	F	37	3.57±2.08	38	3.47±1.29	31	3.13±1.06	26	2.31±1.35

			3 (0-5.5)		**3 (1-4)**		**3 (1-3.6)**		**2.5 (0-3)[e]↓**
	M	34	3.62±2.63	29	3.34±1.32	31	2.97±1.30[b]	28	2.71±1.54
			2.5 (0-6.3)		**4 (1-4)**		**3 (1-4)**		**2 (0-3.4)**
LYM (%)	M&F	71	37.45±17.48	67	43.48±13.58	62	45.10±12.64	54	**37.50±12.87**
			38 (7.6-54)		**47 (9-53)**		**46 (11.8-53.3)**		**40 (5.5-47)[e]↓[f]**
	F	37	37.49±16.80	38	43.97±13.12	31	43.74±10.07	26	33.92±14.99
			38 (6-51)		**47.5 (9-53)**		**46 (6-49)**		**37.5 (4-43.3)[e]↓[f]**
	M	34	37.44±19.12	29	42.83±14.37	31	46.45±14.83	28	40.82±9.66
			47.5 (8-54.3)		**46 (9-53.5)**		**48 (16-58)**		**43 (24-48)*↑**
MON (x 10^9/L)	M&F	71	0.56±0.55	67	0.43±0.56	62	0.29±1.06	54	0.31±0.47
			1 (0-1)		**0 (0-1)**		**0 (0-0.61)[b]↓**		**0 (0-1)**
	F	37	0.68±5.30	38	0.47±0.56	31	0.45±1.46	26	0.23±0.43
			1 (0-1)		**0 (0-1)**		**0 (0-1.1)[b]↓**		**0 (0-0.4)[c]↓**
	M	34	0.44±0.56	29	0.38±0.56	31	0.13±0.34	28	0.39±0.50
			0 (0-1)		**0 (0-1)**		**0 (0-0.3)**		**0 (0-1)**
MON (%)	M&F	71	5.34±3.44	67	5.55±3.26	62	4.24±3.04	54	5.06±3.36
			6 (0-8)		**5 (2-7)**		**4 (0-6)**		**5 (0-6.3)**
	F	37	6.41±3.30	38	5.71±2.89	31	4.74±2.97	26	4.00±3.23
			7 (0-8)*↑		**4.5 (2-8)**		**4 (1-6.1)[b]↓**		**3 (0-5.5)[c]↓**
	M	34	4.18±3.25	29	5.34±3.74	31	3.74±3.09	28	6.04±3.24
			3 (0-8)		**5 (2-6.5)**		**3 (0-6)**		**6 (1-7.5)*↑[f]**
EOS (x10^9/L)	M&F	71	0.17±0.38	67	0.10±0.31	62	0.15±0.36	54	0.35±1.03
			0 (0-0.27)		**0 (0-0.19)**		**0 (0-0.25)**		**0 (0-0.68)[c]↑**
	F	37	0.03±0.16	38	0.05±0.23	31	0.13±0.34	26	0.12±0.33
			0 (0-0.88)		**0 (0-0.70)**		**0 (0-0.28)**		**0 (0-0.27)**
	M	34	0.32±0.48	29	0.17±0.38	31	0.16±0.37	28	0.57±1.37
			0 (0-1)*↑		**0 (0-0.34)**		**0 (0-0.22)**		**0 (0-1.19)**
EOS (%)	M&F	71	2.59±2.49	67	3.57±3.16	62	3.54±3.50	54	2.93±2.54
			1 (0-4)		**3 (0-5)**		**3 (1-4.2)[d]↓**		**2 (0-4)[c]↑**
	F	37	1.73±1.85	38	2.45±2.53	31	2.83±2.45	26	2.54±2.39
			1 (0-2.4)		**1 (0-4)**		**2 (1-4)[b]↑**		**1 (1-3.7)**
	M	34	3.53±2.77	29	5.03±3.34	31	4.23±4.20	28	3.29±2.67
			3.5 (0-7)*↑		**4 (1-7)*↑**		**3 (1-6)**		**2.5 (0-4.8)**
BAS	M&F	71	0.56±0.53	67	0.30±0.46	62	0.37±0.58	54	0.72±1.46

(%)			**1 (0-1)**		**0 (0-1)[a]↓**		**0 (0-1)**		**0 (0-1)**
	F	37	0.51±0.51	38	0.37±0.49	31	0.26±0.45	26	0.38±0.50
			1 (0-1)		**0 (0-1)**		**0 (0-1)**		**0 (0-1)**
	M	34	0.62±0.55	29	0.21±0.41	31	0.48±0.68	28	1.04±1.93
			1 (0-1)		**0 (0-0.4)[a]↓**		**0 (0-1)**		**0 (0-1.9)**
PLT ($x10^9$/L)	M&F	71	331.31±174.42	67	278.75±114.20	62	284±73.74	54	**282**.06±159.64
			317 (22-426)		**288 (29.5-338)**		**292.5 (96.5-332.5)**		**271.5 (16-341.3)**
	F	37	317.59±142.35	38	260.24±109.53	31	275.48±76.10	26	240.40±99.12
			291 (22-420)		**269.5 (5-340.8)**		**294 (105-315)**		**239 (72-289)**
	M	34	346.24±204.90	29	303.00±117.54	31	292.52±71.52	28	321.07±194.**04**
			340 (22-428.8)		**288 (79-354.7)**		**291 (85-339)**		**291 (16-408.1)*↑**
PDW (%)	M&F	71	15.28±1.98	67	13.93±3.34	62	14.73±2.97	54	14.41±3.11
			16 (9-16)		**16 (8-16)**		**16 (6.6-16)**		**16 (6.8-16)**
	F	37	14.81±2.62	38	13.16±3.72	31	14.19±3.41	26	14.54±3.04
			16 (9-16)		**16 (8-16)**		**16 (6-16)**		**16 (8-16)**
	M	34	15.79±0.54	29	14.93±2.48	31	15.26±2.39	28	14.29±3.23
			16 (15-16)		**16 (8-15)**		**16 (9-16.4)**		**16 (6-16)**
MPV (fL)	M&F	71	8.87±0.97	67	8.72±0.83	62	8.97±0.79	54	9.15±1.14
			9 (7-10)		**9 (7-9)**		**9 (8-10)**		**9 (7-10)**
	F	37	8.70±1.00	38	8.79±0.96	31	8.61±0.62	26	9.65±1.26[c]
			9 (7-9.1)		**9 (7-9.2)**		**9 (8-9)**		**10 (7-11)*↑[cef]**
	M	34	9.06±0.92	29	8.62±0.62	31	9.32±0.79	28	8.68±0.77[c]
			9 (7-10)		**9 (8-9)[a]↓**		**9 (8-10)*↑[d]**		**9 (7-9)[f]↓**

Results are expressed as mean ± standard deviation (SD) and median and range (2.5 to 97.5 percentiles) of the number of subjects indicated in the column labeled N. *$\rho < 0.05$ when male reference intervals are compared with female reference intervals per each age category; [a]$\rho < 0.0083$ when reference intervals in age range 1-5 years is compared with reference intervals in age range 6-10 years; [b]$\rho < 0.0083$ when reference intervals in age range 1-5 years is compared with reference intervals in age range 11-15 years; [c]$\rho < 0.00837$ when reference intervals in age range 1-5 years is compared with reference intervals in age range >15-18 years; [d]$\rho < 0.0083$ when reference intervals in age range 6-10 years is compared with reference intervals in age range 11-15 years; [e]$\rho < 0.0083$ when reference intervals in age range 6-10 years is compared with reference intervals in age range >15-18 years; [f]$\rho < 0.0083$ when reference intervals in age range 11-15 years is compared with reference intervals in age range >15-18 year

4.5.2 Comparison of the established age-related reference interval limits for hematological parameters for children and adolescents of Taita-Taveta County, Kenya, with those reported in the medical literature

A comparison of the established age-related reference interval limits for hematological parameters for children and adolescents of Taita-Taveta County, Kenya, with those reported in medical literature are presented in Table 4.12. Results indicate that this study's' developed age and sex-dependent reference intervals for red blood cells, hemoglobin and related indices are generally lower than those of other populations, including those from Ghana, Uganda, Ethiopia, Gabon, Nigeria, England, America^{DL}, America^{S}, and Austria except that of red blood cell distribution width-standard deviation which has not been earlier reported. Further, the developed reference intervals for white blood cells and differential white blood cells for infants, children and adolescents of Taita-Taveta County, Kenya, are generally lower than those of Ghana, Uganda, Ethiopia, Gabon, Nigeria, England, America^{DL}, America^{S}, and Austria in terms of the lower or upper limit or both. In addition, the developed reference intervals for platelets and related indices for infants, children and adolescents of Taita-Taveta County, Kenya were generally lower than those reported earlier by Ghana, Uganda, Ethiopia, Gabon, Nigeria, England, America^{DL}, America^{S}, and Austria in terms of the lower or upper limit or both except those of mean platelet volume which has not been earlier reported (Table 4.12).

Table 4.12: Comparison of the developed age-related reference interval limits for hematological parameters for children and adolescents of Taita-Taveta County, Kenya and those reported in the medical literature

RBC	This study RI	Sex	≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
($x10^{12}$/L)		M&F	**3-5**	**4-5.3↑[a]**	**4-5.2↑[b]**	0-5
		M	3-5	4-5.7↑[b]	4-5.7↑[b]	**0-5.2*↑[c]**
		F	3-5	4-5	4-5	**4-5**
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	↑3.22-5.55↑	↑3.45-5.2		
		M				↑3.79-5.69↑*
		F				↓3.4-5.4
	Uganda		1-5 years	6-12 years		13-18 years
		M&F	↑3.5-5.2	↑3.8-5.4		
		M				↑4.1-5.8↑*
		F				↓3.5-5.4
	England		1-6 years	6-12 years		12-18 years
		M&F	↑3.9-5.3	4-5.2		
		M				↑4.5-5.3*
		F				4.1-5.1
	America[DL]		2-12 years			
		M&F	4-5.2			
	Gabon		1.5-5 years			
		M&F	↑3.66-5.81↑			
	Ethiopia			5-18 years		
		M&F		4.26-5.99↑		
		M		*4.06-6.57*↑		
		F		*4.32-5.63*↑		
	Austria					14-17 years
		M&F				↑**4.62-6.04**↑
		M				
		F				
	America[S]				12-18 years	
		M&F			↑**3.74**-4.93	
	Nigeria[O]		1-17 years			
		M&F	↑4.52-6.98↑			
HB	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(g/dL)		M&F	**7.8-13**	**9.7-13**	**10-14**	6-19.4
		M	9-12.7	11-14	11-14	**6-16*↑[c]**
		F	7-13.5	9-13	10-14	**10-13**
	Nigerian[B]		≥1-5 years	≥5-9 years	≥9-14 years	≥14-17 years
		M&F	↑10.5-14	↑11.5-13.5	↑12.5-14.5	
		M				↑13-16*
		F				↑12-15
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	8.0-12.7	↓9.1-13.5		
		M				↑10.4-14.8↓*
		F				↓9.4-14.2↑
	Uganda		1-5 years	6-12 years		13-18 years
		M&F	6.8-12.5	10-13.7		
		M				↑11.2-15.9*
		F				9.9-14.5↑
	England		1-6 years	6-12 years		12-18 years
		M&F	↑11.5-14	↑11.5-15.5↑		
		M				↑13-17
		F				↑12-16
	America[DL]		2-6 years	6-12 years		
		M&F	↑11-14	↑11.5-15.5↑		
	Gabon		1.5-5 years			
		M&F	8.5-12			
	Ethiopia			5-18 years		
		M&F				

		M		↑*12.04-19.60*↑		
		F		↑*11.57-15.94*↑		
	Austria					14-17 years
		M&F				↑**13.22-16.7**
		M				
		F				
	America[S]				12-18 years	
		M&F			↑**11**-14.3	
	Nigeria[O]		1-17 years			
		M&F	↑9.57-13.73			
MCH	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(pg)		M&F	**19.8-29**	**21-28**	**21-29**	**15-30**
		M	21-30	24-29.5	21-28	15-33.2
		F	19-29	21-28	21-30	20-30
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	↓16.9-29.7	21.4-30.3		↑21.2-32
	America[DL]		2-6 years	6-12 years		
			↑24-30	↑25-33↑		
	Gabon		1.5-5 years			
		M&F	↓17-27.1↓			
	Ethiopia			5-18 years		
		M&F				
		M		↑*25.18-31.05*↑		
		F		↑*25.08-30.8*↑		
	Austria					14-17 years
		M				↑25.90-31.26*
		F				↑20.50-31.10
	America[S]					12-18 years
		M				↑28.2-30.5
		F				↑28.4-30.7*
	Nigeria[O]		1-17 years			
		M&F	↓16.33-24.95↓			
MCHC	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(g/dL)		M&F	**27.6-34**	25-34[a]↓	**26.2-33.3[b]**↓	**0-34**
		M	31-35	**25-36.6***↑	27-33	0-34
		F	26-34	**27-33[a]**↓	25-34	27-34
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	30-36.9↑	↑30.9-36↑[F]		↑30.5-36.6↑
	America[DL]		2-12 years			
		M&F	↑31-37↑			
	Gabon		1.5-5 years			
		M&F	↑30.7-34.8			
	Ethiopia			5-18 years		
		M&F				
		M		↑*32.10-36.20*		
		F		↑*32.07-35.44*		
	Austria					14-17 years
		M				↑33.22-35.83*
		F				↑30.80-35.40
	America[S]					12-18 years
		M				↑34.2-35.6*
		F				↑33.9-35.4
	Nigeria[O]		1-17 years			
		M&F	↑31.48-37.60↑			
MCV	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(fL)		M&F	**63-90**	67-88	**69.7-88.4**	13-90
		M	64-90	**68-86.5[a]**↓	75-90	**13-86***↓
		F	59-88.3	**67-89***↑	68-88	**73-91**

	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	↓56-87	68-89		↑**67-93**↑
	Uganda		1-5 years	6-12 years		13-18 years
		M&F	60.7-82.8↓	↓63.3-83.9↓		
		M				↑65-89.5
		F				↓67.4-89.9*
	America[DL]		2-6 years	6-12 years		
		M&F	↑75-87	↑77-95↑		
	Gabon		1.5-5 years			
		M&F	↓54-80↓			
	Ethiopia			5-18 years		
		M&F				
		M		↑*75.03-93.01*↑		
		F		↑*74.51-91.08*		
	Austria					14-17 years
		M				↑76.39-88.92
		F				↓66.7-95.3↑*
	America[S]					12-18 years
		M				↑80.8-86.56
		F				↑82.1-87.7*
	Nigeria[O]		1-17 years			
		M&F	↓55.38-73.38↓			
RDW-SD	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(%)		M&F	**39-56**	**38-51**	**39.6-59**	**39.1-64[e]**↑
		M	41-53.5	39-52.8[a]↓	42-59[d]↑	42-60[e]↑
		F	39-59	38-53.3	39-56	38-54
RDW-CV	Study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(%)		M&F	**12-15**	**11-14[a]**↓	**11-15**	**12-16**
		M	12-15	11-14[a]↓	12-15	12-18.2
		F	12-15	11-14	11-15	12-15.3
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	12.5-21.6↑	11.5-17.9↑		11.6-16.1
	Ethiopia			5-18 years		
		M		↑12.70-16.07↑		
		F		↑12.30-15.97↑		
WBC	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
($x10^9$/L)		M&F	**3-13**	**3.4-9[a]**↓	**4-8[b]**↓	5-11.6
		M	3-12.7	4-9.5	4-8[b]↓[d]	**5-8[c]**↓[e]
		F	3-13	2-9	4-8.6	**6-9***↑
	Nigerian[B]		≥1-5 years	≥5-9 years	≥9-14 years	≥14-17 years
		M&F	↑6.5-13.2	↑5.3-11↑	4.4-10.7↑	↓3-**10**↑
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	↑5.1-17.6↑	↑4.1-11.9↑		3.7-**9.4**↑
	Uganda		1-5 years	6-12 years		13-18 years
		M&F	↑4.9-13.6	↑4.4-11.5↑		4.1-10.7↑
	England		1-6 years	6-12 years		12-18 years
		M&F	5-17	↑4.5-14.5↑		↓4.5-13↑
	America[DL]		2-6 years	6-12 years		
			↑5-15↑	↑5-13↑		
	Gabon		1.5-5 years			
		M&F	↑5.4-14.8↑			
	Ethiopia			5-18 years		
		M&F		↑4.00-11.67↑		
		M		↑*4.04-11.72*↑		
		F		↑*3.74-11.42*↑		
	Austria					14-17 years

		M&F				↓3.52-11.11
	America[S]					12-18 years
		M				5.24-9.74↑
		F				↓5.52-9.29
	Nigeria[O]		1-17 years			
		M&F	3.23-7.07			
NEU	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(x10^9/L)		M&F	**1.8-7.4**	**1.7-5.3[a]**↓	**1-4.3[b]**↓	2-5
		M	2-8.6	2-4.6	1-4[b]↓	**2-5.2**
		F	1-7.4	1-6.5	2-5.1	**2-6***↑
	Nigerian[B]		≥1-5 years	≥5-9 years	≥9-14 years	≥14-17 years
		M&F	1.9-7.5	1.7-7.3↑	↑1.9-7↑	↑2.4-7.2↑
	Uganda		1-5 years	6-12 years		13-18 years
		M&F	↓1-3.9↓	↓0.9-3.6↓		0.9-3.5↓
	England		1-6 years	6-12 years		12-18 years
		M&F	↓1-8.5↑	↓1-8↑		↓1.5-8↑
	America[DL]		2-6 years	6-12 years		
		M&F	↓1.5-8↑	↑2-8↑		
	Gabon		1.5-5 years			
		M&F	↓1.27-6.85↓			
	Ethiopia			5-18 years		
		M&F				
		M		*↓1.26-7.39*↑		
		F		*↓1.00-6.99*↑		
	Austria					14-17 years
		M				1.79-8.03
		F				1.67-9.22*
	America[S]				12-18 years	
		M			2.73-6.68*	
		F			3.04-6.06	
NEU	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(%)		M&F	**24-66**	**28.7-56**	**25.2-55**	30.3-59.5[e]↑
		M	24-71	30-56	24-55	**28-59**
		F	28-64	28-53	30-53.6	**34-68.3*↑[e]**
	Gabon		1.5-5 years			
		M&F	↓18-54.2↓			
	Austria					14-17 years
		M&F				↑37.24-76.3↑
	America[S]					12-18 years
		M				↑43.2-76.7↑
		F				↑46.4-75.6↑*
LYM	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(x10^9/L)		M&F	**0-4.2**	**1-4**	**1-4.2**	**0-3[e]**↓
		M	0-6.3	1-4	1-4	0-3.4
		F	0-5.5	1-4	1-3.6	0-3[e]↓
	Nigerian[B]		≥1-5 years	≥5-9 years	≥9-14 years	≥14-17 years
		M&F	↑3.1-9↑	↑1.2-6↑	1.1-3.2↓	↑1.1-3.2↑
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	↑2.3-11.9↑	↑1.6-5.8↑		↑1.4-4↑
	Uganda		1-5 years	6-12 years		13-18 years
		M&F	↑2.4-8.4↑	↑2.2-5.9↑		↑1.7-4.7↑
	England		1-6 years	6-12 years		12-18 years
		M&F	↑1.5-9.5↑	↑1.5-7↑		↑1.1-4.5↑
	America[DL]		2-6 years	6-12 years		
		M&F	↑6-9↑	1-5		
	Gabon		1.5-5 years			
		M&F	↑2.34-7.11↑			
	Ethiopia			5-18 years		

		M&F				
		M		*1.50-4.25*		
		F		*1.41-4.47*		
	Austria					14-17 years
		M&F				↑1.27-3.15↑
	America[S]					12-18 years
		M				↑1.03-2.18↓
		F				↑1.17-2.30↓*
LYM	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(%)		M&F	**7.6-54**	**9-53**	**11.8-53.3**	5.5-47[e]↓[f]
		M	8-54.3	9-53.5	16-58	**24-48***↑
		F	6-51	9-53	6-49	**4-43.3[e]↓[f]**
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	↑34.9-75.6↑	↑29.6-62.5		
		M				↑26.5-56.7↑
		F				↑25.7-60.2↑*
	Gabon		1.5-5 years			
		M&F	↑27.4-64.2↑			
	Austria					14-17 years
		M&F				↓16.04-51↑
	America[S]					12-18 years
		M				↓8-41↓*
		F				↑8-39↓
MON	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
($x10^9$/L)		M&F	**0-1**	**0-1**	**0-0.6[b]↓**	**0-1**
		M	0-1	0-1	0-0.3	0-1
		F	0-1	0-1	0-1.1[b]↓	0-0.4[c]↓
	Nigerian[B]		≥1-5 years	≥5-9 years	≥9-14 years	≥14-17 years
		M&F	↑0.1-0.5↓	↑0.2-0.5↓	↑0.1-0.6	↑0.2-0.5↓
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	↑0.2-1	↑0.2-1.1		↑0.2-0.9↓
	Uganda		1-5 years	6-12 years		13-18 years
		M&F	↑0.26-1.04	↑0.24-0.75↓		↑0.21-0.73↓
	England		1-6 years	6-12 years		12-18 years
		M&F	↑0.2-1	↑0.2-1		↑0.2-1
	America[DL]		2-12 years			
		M&F	↑0.2-**1**			
	Gabon		1.5-5 years			
		M&F	↑0.36-1.62↑			
	Ethiopia			5-18 years		
		M&F				
		M		*0.27-1.05*		
		F		*0.27-1.06*		
	Austria					14-17 years
		M&F				↑0.22-0.83↓
	America[S]					12-18 years
		M				↑0.18-0.78↓*
		F				↑0.19-0.72↓
MON	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(%)		M&F	0-8	**2-7**	**0-6**	0-6.3
		M	**0-8**	2-6.5	0-6	**1-7.5*↑[f]**
		F	**0-8***↑	2-8	1-6.1[b]↓	**0-5.5[c]↓**
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	↑4.9-13.6↑	↑5-13.3↑		↑4.9-14.4↑
	Gabon		1.5-5 years			
		M&F	↑4.7-13↑			
	Austria					14-17 years
		M&F				↑4.32-9.28↑

	America[S]					12-18 years
		M				↑4-8↑*
		F				↑4-7↓
EOS	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(x10^9/L)		M&F	0-0.27	**0-0.19**	**0-0.25**	**0-0.68**[c]↑
		M	**0-1***↑	0-0.34	0-0.22	0-1.19
		F	**0-0.88**	0-0.70	0-0.28	0-0.27
	Nigerian[B]		≥1-5 years	≥5-9 years	≥9-14 years	≥14-17 years
		M&F	↑0.2-0.4↑	↑0.2-0.5↑	↑0.1-1↑	↑0.2-0.6↓
	Uganda		1-5 years	6-12 years		13-18 years
		M&F	↑0.14-2.03↑	↑0.20-3.14↑		↑0.26-2.77↑
	America[DL]		2-12 years			
		M&F	↑0.1-1↑			
	Gabon		1.5-5 years			
		M&F	↑0.12-3.05↑			
	Ethiopia			5-18 years		
		M&F				
		M		↑*0.048-1.49*↑		
		F		↑*0.055-1.31*↑		
	Austria					14-17 years
		M				↑0.07-1.1↑*
		F				↑0.03-0.59↓
	America[S]					12-18 years
		M				↑0.04-0.20↓
		F				↑0.05-0.17↓
EOS	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(%)		M&F	0-4	0-5	**1-4.2**[d]↓	**0-4**[c]↑
		M	**0-7***↑	**1-7***↑	1-6	0-4.8
		F	**0-2.4**	**0-4**	1-4[b]↑	1-3.7
	Gabon		1.5-5 years			
		M&F	↑1.3-24.4↑			
	Austria					14-17 years
		M				↑0.76-14.41↑*
		F				↑0.70-9.40↑
	America[S]					12-18 years
		M				↑2-4*
		F				↑1-3↓
BAS	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(%)		M&F	**0-1**	**0-1**[a]↓	**0-1**	**0-1**
		M	0-1	0-0.4[a]↓	0-1	0-1.9
		F	0-1	0-1	0-1	0-1
	Gabon		1.5-5 years			
		M&F	↑0.6-1.9↑			
	Austria					14-17 years
		M&F				↑0.1-1.38↑
	America[S]					12-18 years
		M&F				0-1
PLT	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(x10^9/L)		M&F	**22-426**	**29.5-338**	**96.5-332.5**	16-341
		M	22-428.8	79-354.7	85-339	**16-408***↑
		F	22-420	5-340.8	105-315	**72-289**
	Nigerian[B]		≥1-5 years	≥5-9 years	≥9-14 years	≥14-17 years
		M&F	↑117-365↓	↑120-376↑	130-405	
		M				↑98-427
		F				↑100-443↑*
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	↑110-637↑	↑117-417↑		
		M				↑108-326↓

		F				↑143-390↑*
	Uganda		1-5 years	6-12 years		13-18 years
		M&F	↑126-376↓	↑134-355↑		
		M				↑110-327↓
		F				↑124-353↑*
	England		1-6 years	6-12 years		12-18 years
		M&F	↑150-400↓	↑150-400↑		↑150-**400**↑
	America[DL]		2-6 years	6-12 years		
		M&F	↑200-490↑	↑170-450↑		
	Gabon		1.5-5 years			
		M&F	↑192-646↑			
	Austria					14-17 years
		M&F				↑157.2-**354.4**↑
	America[S]					12-18 years
		M				↑180-299↓
		F				↑192-307↑*
	Nigeria[O]		1-17 years			
		M&F	↑138.9-248.16↓			
	Ethiopia			5-18 years		
		M&F		↑188-463.5↑		
		M		↑*158.5-469.9*↑		
		F		↑*197.7-460.4*↑		
PDW	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(%)		M&F	**9-16**	**8-16**	**6.6-16**	**6.8-16**
		M	15-16	8-15	9-16.4	6-16
		F	9-16	8-16	6-16	8-16
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	8.8-25.4↑	↑12.1-20.5↑		
		M				↑11.9-20.8↑
		F				↑12.4-24.1↑*
MPV	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(fL)		M&F	**7-10**	**7-9**	8-10	7-10
		M	7-10	8-9[a]↓	**8-10*↑[d]**	**7-9[f]↓**
		F	7-9.1	7-9.2	**8-9[a]**	**7-11*↑[cef]**

Uganda by Lugada et al. (2004) [90% RI], Port Harcourt, Nigeria[B] by Buseri et al. (2010), Kintampo, Ghana by Dosoo et al. (2014), Moyen-Ogooue´ province, Gabon by Humberg et al. (2011), Nnewi and environs, Anambra State, Nigeria[O] by Onwurah et al. (2018), southwest Ethiopia by Bimerew et al. (2018), Austria by Bogner et al. (2019), America[DL] by Dacie and Lewis (2017), America by Soldin et al. (2011), and North Bristol NHS Trust Version No: 4, England. Italicized values are similar values.

4.6 Established reference intervals for liver and kidney function tests and electrolytes for children and adolescents of Taita-Taveta County, Kenya

The established reference intervals for male children and adolescents of Taita-Taveta County, Kenya, for total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total bilirubin (T-BIL), direct bilirubin (D-BIL), creatinine (CREAT), blood urea nitrogen (BUN), potassium (K), sodium (NA), chloride (CL), and calcium (CA) were similar to those of the female population of the same County ($\rho > 0.05$). Therefore, combined reference intervals for these parameters for this population were established. The established reference interval limits for total protein (TP) are 73 (45-84.6) g/L, albumin (ALB) is 45 (28-52) g/L, alanine aminotransferase (ALT) is 14 (4-61.5) U/L, aspartate aminotransferase (AST) is 26.5 (11-128) U/L, alkaline phosphatase (ALP) is 55 (0-492) U/L, gamma-glutamyltransferase (GGT) is 16 (4-175) U/L, total bilirubin (T-BIL) is 4 (1-166) µmol/L, direct bilirubin (D-BIL) is 2 (0-15.9) µmol/L, creatinine (CREAT) is 48.5 (18-131) µmol/L, blood urea nitrogen (BUN) is 3 (2-13) mmol/L, potassium (K) is 4 (4-7) mmol/L, sodium (NA) is 138 (122-149) mmol/L, chloride (CL) is 98 (85-116) mmol/L and calcium (CA) is 2 (1-2) mmol/L (Table 4.13).

Table 4.13: Established reference interval limits for serum biochemistry analytes for children and adolescents of Taita-Taveta County, Kenya

Analyte (unit)	Sex	N	Percentile			Reference Interval	IV	Difference between M&F	
			Median	2.5^{th}	97.5^{th}			Z value	Sig
TP (g/L)	**M&F**	**254**	71.45±10.71					-0.227	ρ = 0.820
			73	**45**	**84.63**	**45-84.6**	**39.6**		
	F	132	71.98±9.25						
			73	**46.95**	**83.35**	**47-83.4**	**36.4**		
	M	122	70.87±12.10						
			73.5	**33.68**	**85**	**33.7-85**	**51.3**		
ALB (g/L)	M&F	**254**	43.94±5.68						
			45	**28**	**52**	**28-52**	**24**		
	F	132	43.91±5.28						
			45	**28**	**52**	**28-52**	**24**	-0.725	ρ = 0.468
	M	122	44.02±6.11						
			45	**19.23**	**50.93**	**19.2-50.9**	**31.7**		
ALT (U/L)	M&F	**254**	24.94±103.34						
			14	**4**	**61.5**	**4-61.5**	**57.5**		
	F	132	19.69±31.45						
			13	**4**	**162.45**	**4-162.5**	**158.5**	-1.363	ρ = 0.173
	M	122	30.63±145.58						
			14.5	**1.38**	**65.1**	**1.4-65.1**	**63.7**		
AST (U/L)	M&F	**254**	33.07±28.75						
			26.5	**11**	**128.13**	**11-128.1**	**117.1**		
	F	132	33.38±31.64						
			26.5	**10**	**179.05**	**10-179.1**	**169.1**	-0.571	ρ = 0.568
	M	122	32.75±25.38						
			26.5	**11**	**93.43**	**11-93.4**	**82.4**		
ALP (U/L)	**M&F**	**254**	119.15±148.76						
			55	**0**	**492.5**	**0-492.5**	**492.5**		
	F	132	99.38±133.15					-1.372	ρ = 0.170
			1.5	**0**	**463.18**	**0-463.2**	**463.2**		
	M	122	140.53±161.83						

			100	**0**	**652.08**	**0-652.1**	**652.1**		
GGT (U/L)	M&F	**254**	35.12±51.24					-0.199	ρ = 0.842
			16	**4**	**175**	**4-175**	**171**		
	F	132	34.67±54.46						
			16	**5**	**242.02**	**5-242**	**237**		
	M	122	35.61±47.73						
			16	**4**	**175**	**4-175**	**171**		
T-BIL (µmol/L)	M&F	**254**	13.76±36.41					-0.829	ρ = 0.407
			4	**1**	**166**	**1-166**	**165**		
	F	132	15.75±42.67						
			4	**33**	**168.03**	**33-168**	**135**		
	M	122	11.61±28.11						
			4.5	**1**	**141.88**	**1-141.9**	**140.9**		
D-BIL (µmol/L)	M&F	**254**	3.48±13.45					-0.416	ρ = 0.677
			2	**0**	**15.88**	**0-15.9**	**15.9**		
	F	132	4.48±18.05						
			2	**0**	**41.55**	**0-41.6**	**41.6**		
	M	122	2.39±4.80						
			2	**0**	**7.77**	**0-7.8**	**7.8**		
CREAT (µmol/L)	M&F	**254**	54.98±24.71					-0.545	ρ = 0.586
			48.5	**18**	**131**	**18-131**	**113**		
	F	132	53.68±22.28						
			49	**18**	**130**	**18-130**	**112**		
	M	122	56.39±27.11						
			47	**19.45**	**131**	**19.5-131**	**111.5**		
BUN (mmol/L)	M&F	**254**	3.64±3.22					-1.278	ρ = 0.201
			3	**2**	**13**	**2-13**	**11**		
	F	132	4.06±4.18						
			3	**2**	**22.42**	**2-22.4**	**20.4**		
	M	122	3.19±1.51						
			3	**1**	**9.77**	**1-9.8**	**8.8**		
K (mmol/L)	M&F	**254**	5.45±11.66					-0.497	ρ = 0.619
			4	**4**	**7**	**4-7**	**3**		
	F	132	4.54±0.80						
			4	**4**	**5**	**4-5**	**1**		
	M	122	4.42±0.95						
			4	**4**	**6**	**4-6**	**2**		
NA (mmol/L)	M&F	**254**	136.34±13.51					-0.142	ρ = 0.887

			138	**122.13**	**149**	**122.1-149**	**26.9**		
	F	132	136.26±13.93						
			138	**121.98**	**150**	**122-150**	**28**		
	M	122	136.43±13.09						
			137.5	**120.6**	**147**	**120.6-147**	**26.4**		
CL (mmol/L)	M&F	**254**	97.70±10.63						
			98	**85**	**116**	**85-116**	**31**		
	F	132	97.62±10.75						
			98	**85**	**116.68**	**85-116.7**	**31.7**	-0.500	ρ = 0.617
	M	122	97.79±10.55						
			98	**86**	**112**	**86-112**	**26**		
CA (mmol/L)	M&F	254	1.95±0.30						
			2	**1**	**2**	**1-2**	**1**		
	F	**132**	1.93±0.38						
			2	**0**	**2**	**0-2**	**2**	-0.530	ρ = 0.596
	M	**122**	1.98±0.20						
			2	**1**	**2**	**1-2**	**1**		

Results are expressed as mean ± standard deviation, and median and 95% range (in brackets) for the number of referent participants in the column labeled N. Statistical comparisons of the median values between male and female referent participants were carried out using Mann-Whitney U test. Differences were considered significant at $\rho < 0.05$

4.6.1 Effects of age on the established reference intervals for serum biochemistry analytes for children and adolescents of Taita-Taveta County, Kenya

The effects of age on the established reference intervals male and female children and adolescents' population of Taita-Taveta County, Kenya are depicted in Table 4.14 The different age categories were as follows: (a) category ≥ 1-5 years, (b) category ≥ 5-10 years, (c) category ≥ 10-15 years, and (d) category ≥ 15-18 years. Median reference interval limits differences between male and female participants were estimated within each age category using the Mann-Whitney U test where ρ-value less than 0.05 was considered statistically significant. Median reference interval limits differences within and between the four age groups for each sex were done using the Kruskal-Walis U test followed by the Mann-Whitney U test with an adjusted statistically significant ρ-value of less than 0.0083. The mean ± standard deviation (SD), median and 95% range, sex and age groups are presented in Tables 4.14

A Kruskal-Wallis H test on the effects of age on total protein (TP) (χ^2 (3) = 48.844, $\rho < 0.001$), albumin (ALB) (χ^2 (3) = 18.184, $\rho < 0.001$), alanine aminotransferase (ALT) (χ^2 (3) = 9.852, $\rho = 0.020$), aspartate aminotransferase (AST) (χ^2 (3) = 67.672, $\rho < 0.001$), alkaline phosphatase (ALP) (χ^2 (3) = 26.515, $\rho < 0.001$), gamma-glutamyltransferase (γ-GT) (χ^2 (3) = 19.013, $\rho < 0.001$), total bilirubin (T-BIL) (χ^2 (3) = 15.368, $\rho = 0.002$), direct bilirubin (D-BIL) (χ^2 (3) = 22.262, $\rho < 0.001$), creatinine (CREAT) (χ^2 (3) = 56.313, $\rho < 0.001$), blood urea nitrogen (BUN) (χ^2 (3) = 16.661, $\rho = 0.001$), potassium (K)

(χ^2 (3) = 12.998, ρ = 0.005), sodium (NA) (χ^2 (3) = 17.086, ρ = 0.001), and chloride (CL) (χ^2 (3) = 23.542, ρ < 0.001) for the combined male and female children and adolescents of Taita-Taveta County, Kenya indicates that these measured parameters were statistically significantly affected by advancement in age. Mann-Whitney U test was used as a follow-up test for pairwise comparisons to identify the significant age group pairs with adjusted significant ρ-value of less than 0.0083.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for total protein (TP g/L) for combined male and female children and adolescents of age category ≥1-5 years (66 (33-84) g/L) with a mean rank of 49.67 is significantly lower than that of age category ≥5-10 years (74 (60-83) g/L) with a mean rank of 90.51 (U = 970.5, z = -6.009, ρ < 0.001, **r = 0.5115**), the reference interval limits for total protein (TP g/L) for combined male and female children and adolescents of age category ≥1-5 years (66 (33-84) g/L) with a mean rank of 49.01 is significantly lower than that of age category ≥10-15 years (74 (56-139) g/L) with a mean rank of 87.60 (U = 923.5, z = -5.773, ρ < 0.001, **r = 0.5006**). In summary, the reference interval limits for total protein (TP g/L) for combined male and female children and adolescents of age category ≥1-5 years was lower than those of age categories ≥5-10 and ≥10-15 years which were similar but reverted to that of age category ≥1-5 years in age category ≥15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for albumin (ALB g/L) for combined male and female children and adolescents of age category ≥1-5 years (44 (19-50) g/L) with a mean rank of 58.08 is significantly lower than that of age category ≥5-10 years (46 (26-52) g/L) with a mean rank of 81.60 (U = 1568, z = -3.480, ρ < 0.001, r = 0.2962). In summary, the reference interval limits for albumin (ALB g/L) for combined male and female children and adolescents of age category ≥1-5 years was lower than that of age category ≥5-10 years but reverted to the levels of age category ≥1-5 years in age categories ≥10-15 and ≥15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for alanine aminotransferase (ALT U/L) for combined male and female children and adolescents (14 (4-62) U/L) was not affected by advancement in age.

Results for the pairwise comparison using Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for aspartate aminotransferase (AST U/L) for combined male and female children and adolescents of age category ≥1-5 years (36 (13-167) U/L) with mean rank of 86.67 is significantly lower than that of age category ≥5-10 years (27 (9-59) U/L) with mean rank of 51.31 (U = 1159.5, z = -5.198, ρ < 0.001, r = 0.4425), the reference interval limits for aspartate aminotransferase (AST U/L) for combined male and female children and

adolescents of age category ≥1-5 years (36 (13-167) U/L) with mean rank of 86.42 is significantly lower than that of age category ≥10-15 years (24.5 (9.5-44) U/L) with mean rank of 44.76 (U = 822, z = -6.224, ρ < 0.001, **r = 0.5397**), the reference interval limits for aspartate aminotransferase (AST U/L) for combined male and female children and adolescents of age category ≥5-10 years (27 (9-59) U/L) with mean rank of 72.99 is significantly lower than that of age category ≥15-18 years (21.5 (10-194) U/L) with mean rank of 46.12 (U = 1005.5, z = -4.196, ρ < 0.001, r = 0.3815), the reference interval limits for aspartate aminotransferase (AST U/L) for combined male and female children and adolescents of age category ≥10-15 years (24.5 (9.5-44) U/L) with mean rank of 66.61 is significantly lower than that of age category ≥15-18 years (21.5 (10-194) U/L) with mean rank of 49.19 (U = 1171, z = -2.788, ρ = 0.005, r = 0.2589). In summary, the reference interval limits for aspartate aminotransferase (AST U/L) for combined male and female children and adolescents of age category ≥1-5 years continuously decreased through age categories ≥5-10, ≥10-15 and ≥15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for alkaline phosphatase (ALP U/L) for combined male and female children and adolescents of age category ≥1-5 years (187 (0-451) U/L) with a mean rank of 79.77 is significantly higher than that of age category ≥5-10 years (1 (0-488) U/L) with a mean rank of 58.60 (U = 164.9, z = -3.129, ρ = 0.002, r = 0.2664), and the reference interval limits for alkaline

phosphatase (ALP U/L) for combined male and female children and adolescents of age category ≥1-5 years (187 (0-451) U/L) with a mean rank of 81.44 is significantly higher than that of age category ≥10-15 years (1 (0-661) U/L) with a mean rank of 50.49 (U = 1176, z = -4.659, ρ < 0.001, r = 0.4040). In summary, the reference interval limits for alkaline phosphatase (ALP U/L) for combined male and female children and adolescents of age category ≥1-5 years decreased through age categories ≥5-10 and ≥10-15 years which were similar and reverted to levels of age category ≥1-5 years in age category ≥15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for gamma-glutamyltransferase (γ-GT U/L) for combined male and female children and adolescents of age category ≥1-5 years (27 (4-264) U/L) with a mean rank of 81.29 is significantly higher than that of age category ≥5-10 years (14 (4-282) U/L) with a mean rank of 57.01 (U = 1541.5, z = -3.570, ρ = 0.002, r = 0.3039), and the reference interval limits for gamma-glutamyltransferase (γ-GT U/L) for combined male and female children and adolescents of age category ≥1-5 years (27 (4-264) U/L) with a mean rank of 78.89 is significantly higher than that of age category ≥10-15 years (13.5 (6-65) U/L) with a mean rank of 53.38 (U = 1356.5, z = -3.815, ρ < 0.001, r = 0.3080). In summary, the reference interval limits for gamma-glutamyltransferase (γ-GT U/L) for combined male and female children and adolescents of age category ≥1-5 years decreased through age categories ≥5-10

and ≥10-15 years which were similar and reverted to levels of age category ≥1-5 years in age category ≥15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for total bilirubin (T-BIL μmol/L) for combined male and female children and adolescents of age category ≥1-5 years (6 (0.8-265) μmol/L) with a mean rank of 80.48 is significantly higher than that of age category ≥5-10 years (3 (1-36) μmol/L) with a mean rank of 57.87 (U = 1599, z = -3.360, ρ = 0.001, r = 0.2860).

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for direct bilirubin (D-BIL μmol/L) for combined male and female children and adolescents of age category ≥1-5 years (2 (0-81) μmol/L) with a mean rank of 83.66 is significantly higher than that of age category ≥5-10 years (1 (0-3.9) μmol/L) with a mean rank of 54.49 (U = 1373, z = -4.522, ρ < 0.001, r = 0.3849). In summary, the reference interval limits for total bilirubin (T-BIL μmol/L) and direct bilirubin (D-BIL μmol/L) for combined male and female children and adolescents of age category ≥1-5 years decreased to age categories ≥5-10 years and reverted to levels of age category 1-5 years in age category ≥10-15 and ≥15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for creatinine (CREAT μmol/L) for combined male

and female children and adolescents of age category ≥1-5 years (41 (18-131) μmol/L) with a mean rank of 51.84 is significantly lower than that of age category ≥10-15 years (53 (38-130) μmol/L) with a mean rank of 84.36 (U = 1124, z = -4.859, ρ < 0.001, r = 0.4213), and the reference interval limits for creatinine (CREAT μmol/L) for combined male and female children and adolescents of age category ≥5-10 years (44 (22-138) μmol/L) with a mean rank of 43.92 is significantly lower than that of age category ≥15-18 years (56 (44-118) μmol/L) with a mean rank of 82.19 (U = 664.5, z = -5.974, ρ < 0.001, **r = 0.5431**). In summary, the reference interval limits for creatinine (CREAT μmol/L) for combined male and female children and adolescents of age category ≥1-5 years increased through age categories ≥5-10, ≥10-15 years and was highest in age category ≥15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for blood urea nitrogen (BUN μmol/L) for combined male and female children and adolescents of age category ≥5-10 years (3 (2-5) μmol/L) with a mean rank of 72.83 is significantly higher than that of age category ≥15-18 years (3 (2-5.9) μmol/L) with a mean rank of 46.32 (U = 1016.5, z = -4.393, ρ < 0.001, r = 0.3994). Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for potassium (K mmol/L) for combined male and female children and adolescents of age category ≥5-10 years (4 (4-45) mmol/L) with a mean rank of 67.35 is

significantly higher than that of age category ≥15-18 years (4 (4-5.6) mmol/L) with a mean rank of 53.12 (U = 1383.5, z = -2.841, ρ = 0.004, r = 0.2583). In summary, the reference interval limits for blood urea nitrogen (BUN μmol/L) and potassium (K mmol/L) for combined male and female children and adolescents of age category ≥1-5 years is similar to that of age categories ≥5-10, ≥10-15 and ≥15-18 years; however, age category ≥5-10 years is lower than age category ≥15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for sodium (NA mmol/L) for combined male and female children and adolescents of age category ≥1-5 years (135 (126-160) mmol/L) with a mean rank of 57.34 is significantly lower than that of age category ≥5-10 years (138 (107-148) mmol/L) with a mean rank of 82.39 (U = 1515, z = -3.694, ρ < 0.001, r = 0.3145), and the reference interval limits for sodium (NA mmol/L) for combined male and female children and adolescents of age category ≥1-5 years (135 (126-160) mmol/L) with a mean rank of 57.31 is significantly lower than that of age category ≥10-15 years (138 (5-149) mmol/L) with a mean rank of 78.10 (U = 1513, z = -3.111, ρ = 0.002, r = 0.2698).

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for chloride (CL mmol/L) for combined male and female children and adolescents of age category ≥1-5 years (101 (88-125)

mmol/L) with a mean rank of 83.17 is significantly higher than that of age category ≥5-10 years (95 (75-110) mmol/L) with a mean rank of 55.01 (U = 1408, z = -4.142, $\rho < 0.001$, r = 0.3526), and the reference interval limits for chloride (CL mmol/L) for combined male and female children and adolescents of age category ≥1-5 years (101 (88-125) mmol/L) with a mean rank of 78.92 is significantly higher than that of age category ≥10-15 years (96 (17-110) mmol/L) with a mean rank of 53.35 (U = 1355, z = -3.827, $\rho < 0.001$, r = 0.3318). In summary, the reference interval limits for sodium (NA mmol/L), and chloride (CL mmol/L) for combined male and female children and adolescents of age category ≥1-5 years decreases in age categories 5-10 and 10-15 years which are similar and reverts to the levels of age category 1-5 years in the age category 15-18 years.

A Kruskal-Wallis H test on the effects of age on total protein (TP) (χ^2 (3) = 15.333, $\rho = 0.002$), albumin (ALB) (χ^2 (3) = 20.562, $\rho < 0.001$), alanine aminotransferase (ALT) (χ^2 (3) = 11.986, $\rho = 0.007$), aspartate aminotransferase (AST) (χ^2 (3) = 34.163, $\rho < 0.001$), alkaline phosphatase (ALP) (χ^2 (3) = 16.544, $\rho = 0.001$), gamma-glutamyltransferase (γ-GT) (χ^2 (3) = 20.754, $\rho < 0.001$), total bilirubin (T-BIL) (χ^2 (3) = 12.287, $\rho = 0.006$), direct bilirubin (D-BIL) (χ^2 (3) = 19.660, $\rho < 0.001$), creatinine (CREAT) (χ^2 (3) = 42.183, $\rho < 0.001$), blood urea nitrogen (BUN) (χ^2 (3) = 12.908, $\rho = 0.005$), potassium (K) (χ^2 (3) = 10.243, $\rho = 0.017$), sodium (NA) (χ^2 (3) = 23.260, $\rho < 0.001$), and chloride (CL) (χ^2 (3) = 8.418, $\rho < 0.038$) for the male children and adolescents of Taita-Taveta County, Kenya indicates that these measured

parameters were statistically significantly affected by advancement in age. Mann-Whitney U test was used as a follow-up test for pairwise comparisons to identify the significant age group pairs with adjusted significant ρ-value of less than 0.0083.

A Kruskal-Wallis H test on the effects of age on total protein (TP) (χ^2 (3) = 39.262, ρ < 0.001), albumin (ALB) (χ^2 (3) = 8.461, ρ = 0.037), aspartate aminotransferase (AST) (χ^2 (3) = 35.375, ρ < 0.001), alkaline phosphatase (ALP) (χ^2 (3) = 10.400, ρ = 0.015), creatinine (CREAT) (χ^2 (3) = 12.565, ρ = 0.006), blood urea nitrogen (BUN) (χ^2 (3) = 8.519, ρ = 0.036) and chloride (CL) (χ^2 (3) = 19.438, ρ < 0.001) for the female children and adolescents of Taita-Taveta County, Kenya indicates that these measured parameters were statistically significantly affected by advancement in age. Mann-Whitney U test was used as a follow-up test for pairwise comparisons to identify the significant age group pairs with adjusted significant ρ-value of less than 0.0083.

Results for the pairwise comparison using Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for total protein (TP g/L) for female children and adolescents of age category ≥1-5 years (66 (45-72) g/L) with mean rank of 24.04 is significantly lower than that of age category ≥5-10 years (74 (69-76) g/L) with mean rank of 51.59 (U = 186.5, z = -5.492, ρ < 0.001, **r = 0.6342**), the reference interval limits for total protein (TP g/L) for female children and adolescents of age category ≥1-5 years (66 (45-72) g/L) with mean rank of

24.03 is significantly lower than that of age category ≥10-15 years (75 (63-82) g/L) with mean rank of 47 (U = 186, z = -4.792, ρ < 0.001, **r = 0.5811**), and the reference interval limits for total protein (TP g/L) for female children and adolescents of age category ≥1-5 years (66 (45-72) g/L) with mean rank of 25.39 is significantly lower than that of age category ≥15-18 years (72 (66-76) g/L) with mean rank of 41.40 (U = 236.5, z = -3.428, ρ = 0.001, r = 0.4319). The reference interval limits for total protein (TP g/L) for female children and adolescents of age category ≥1-5, ≥5-10, ≥10-15 and ≥15-18 years were similar.

Results for the pairwise comparison using Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for total protein (TP g/L) for male children and adolescents of age category ≥1-5 years (66 (33-74) g/L) with mean rank of 25.75 is significantly lower than that of age category ≥5-10 years (74 (42-77) g/L) with mean rank of 39.33 (U = 280.5, z = -2.935, ρ = 0.003, r = 0.3698), the reference interval limits for total protein (TP g/L) for male children and adolescents of age category ≥1-5 years (66 (33-74) g/L) with mean rank of 25.51 is significantly lower than that of age category ≥10-15 years (74 (33-74) g/L) with mean rank of 41.21 (U = 272.5, z = -3.352, ρ = 0.001, r = 0.4158), and the reference interval limits for total protein (TP g/L) for male children and adolescents of age category ≥1-5 years (66 (33-74) g/L) with mean rank of 25.38 is significantly lower than that of age category ≥15-18 years (74 (52-78) g/L) with mean rank of 38.93 (U = 268, z = -2.949, ρ = 0.003, r = 0.3745). The

reference interval limits for total protein (TP g/L) for male children and adolescents of age category ≥5-10, ≥10-15 and ≥15-18 years are similar.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for albumin (ALB g/L) for male children and adolescents of age category ≥1-5 years (44 (19-45) g/L) with a mean rank of 23.40 is significantly lower than that of age category ≥15-18 years (48 (34-49) g/L) with a mean rank of 41.34 (U = 200.5, z = -3.921, $\rho < 0.001$, r = 0.4980) and the reference interval limits for albumin (ALB g/L) for male children and adolescents of age category ≥10-15 years (44 (34-46) g/L) with a mean rank of 23.13 is significantly lower than that of age category ≥15-18 years (48 (34-49) g/L) with a mean rank of 37.61 (U = 221, z = -3.259, $\rho = 0.001$, r = 0.4243). The reference interval limits for albumin (ALB g/L) for male children and adolescents of age category ≥1-5, ≥5-10, and ≥10-15 years are similar.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for alanine aminotransferase (ALT U/L) for male children and adolescents of age category ≥1-5 years (18 (6-24) U/L) with a mean rank of 38.06 is significantly higher than that of age category ≥15-18 years (11.5 (1-16) U/L) with a mean rank of 23.54 (U = 253, z = -3.171, $\rho = 0.002$, r = 0.4027). The reference interval limits for alanine aminotransferase (ALT U/L) for male children and adolescents of age category ≥1-5, ≥5-10, and ≥10-15 years are similar.

Results for the pairwise comparison using Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for aspartate aminotransferase (AST U/L) for female children and adolescents of age category ≥1-5 years (34 (13-57) U/L) with mean rank of 47.2 is significantly higher than that of age category ≥5-10 years (27 (2-32) U/L) with mean rank of 29.04 (U = 362.5, z = -3.613, ρ < 0.001, r = 0.4172), the reference interval limits for aspartate aminotransferase (AST U/L) for female children and adolescents of age category ≥1-5 years (34 (13-57) U/L) with mean rank of 44.70 is significantly higher than that of age category ≥10-15 years (24 (13-28) U/L) with mean rank of 22.32 (U = 196, z = -4.655, ρ < 0.001, **r = 0.5645**), and the reference interval limits for aspartate aminotransferase (AST U/L) for female children and adolescents of age category ≥1-5 years ((34 (13-57) U/L) with mean rank of 40.09 is significantly higher than that of age category ≥15-18 years (18 (10-65) U/L) with mean rank of 20.48 (U = 181.5, z = -4.186, ρ < 0.001, **r = 0.5274**). The reference interval limits for aspartate aminotransferase (AST U/L) for female children and adolescents of age category ≥5-10, ≥10-15 and ≥15-18 years were similar.

Interestingly, the reference interval limits for aspartate aminotransferase (AST U/L) for female children and adolescents of age category ≥5-10 years (27 (2-32) U/L) and age category ≥10-15 years (24 (13-28) U/L) are similar while the reference interval limits for aspartate aminotransferase (AST U/L) for female children and adolescents of age category ≥5-10 years (27 (2-32) U/L) with a mean rank of 39.01 is significantly higher than that of age category ≥15-18

years (18 (10-65) U/L) with a mean rank of 22.98 (U = 246.5, z = -3.391, ρ = 0.001, r = 0.4239).

Results for the pairwise comparison using Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for aspartate aminotransferase (AST U/L) for male children and adolescents of age category ≥1-5 years (46.5 (21-68) U/L) with mean rank of 40.03 is significantly higher than that of age category ≥5-10 years (28 (12-35) U/L) with mean rank of 22.59 (U = 220, z = -3.771, ρ < 0.001, r = 0.4751), the reference interval limits for aspartate aminotransferase (AST U/L) for male children and adolescents of age category ≥1-5 years (46.5 (21-68) U/L) with mean rank of 41.29 is significantly higher than that of age category ≥10-15 years (25 (6-31) U/L) with mean rank of 22.81 (U = 211, z = -4.155, ρ < 0.001, **r = 0.5154**), and the reference interval limits for aspartate aminotransferase (AST U/L) for male children and adolescents of age category ≥1-5 years (46.5 (21-68) U/L) with mean rank of 41.81 is significantly higher than that of age category ≥15-18 years (22 (11-26) U/L) with mean rank of 18.98 (U = 125.5, z = -4.971, ρ < 0.001, **r = 0.6313**). The reference interval limits for aspartate aminotransferase (AST U/L) for male children and adolescents of age category ≥5-10, ≥10-15 and ≥15-18 years were similar.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for alkaline phosphatase (ALP U/L) for female children and adolescents of age category ≥1-5 years (182 (0-239) U/L) with a

mean rank of 40.53 is significantly higher than that of age category ≥10-15 years (1(0-113) U/L) with a mean rank of 27.31 (U = 350.5, z = -2.772, ρ = 0.006, r = 0.3362), and the reference interval limits for aspartate alkaline phosphatase (ALP U/L) for female children and adolescents of age category ≥1-5 years (182 (0-239) U/L) with a mean rank of 37.04 is significantly higher than that of age category ≥15-18 years (28.5 (0-128) U/L) with a mean rank of 24.83 (U = 294.5, z = -2.636, ρ = 0.008, r = 0.3321). The reference interval limits for alkaline phosphatase (ALP U/L) for female children and adolescents of age category ≥1-5 years differs from that of age category ≥10-15 and ≥15-18 years, both of which are similar, age category ≥1-5 and ≥5-10 years are similar, and age category ≥5-10, ≥10-15 and ≥15-18 years are similar.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for creatinine (CREAT μmol/L) for female children and adolescents of age category ≥5-10 years (47.5 (11-55) U/L) with a mean rank of 27.28 is significantly lower than that of age category ≥15-18 years (50 (46-69) μmol/L/L) with a mean rank of 40.13 (U = 295.5, z = -2.719, ρ = 0.007, r = 0.3399). In summary, the reference interval limits for creatinine (CREAT μmol/L) for female children and adolescents of age category ≥1-5 years is similar to that of age category ≥5-10, ≥10-15 and ≥15-18 years but that of age category 5-10 years is significantly lower than that of age category 15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for blood urea nitrogen (BUN μmol/L) for female children and adolescents of age category ≥1-5 years (3 (2-9) μmol/L) with a mean rank of 37.61 is significantly lower than that of age category ≥15-18 years (3 (2-4) μmol/L) with a mean rank of 25.04 (U = 300, z = -2.849, ρ = 0.004, r = 0.3561). In summary, the reference interval limits for blood urea nitrogen (BUN μmol/L) for female children and adolescents of age category ≥1-5 years differs from that of age category 15-18 years. However, it is similar to the age category ≥5-10 and ≥10-15 years, both of which are similar to the age category ≥15-18 years.

Results for the pairwise comparison using Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for chloride (Cl mmol/L) for female children and adolescents of age category ≥1-5 years (101 (88-105) mmol/L) with mean rank of 46.95 is significantly higher than that of age category ≥5-10 years (95 (50-98) mmol/L) with mean rank of 29.29 (U = 372, z = -3.517, ρ < 0.001, r = 0.4061), the reference interval limits for chloride (Cl mmol/L) for female children and adolescents of age category ≥1-5 years (101 (88-105) mmol/L) with mean rank of 40.85 is significantly higher than that of age category ≥10-15 years (94 (23-100) mmol/L) with mean rank of 26.92 (U = 338.5, z = -2.904, ρ = 0.004, r = 0.3522), the reference interval limits for chloride (Cl mmol/L) for female children and adolescents of age category ≥5-10 years (95 (50-98) mmol/L)

with mean rank of 26.37 is significantly lower than that of age category ≥15-18 years (101 (88-106) mmol/L) with mean rank of 41.46 (U = 261, z = -3.196, ρ = 0.001, r = 0.3995), and the reference interval limits for chloride (Cl mmol/L) for female children and adolescents of age category ≥10-15 years (94 (23-100) mmol/L) with mean rank of 23.71 is significantly lower than that of age category ≥15-18 years (101 (88-106) mmol/L) with mean rank of 35.31 (U = 239, z = -2.642, ρ = 0.008, r = 0.3499). In summary, the reference interval limits for chloride (Cl mmol/L) for female children and adolescents of age category ≥1-5 years is significantly higher than age categories ≥5-10 and ≥10-15 years, both of which are similar, age category 5-10 and 10-15 years are similar, age category 5-10 years is significantly lower than age category 15-18 years and age category 10-15 years is significantly lower than age category ≥15-18 years which is similar to age category 1-5 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for alkaline phosphatase (ALP U/L) for male children and adolescents of age category ≥1-5 years (239.5 (0-308) U/L) with a mean rank of 41.28 is significantly higher than that of age category ≥10-15 years (1 (0-183) U/L) with a mean rank of 23.92 (U = 245.5, z = -3.723, ρ < 0.001, r = 0.4618), and the reference interval limits for aspartate alkaline phosphatase (ALP U/L) for male children and adolescents of age category ≥1-5 years (239.5 (0-308) U/L) with a mean rank of 38.50 is significantly higher than that of age category ≥15-18 years (71.5 (0-186) U/L) with a mean rank of 23 (U = 238, z =

-3.385, ρ = 0.001, r = 0.4300). The reference interval limits for alkaline phosphatase (ALP U/L) for male children and adolescents of age category ≥1-5 years differs from that of age category ≥10-15 and ≥15-18 years, both of which are similar, age category ≥1-5 and ≥5-10 years are similar, and age category ≥5-10, ≥10-15 and ≥15-18 years are similar.

Results for the pairwise comparison using Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for gamma-glutamyltransferase (γ-GT U/L) for male children and adolescents of age category ≥1-5 years (50 (6-132) U/L) with mean rank of 40.28 is significantly higher than that of age category ≥5-10 years (14 (4-28) U/L) with mean rank of 22.29 (U = 211.5, z = -3.888, ρ < 0.001, r = 0.4898), the reference interval limits for gamma-glutamyltransferase (γ-GT U/L) for male children and adolescents of age category ≥1-5 years (50 (6-132) U/L) with mean rank of 40.57 is significantly higher than that of age category ≥10-15 years (14 (8-26) U/L) with mean rank of 24.69 (U = 269.5, z = -3.387, ρ < 0.001, r = 0.4201), and the reference interval limits for gamma-glutamyltransferase (γ-GT U/L) for male children and adolescents of age category ≥1-5 years (50 (6-132) U/L) with mean rank of 38.34 is significantly higher than that of age category ≥15-18 years (14 (6-26) U/L) with mean rank of 23.20 (U = 243.5, z = -3.300, ρ = 0.001, r = 0.4191). In summary, the reference interval limits for gamma-glutamyltransferase (γ-GT U/L) for male children and adolescents of age category ≥1-5 years is significantly higher than age categories ≥5-10, ≥10-15 and ≥15-18 years which are similar.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for total bilirubin (T-BIL μmol/L) for male children and adolescents of age category ≥1-5 years (8.5 (1-49) μmol/L) with a mean rank of 38.40 is significantly higher than that of age category ≥5-10 years (3 (1-6.5) μmol/L) with a mean rank of 24.50 (U = 275.5, z = -3.041, ρ = 0.002, r = 0.3831). In summary, the reference interval limits for total bilirubin (T-BIL μmol/L) for male children and adolescents increased from levels of age category 1-5 years to levels in the age category 5-10 years and reverted back to levels of age category 1-5 years in the age category 10-15 and 15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for direct bilirubin (D-BIL μmol/L) for male children and adolescents of age category ≥1-5 years (2.5 (1-7.7) μmol/L) with a mean rank of 40.38 is significantly higher than that of age category ≥5-10 years (1 (0-2) μmol/L) with a mean rank of 22.17 (U = 208, z = -4.205, ρ < 0.001, **r = 0.5298**). In summary, the reference interval limits for total bilirubin (T-BIL μmol/L) for male children and adolescents increased from levels of age category 1-5 years to levels in the age category 5-10 years and reverted back to levels of age category 1-5 years in the age category 10-15 and 15-18 years.

Results for the pairwise comparison using Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for creatinine (CREAT μmol/L) for male children and

adolescents of age category ≥1-5 years (37 (18-58) μmol/L) with mean rank of 23.44 is significantly lower than that of age category ≥10-15 years (54 (38-85) μmol/L) with mean rank of 43.48 (U = 202, z = -4.273, ρ < 0.001, r = **0.5300**), the reference interval limits for creatinine (CREAT μmol/L) for male children and adolescents of age category ≥1-5 years (37 (18-58) μmol/L) with mean rank of 21.79 is significantly lower than that of age category ≥15-18 years (67.5 (44-76) μmol/L) with mean rank of 43.29 (U = 146, z = -4.673, ρ < 0.001, r = **0.5935**), the reference interval limits for creatinine (CREAT μmol/L) for male children and adolescents of age category ≥5-10 years (41 (27-60.38) μmol/L) with mean rank of 21.38 is significantly lower than that of age category ≥10-15 years (54 (38-85) μmol/L) with mean rank of 39.03 (U = 185, z = -3.923, ρ < 0.001, r = **0.5066**) and the reference interval limits for creatinine (CREAT μmol/L) for male children and adolescents of age category ≥5-10 years (41 (27-60.38) μmol/L) with mean rank of 18.09 is significantly lower than that of age category ≥15-18 years (67.5 (44-76) μmol/L) with mean rank of 40.30 (U = 89.5, z = -5.062, ρ < 0.001, r = **0.6705**). In summary, the reference interval limits for creatinine (CREAT μmol/L) for male children and adolescents increased from age category ≥1-5 years to 5-10 years but were more pronounced through age category 10-15 years to 15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for blood urea nitrogen (BUN μmol/L) for male children and adolescents of age category ≥5-10 years (4 (2-4) μmol/L) with a

mean rank of 35.53 is significantly higher than that of age category ≥15-18 years (3 (2-3.3) μmol/L) with a mean rank of 22.23 (U = 216.5, z = -3.194, ρ = 0.001, r = 0.4231). In summary, the reference interval limits for blood urea nitrogen (BUN μmol/L) for male children and adolescents of age category ≥1-5 years were similar to those of age category ≥5-10, ≥10-15 and ≥15-18 years but age category ≥5-10 years was significantly higher than that of age category ≥15-18 years.

Results for the pairwise comparison using the Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for potassium (K mmol/L) for male children and adolescents of age category ≥1-5 years (4 (4-5) mmol/L) with a mean rank of 36.76 is significantly higher than that of age category ≥15-18 years (4 (4-4.3) μmol/L) with a mean rank of 25.11 (U = 297, z = -3.135, ρ = 0.002, r = 0.3981). In summary, the reference interval limits for potassium (K mmol/L) for male children and adolescents of age category ≥1-5 years were similar to those of age categories ≥5-10 and ≥10-15 years but significantly higher than that of age category ≥15-18 years.

Results for the pairwise comparison using Mann-Whitney U test with adjusted statistically significant ρ-value of less than 0.0083 indicate that the reference interval limits for sodium (NA mmol/L) for male children and adolescents of age category ≥1-5 years (134 (128-138) mmol/L) with mean rank of 23.79 is significantly lower than that of age category ≥5-10 years (138 (120-140) mmol/L) with mean rank of 41.62 (U = 214, z = -3.865, ρ < 0.001, r = 0.4869),

the reference interval limits for sodium (NA mmol/L) for male children and adolescents of age category ≥1-5 years (134 (128-138) mmol/L) with mean rank of 24.56 is significantly lower than that of age category ≥10-15 years (139 (4-146) mmol/L) with mean rank of 42.26 (U = 240, z = -3.786, ρ < 0.001, r = 0.4808), the reference interval limits for sodium (NA mmol/L) for male children and adolescents of age category ≥1-5 years (134 (128-138) mmol/L) with mean rank of 23.60 is significantly lower than that of age category ≥15-18 years (138.5 (132-140) mmol/L) with mean rank of 41.09 (U = 207.5, z = -3.817, ρ < 0.001, r = 0.4848). In summary, the reference interval limits for sodium (NA mmol/L) for male children and adolescents of age category ≥5-10 years were significantly lower than those of age category ≥5-10, ≥10-15, and ≥15-18 years which were all similar.

An investigation to assess the effect of gender on the developed reference interval limits for liver and kidney function tests at the four studied age categories indicates that: the reference interval limits for alanine aminotransferase (ALT) of males (18 (6-24) U/L) with a mean rank of 41.82 is higher than that of females (14 (6-19) U/L) with a mean rank of 30.65 (U = 431, z = -2.286, ρ = 0.022, r = 0.2713) and alkaline phosphatase (ALP) of males (239.5 (0-308)) with a mean rank of 41.35 is higher than that of females (182 (0-239) U/L) with a mean rank of 31.08 (U = 447, z = -2.099, ρ = 0.036, r = 0.2491) while the blood urea nitrogen (BUN) of males (2 (1-4) μmol/L) with a mean rank of 30.93 is lower than that of females (3 (2-9) μmol/L) with a mean rank of 40.66 (U = 456.5, z = -2.091, ρ = 0.037, r = 0.2482) at age

category ≥1-5 years. The reference intervals for creatinine (CREAT) of males (41 (27-60) μmol/L) with a mean rank of 27.41 is lower than that of females (47.5 (11-55) μmol/L) with a mean rank of 39.03 (U = 360, z = -2.424, ρ = 0.015, r = 0.2961) in age category ≥5-10 years. The reference interval limits for albumin (ALB) of males (48 (34-49) g/L) with a mean rank of 34.79 is higher than that of females (44.5 (34-47) g/L) with a mean rank of 19.65 (U = 160, z = -3.55, ρ < 0.001, r = 0.4831) and creatinine (CREAT) of males (67.5 (44-76) μmol/L) with a mean rank of 32.80 is higher than that of females (50 (46-69) μmol/L) with a mean rank of 21.79 (U = 215.5, z = -2.576, ρ = 0.010, r = 0.3505) in age category ≥15-18 years.

Table 4.14: Effects of age and sex on the established reference intervals for serum biochemistry analytes for children and adolescents of Taita-Taveta County, Kenya

Analyte (units)	Sex	N	≥1-5 years	N	≥5-10 years	N	≥10-15 years	N	≥15-18 years
TP	M&F	71	64.41±11.52	67	73.91±5.28	62	75.89±12.86	54	72.56±6.96
(g/L)			**66 (33-84)**		**74 (60-83)[a]↑**		**74 (56-139)[b]↑**		**74 (52-85)**
	F	37	65.08±9.04	38	74.89±3.07	31	76.52±12.47	26	72.15±4.63
			66 (45-72)		**74 (69-76)[a]↑**		**75 (63-82)[b]↑**		**72 (66-76)[c]↑**
	M	34	63.68±13.84	29	72.62±7.09	31	75.26±13.42	28	72.93±8.65
			66 (33-74)		**74 (42-77)[a]↑**		**74 (33-74)[b]↑**		**74 (52-78)[c]↑**
ALB	M&F	71	41.61±7.01	67	44.93±5.70	62	44.34±4.45	54	45.43±3.89
(U/L)			**44 (19-50)**		**46 (26-52)[a]↑**		**45 (34-52)**		**46.5 (34-51)**
	F	37	42.59±5.46	38	44.97±6.11	31	44.35±4.90	26	43.69±3.83
			44 (28-46)		**47 (28-48)**		**45 (34-48)**		**44.5 (34-47)**
	M	34	40.53±8.33	29	44.86±5.21	31	44.32±4.05	28	47.04±3.25
			44 (19-45)		**45 (22-48)**		**44 (34-46)**		**48 (34-49)*↑[cf]**
ALT	M&F	71	**40.87±190.25**	67	18.16±15.44	62	14.85±6.42	54	24.00±**48.04**
(U/L)			**16 (0-451)**		**13 (7-72)**		**14 (0.6-33)**		**11.5 (1-219)**
	F	37	15.62±6.56	38	17.53±10.52	31	13.58±5.08	26	35.92±**67.64**
			14 (6-19)		**14 (6-22)**		**13 (1-17)**		**11.5 (4-70)[e]↓**
	M	34	68.35±**274.29**	29	19.00±20.35	31	16.13±7.38	28	12.93±5.52
			18 (6-24)*↑		**13 (8-28)**		**16 (0-19)**		**11.5 (1-16)[c]↓**
AST	M&F	71	48.21±34.82	67	27.54±11.01	62	24.56±8.02	54	29.81±40.97
(U/L)			**36 (13-167)**		**27 (9-59)[a]↓**		**24.5 (9.5-44)[b]↓**		**21.5 (10-194)[e]↓[f]**
	F	37	44.84±30.73	38	26.97±7.03	31	23.55±8.48	26	38.15±57.68
			34 (13-57)		**27 (2-32)[a]↓**		**24 (13-28)[b]↓**		**18 (10-65)[ce]↓**
	M	34	51.88±38.92	29	28.28±14.82	31	25.58±7.54	28	22.07±9.25
			46.5 (21-68)		**28 (12-35)[a]↓**		**25 (6-31)[b]↓**		**22 (11-26)[c]↓**
ALP	M&F	71	177.86±122.27	67	110.36±155.54	62	84.16±166.90	54	93.02±129.84[c]
(U/L)			**187 (0-451)**		**1 (0-488)[a]↓**		**1 (0-661)[b]↓**		**49.5 (0-553)**
	F	37	149.14±115.83	38	91.08±154.62	31	67.81±134.10	26	78.35±105.63
			182 (0-239)		**1 (0-147)**		**1(0-113)[b]↓**		**28.5 (0-128)[c]↓**
	M	34	209.12±123.06	29	135.62±155.79	31	100.52±195.20	28	106.64±149.54
			239.5 (0-308)*↑		**1 (0-241)**		**1 (0-183)[b]↓**		**71.5 (0-186)[c]↓**
GGT	M&F	71	64.03±70.07	67	27.66±49.86	62	18.06±13.24	54	25.94±33.65
(U/L)			**27 (4-264)**		**14 (4-282)[a]↓**		**13.5 (6-65)[b]↓**		**15 (6-156)**
	F	37	51.08±67.88	38	34.92±62.88	31	16.39±10.00	26	32.73±45.99
			18 (4-77)		**14.5 (5-60)**		**12 (5-21)**		**17 (7-54)**
	M	34	78.12±70.69	29	18.14±21.69	31	19.74±15.83	28	19.64±13.38
			50 (6-132)		**14 (4-28)[a]↓**		**14 (8-26)[b]↓**		**14 (6-26)[c]↓**
T-BIL	M&F	71	35.65±62.97	67	5.19±11.48	62	5.56±4.98	54	5.04±2.80
(µmol/L)			**6 (0.8-265)**		**3 (1-36)[a]↓**		**4 (0-23)**		**4.5 (1-12)**
	F	37	41.38±73.62	38	6.05±15.12	31	6.10±5.95	26	4.96±3.30[c]
			5 (0-44)		**3 (1-12)**		**5 (0-8.77)**		**3 (1-7)**
	M	34	29.41±49.21	29	4.07±2.52	31	5.03±3.80	28	5.11±2.28

			8.5 (1-49)		**3 (1-6.5)[a]↓**		**4 (0-7)**		**5 (2-6.8)**
D-BIL (µmol/L)	M&F	71	7.69±24.81	67	1.37±0.85	62	2.32±3.35	54	1.87±1.64
			2 (0-81)		**1 (0-3.9)[a]↓**		**2 (0-17)**		**2 (0-8)**
	F	37	10.86±33.28	38	1.47±1.03	31	2.65±4.22	26	2.00±2.15
			2 (0-13)		**1 (0-2)**		**2 (0-4.5)**		**1 (0-3)**
	M	34	4.248±8.60	29	1.24±0.51	31	2.00±2.18	28	1.75±0.97[c]
			2.5 (1-7.7)		**1 (0-2)[a]↓**		**2 (0-2.9)**		**2 (0-2.2)**
CREAT (µmol/L)	M&F	71	48.85±31.35	67	48.90±19.70	62	61.42±22.65	54	63.22±18.10
			41 (18-131)		**44 (22-138)**		**53 (38-130)[b]↑**		**56 (44-118)[e]↑**
	F	37	51.70±31.93	38	48.84±13.51	31	57.03±17.22	26	59.58±20.42
			43 (18-64)		**47.5 (11-55)*↑**		**52 (38-65)**		**50 (46-69)[e]↑**
	M	34	45.74±30.88	29	48.97±25.96	31	65.81±26.58	28	66.61±15.24
			37 (18-58)		**41 (27-60)**		**54 (38-85)[b]↑[d]**		**67.5 (44-76)*↑[ce]**
BUN (mmol/L)	M&F	71	4.76±5.71	67	3.48±0.73	62	3.23±1.42	54	2.85±0.94
			3 (1-28)		**3 (2-5)**		**3 (1.2-8)**		**3 (2-5.9)[e]↓**
	F	37	6.14±7.33	38	3.50±0.69	31	3.26±1.79	26	2.88±0.82
			3 (2-9)*↑		**3 (2-4)**		**3 (2-4)**		**3 (2-4)[e]↓**
	M	34	3.26±2.44	29	3.45±0.78	31	3.19±0.95	28	2.82±1.06
			2 (1-4.3)		**4 (2-4)**		**3 (0-4)**		**3 (2-3.3)[e]↓**
K (mmol/L)	M&F	71	4.56±0.75	67	6.37±16.33	62	4.56±1.53	54	4.17±0.42
			4 (4-7)		**4 (4-45)**		**4 (4-12)**		**4 (4-5.6)[e]↓**
	F	37	4.54±0.80	38	7.92±21.68	31	4.55±1.46	26	4.24±0.51
			4 (4-5)		**4 (4-7)**		**4 (4-5.2)**		**4 (4-4.5)**
	M	34	4.59±0.70	29	4.34±0.48	31	4.58±1.63	28	4.11±0.32
			4 (4-5)		**4 (4-5)**		**4 (4-5.3)**		**4 (4-4.3)[c]↓**
NA (mmol/L)	M&F	71	136.32±7.07	67	136.97±8.68	62	134.42±24.48	54	137.78±3.84
			135 (126-160)		**138 (107-148)[a]↑**		**138 (5-149)[b]↑**		**138 (127-144)**
	F	37	137.46±8.32	38	136.47±10.47	31	133.68±24.54	26	137.31±4.58
			136 (124-141)		**138 (76-140)**		**138 (5-144)**		**137.5 (124-140)**
	M	34	135.09±5.25	29	137.62±5.64	31	135.16±24.80	28	138.21±3.04
			134 (128-138)		**138 (120-140)[a]↑**		**139 (4-146)[b]↑**		**138.5 (132-140)[c]↑**
CL (mmol/L)	M&F	71	101.35±8.00	67	95.91±7.92	62	94.21±15.91	54	99.13±6.90
			101 (88-125)		**95 (75-110)[a]↓**		**96 (17-110)[b]↓**		**98 (86-116)**
	F	37	101.43±8.32	38	94.55±8.81	31	93.84±14.85	26	101.19±7.72
			101 (88-105)		**95 (50-98)[a]↓**		**94 (23-100)[b]↓**		**101 (88-106)[e]↑[f]**
	M	34	101.26±7.78	29	97.69±6.28	31	94.58±17.13	28	97.21±5.51
			101 (88-104)		**97 (89-100)**		**97 (9-102)**		**98 (86-101)**

Results are expressed as mean ± standard deviation (SD) and median and 95% range (in brackets) of the number of subjects indicated in the column labeled N. *$\rho < 0.05$ when male reference interval limits are compared to female reference interval limits for each of the four age categories using Mann-Whitney U test. Kruskal-Wallis H test, followed by Mann-Whitney with an adjusted significant p-value of less than 0.0083, was used to compare reference interval limits within and between each age group for each sex and/or combined sex. [a]$\rho < 0.0083$ when reference interval limits in age range ≥ 1-5 years is compared with reference interval limits in age range > 5-10 years; [b]$\rho < 0.0083$ when reference interval limits in age range ≥ 1-5 years is compared with reference interval limits in age range >10-15 years; [c]$\rho < 0.0083$ when reference interval limits in age range ≥ 1-5 years is compared to reference interval limits in age range > 15-18 years; [d]$\rho < 0.0083$ when reference interval limits in age range ≥ 5-10 years is compared to reference interval limits in age range > 10-15 years; [e]$\rho < 0.0083$ when reference interval limits in age range > 5-10 years is compared to reference interval limits in age range > 15-18 years; [f]$\rho < 0.0083$ when reference interval limits in age range >10-15 years is compared to reference interval limits in age range > 15-18 years.

4.6.2 Comparison of the developed reference intervals for serum biochemistry analytes for children and adolescents of Taita-Taveta County, Kenya, with those reported in the medical literature

A comparison of the established age-related reference interval limits for serum biochemistry parameters for children and adolescents of Taita-Taveta County, Kenya, with those reported in the medical literature is presented in Table 4.15. Results indicate that this study's developed age and sex-independent reference intervals: for total protein are lower than those reported for Tanzania children and adolescents, lower than those of Ghanaians between 1 and 5 years, but higher between 5 and 18 years; for albumin, the Taita-Taveta median values are lower than those reported for Tanzania and Ghana for children and adolescents.

Further, the developed age and sex-specific median 95% reference interval values for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in this study are lower than those reported for Tanzania for children and adolescents; for alanine aminotransferase (ALT), the developed median reference intervals are lower than those reported for Ghana between 1 and 5 years, but higher between 5-18 years. However, the reported Ghanaian median values for aspartate aminotransferase (AST) are higher than those for Taita-Taveta children and adolescents.

In addition, the established age and sex-specific reference intervals for alkaline phosphatase (ALP) for children and adolescents in this study for the Taita-Taveta population is lower than that reported for the Tanzanian population.

Interestingly, the developed reference intervals for γ-glutamyltransferase (γ-GT) for children and adolescents are higher than that reported for the Ghanaian population. The reference intervals for total (T-BIL) and direct (D-BIL) bilirubin established in this study for children and adolescents of Taita-Taveta County, Kenya, are lower than those reported for Tanzanian and Ghanaian populations.

For the kidney function tests, the established age and sex-specific reference intervals for creatinine (CREAT) for children and adolescents of Taita-Taveta County, Kenya in this study are higher than those reported for Tanzania, higher than those reported for Ghanaians between 1-5 years, and lower than those reported for Ghanaians between 5 and 18 years. The established age and sex-specific reference intervals for blood urea nitrogen (BUN) for children and adolescents of Taita-Taveta County, Kenya, are similar to the age-dependent and sex-independent reference intervals for Tanzanians and similar to the sex-independent reference intervals for Ghanaian children and higher than the sex-dependent intervals for Ghanaian adolescents. The age-dependent and sex-independent reference intervals for potassium for children and adolescents of Taita-Taveta County, Kenya, are higher than those reported for Tanzania and Ghana populations.

Further, the age-dependent and sex-independent reference intervals for sodium for children and adolescents of Taita-Taveta County, Kenya in this study are higher than those reported for Tanzania and Ghana populations. The age-dependent and sex-independent reference interval limits for chloride for

children and adolescents of Taita-Taveta County, Kenya, are lower than those reported for Tanzanian and Ghanaian populations (Table 4.15).

Table 4.15: Comparison of the developed reference intervals for serum biochemistry analytes for children and adolescent population of Taita-Taveta County, Kenya, with those reported in the medical literature

TP	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(g/L)		M&F	**33-84**	**60-83[a]↑**	**56-139[b]↑**	**52-85**
		M	33-74	42-77[a]↑	33-74[b]↑	52-78[c]↑
		F	45-72	69-76[a]↑	63-82[b]↑	66-76[c]↑
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	†60-76↓	†66-80		
		M			†68-84*	
		F			†67-84	
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	†56-87	↓54-87.9†	↓46.4-86.5AID	
		M				
		F				
	Canadians		3-5 years			6-19 years
		M&F				
		M	†63-81			†68-82
		F	63-81			68-82
	Koreans		1-6 years		7-12 years	13-18 years
		M&F	†64-78			†68.7-80.9
		M			†68.5-76.2	
		F			†65-78.8	
ALB	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(g/L)		M&F	**19-50**	**26-52[a]↑**	**34-52**	34-51
		M	19-45	22-48	34-46	**34-49*↑[cf]**
		F	28-46	28-48	34-48	**34-47**
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	†40-49	†40-48↓		
		M			†41-51*	
		F			†40-49	
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	†35.9-50	†34.2-49.8		
		M			34.3-48.9	
		F			†37.8-50.5*†	
	Canadians		3-5 years		6-15 years	16-29 years
		M&F				
		M	†39-50		†41-51	†46-53*†
		F	39-50		41-51	†39-50
	Koreans		1-18 years			
		M&F	†40-50			
		M				
		F				
	Austrian[B]					14-17 years
		M&F				
		M				†44.20-58.68*†
		F				†40.30-54.80†
	America[S]				10-19 years	
		M&F				
		M			37-56	
		F			37-56	
ALT	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(U/L)		M&F	0-451	**7.4-72**	**0.6-33**	**1-219**
		M	**6-24*↑**	8-28	0-19	1-16[c]↓
		F	**6-19**	6-22	1-17	4-70[e]↓
	Kenya[W]					13-17 years
		M&F				
		M				5-42↓

		F				4-65*↓
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	†10-28†	†9-35↓		
		M			†10-36*↓	
		F			†7-33↓	
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	7-55†	↓5-53↓	8-55AID	
		M			†10-61*↓	
		F			†7-48↓	
	Canadians		3-5 years	6-8 years	9-11 years	12-17 years
		M&F				
		M	†15-33†	†16-37↓	†18-39†	†17-50*↓
		F	15-33	16-37	18-39	†14-41↓
	Koreans		1-18 years			
		M&F	7.2-27			
		M				
		F				
	Gabon		1.5-5 years			
		M&F	6-32†			
	Austrian[B]					14-17 years
		M&F				
		M				†9.72-36.85↓
		F				†10-32↓
	America[S]				10-18 years	
		M&F				
		M			†5-30*↓	
		F			†5-20↓	
AST	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(U/L)		M&F	**13-167**	**9-59**[a]↓	**9.5-44**[b]↓	**10-194**[e]↓[f]
		M	21-68	12-35[a]↓	6-31[b]↓	11-26[c]↓
		F	13-57	2-32[a]↓	13-28[b]↓	10-65[c]↓
	Kenya[W]					13-17 years
		M&F				
		M				†17-59*↓
		F				12-43↓
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	†27-55↓	†21-51↓		
		M			†19-42*↓	
		F			†17-36↓	
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	†23-72↓	†19-57	14-62 PGDA	
		M			†18-67*↓	
		F			11-49↓	
	Canadians		3-5 years	6-11 years		12-17 years
		M&F				
		M	†28-52↓	†25-47*↓		†18-36↓*
		F	28-52	†23-44↓		†15-34↓
	Koreans		1-6 years	7-12 years		13-18 years
		M&F	†22.8-48↓			10.2-25.2↓
		M		16.2-37.8*†		
		F		13.2-30.6		
	Austrian					14-17 years
		M&F				
		M				†17.72-45.57↓
		F				†14-37↓
	America[S]				12-18 years	
		M&F				
		M			< 39↓	
		F			< 32↓	
ALP	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(U/L)		M&F	0-451	**0-489**[a]↓	**0-661**[b]↓	**0-553**
		M	**0-308***↑	0-241	0-183[b]↓	0-186[c]↓
		F	**0-239**	0-147	0-113[b]↓	0-128[c]↓
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	†153-410†	†174-460↓		
		M			†124-537*↓	

		F			†68-498↓	
	Canadians		3-5 years	6-10 years	11-15 years	16-21 years
		M&F				
		M	†144-327†	†153-367↓	†113-438*↓	†56-167*↓
		F	144-327	153-367	†64-359↓	†44-107↓
	Koreans		0-12 years		13-15 years	16-18 years
		M&F	†120.6-345†			
		M			†62.4-339.6↓	†57.6-113.4↓
		F			†55.2-165.6↓	†34.2-96.6↓
GGT	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(U/L)		M&F	**4-264**	**4-282[a]↓**	**6-65[b]↓**	**6-156**
		M	6-132	4-28[a]↓	8-26[b]↓	6-26[c]↓
		F	4-77	5-60	5-21	7-54
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	3-34↓	†7-31↓	6-45↓ AID	
		M				
		F				
	Austrian					14-17 years
		M&F				
		M				5.72-26.57↓
		F				5-27↓
	America[S]				13-18 years	
		M&F				
		M			↓2-42↓	
		F			↓4-24↓	
	Canadians		3-5 years	6-14 years		15-19 years
		M&F				
		M	†11-20↓	†10-26*↓		†10-33↓
		F	11-20	†9-24↓		†12-38*↓
T-BIL	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(μmol/L)		M&F	**0.9-265**	**1-36[a]↓**	**0-23**	**1-12**
		M	1-49	1-6.5[a]↓	0-7	2-7
		F	6-44	1-12	0-9	1-7
	Kenya[W]					13-17 years
		M&F				
		M				5.7-62.6†*
		F				3.7-38.5†
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	†2-9↓	†2-11↓		
		M			↑2-15†	
		F			↑3-22*****†	
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	†1.8-21↓	1.7-18.9	†3.3-21.6† AID	
		M				
		F				
	Canadians		3-5 years	6-15 years	16-48 years	
		M&F				
		M	†1.71-8.55	†1.71-15.39†	†3.42-18.81*†	
		F	1.71-8.55	1.71-15.39	1.71-15.39	
	Koreans		1-18 years			
		M&F	3.4-13.7			
		M				
		F				
	Gabon		1.5-5 years			
		M&F	↓0.44-22.12↓			
D-BIL	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(μmol/L)		M&F	**0-81**	**0-3.9[a]↓**	**0-17**	**0-8**
		M	1-8	0-2	0-3	0-2
		F	0-13	0-2[a]↓	0-5	0-3
	Ghana		0.5-4.9 years	5-12 years		13-17 years
		M&F	†0.4-3.6↓	†0.6-3.9		PGDA
		M				†1.2-4.0*↓
		F				†0.8-3.9↓
CREAT	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(μmol/L)		M&F	**18-131**	22-138	**38-130[b]↑**	44-118[e]↑
		M	18-58	**27-60**	38-85	**44-76↑[ce]**

		F	18-64	**11-55*↑**	38-65	**46-69*ᵉ↑**
	Kenya[W]					13-17 years
		M&F				
		M				†49.6-103.7†*
		F				†48.0-87.6†
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	15-50↓	↓24-49↓		
		M			↓36-80*†	
		F			↓32-64↓	
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	17-52↓	†33-74†	PGIA	
		M			42-79*	
		F			↓33-78†	
	Canadians		3-5 years	6-9 years	10-11 years	12-15 years
		M&F				
		M	†26.52-44.20	†35.36-53.04	†35.36-61.88	44.20-79.56*†
		F	26.52-44.20	35.36-53.04	35.36-61.88	44.20-70.72
	Canadians					16-79 years
		M&F				
		M				61.88-106.08*†
		F				53.04-88.40
	Koreans		1-6 years	7-12 years	13-15 years	16-18 years
		M&F	†23-46.5	†30.2-66.2		PGIA
		M			38.7-97.1*	†61.7-102.8*
		F			†44.4-71.7	†48.9-77.8
	Gabon		1.5-5 years			
		M&F	↓7.3-40.5↓			
	Austrian[B]					14-17 years
		M&F				
		M				†53.93-91.94†
		F				†51.27-87.52†
	America[S]					15-20 years
		M&F				
		M				↑53.04-88.40*†
		F				53.04-79.56
	India				11-17 years	
		M&F				
		M			↓25.64-78.36*†	
		F			↓22.11-69.85	
BUN	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(mmol/L)		M&F	1-28	**2-5**	**1-8**	**2-6ᵉ↓**
		M	**1-4**	2-4	0-4	2-3ᵉ↓
		F	**2-9*↑**	2-4	2-4	2-4ᵉ↓
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	1.3-4.2	1.4-4.4		
		M			1.5-4.5↓	
		F			1.6-4.4↓	
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	1-4.2	1-4.5	PGIA	
		M			↓1-5.5*	
		F			↓1-3.6↓	
	Canadians		3-5 years	6-7 years		8-19 years
		M&F				
		M	†3.21-6.79	†2.86-7.50†		†2.86-7.14*
		F	3.21-6.79	2.86-7.50		2.86-6.79†
	Koreans		1-18 years			
		M&F	↓0.81-2.17↓			
		M				
		F				
	Austrian[B]					14-17 years
		M&F				
		M				†6.03-12.25*
		F				4.43-10.82↓
	India				11-17 years	
		M&F				
		M			†3.91-11.63†	

		F			†3.35-11.21†	
K	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(mmol/L)		M&F	**4-7**	**4-45**	**4-12**	**4-5.6[e]↓**
		M	4-5	4-5	4-5.3	4-4.3[c]↓
		F	4-5	4-7	4-5.2	4-4.5
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	3.8-5.8↓	3.2-5.2↓		
		M			↓3.6-5.1↓	
		F			↓3.6-5.0↓	
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	↓3.6-5.8↓	↓3.6-5.6↓	↓3.6-5.9 PGDA	
		M				
		F				
	Canadians		3-5 years	6-79 years		
		M&F				
		M	3.9-4.6	3.8-4.9		
		F	3.9-4.6	3.8-4.9		
	Koreans		1-18 years			
		M&F	3.7-4.8			
		M				
		F				
NA	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(mmol/L)		M&F	**126-160**	**107-148[a]↑**	**4.6-149[b]↑**	**127-144**
		M	128-138	120-140[a]↑	4-146[b]↑	132-140[c]↑
		F	124-141	76-140	5-144	124-140
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	133-141↓	†134-141↓		
		M			†134-140↓	
		F			134-140	
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	131-149↓	†135-151	†132-152	
		M				
		F				
	Canadians		3-5 years	6-15 years		16-49 years
		M&F				
		M	†135-142↓	†136-143↓		†137-143
		F	135-142	136-143		137-142
	Koreans		0-6 years			7-18 years
		M&F	†134-141↓			†136-142
		M				
		F				
CL	This study RI		≥1-5 years	≥5-10 years	≥10-15 years	≥15-18 years
(mmol/L)		M&F	**88-125**	**75-110[a]↓**	**17-110[b]↓**	**86-116**
		M	88-104	89-100	↓9-102	86-101
		F	88-105	50-98[a]↓	23-100[b]↓	88-106[e]↑[f]
	Canadians		3-5 years	6-11 years		12-29 years
		M&F				
		M	†100-107↓	†101-107↓		†101-106↓
		F	100-107	101-107		†100-107*↓
	Koreans		1-18 years			
		M&F	†100-108↓			
		M				
		F				
	Tanzania		≥1-5 years	≥5-13 years	≥13-18 years	
		M&F	†100-108↓	†98-108↓		
		M			†98-105↓	
		F			†99-106*↓	
	Ghana		0.5-4.9 years	5-12 years	13-17 years	
		M&F	†98-115↓	†99-114↓	†96-116	

Moyen-Ogooue´ province, Gabon by Humberg et al. (2011), Kintampo, Ghana by Dosoo et al. (2014), Western Kenya[W] by Zeh et al. (2011), Kilimanjaro Region, Tanzania by Buchanan et al. (2015), Austrian by Bogner et al. (2019), Indo-European Indians by Bandesh et al. (2019), and America[S] by Soldin et al. (2011), Canadian by Adeli et al. (2015), and Koreans by Sung et al. (2021). Symbols † and ↓ stands for literature values of the measured analytes, which are higher and lower, respectively, from the reference intervals established here. ↓ stands for decreasing values in the reference intervals developed in this study. PGDA means progressively decreasing with age; PGIA means progressively increasing with age; AID means age-independent.

4.7.1 Established trimester-specific reference intervals for hematological parameters for pregnant women of Taita Taveta County, Kenya

The established reference intervals for hematological parameters for pregnant women in their second and third trimesters of Taita Taveta County, Kenya, are reported in Table 4.16. Results indicate that the established reference intervals for hematological parameters for the pregnant women of Taita-Taveta County, Kenya, for RBC ($x10^{12}$/L), HB (g/dL), MCH (pg), MCHC (g/dL), MCV (fL), RDW-CV (%), RDW-SD (%), WBC ($x10^9$/L), NEU ($x10^9$/L), NEU (%), LYM ($x10^9$/L), LYM (%), MON ($x10^9$/L), MON (%), BAS $x10^9$/L, EOS ($x10^9$/L), EOS (%), PLT (10^9/L), PCT (%), PDW (%) and MPV (fL) in their second trimester were similar to those of pregnant women in their third trimester ($\rho > 0.05$). Therefore, trimester-independent reference intervals of these parameters for this population were established. The established trimester independent reference interval for the pregnant women of Taita-Taveta County, Kenya, for RBC is 4.0 (3.2-5.2) $x10^{12}$/L, HB is 11.1 (7.8-14.3) g/dL, MCH is 28.2 (20.8-33.2) pg, MCHC is 33.8 (28-36.5) g/dL, MCV is 82.9 (61.9-94.8) fL, RDW-CV is 12.8 (11.3-20.85) %, RDW-SD is 45.1 (38-69.5) %, WBC is 7.8 (4.4-15.11) $x10^9$/L, NEU is 5.23 (2.3-12.5) $x10^9$/L, NEU is 67.4 (39.08-85.9) %, LYM is 2.04 (1.0-4.4) $x10^9$/L, LYM is 25.8 (10.8-44) %, MON is 0.3 (0.1-1.0) $x10^9$/L, MON is 3.8 (1.gf8-10.1) %, EOS is 0.14 (0.07-0.63) $x10^9$/L, EOS is 1.8 (0.3-6.21) %, BAS is 0.14 (0.1-0.6) $x10^9$/L, PLT is 237 (128-388.4) $x10^9$/L, PCT is 0.211 (0.2-0.3) %, PDW is 45.1 (38-69.5) %, and MPV is 8.9 (7.5-11) fL.

The established reference intervals for BAS ($x10^9$/L), BAS % and PVC (%) for pregnant women in their second trimester of Taita-Taveta County, Kenya, significantly differed from those of pregnant women in their third trimester of the same County ($\rho < 0.05$). The established trimester dependent reference intervals for this population of Taita-Taveta County, Kenya, for BAS is 0.03 (0.01-0.14) $x10^9$/L for pregnant women in their second trimester and 0.03 (0-0.60) $x10^9$/L for pregnant women in their third trimester, BAS is 0.5 (0.2-0.99) % for pregnant women in their second trimester and 0.4 (0.1-0.90) % for pregnant women in their third trimester, and PCV is 0.3 (0.3-71.2) % for pregnant women in their second trimester and 0.3 (0.3-45) % for pregnant women in their third trimester (Table 4.16).

Table 4.16: Established trimester-specific reference intervals for hematological parameters for pregnant women of Taita Taveta County, Kenya

Analyte (Unit)	Sex	N	Median	Percentiles		Reference Interval	IV	Difference between M&F	
				2.5^{th}	97.5^{th}			Z value	Sig
RBC ($x10^{12}$/L)	**2&3**	**296**	**4.0**	**3.2**	**5.2**	**3.2-5.2**	**2**	1.079	ρ = 0.2805
	2	124	4.2	3.2	5.2	3.2-5.2	2		
	3	172	4.0	3.2	5.3	3.2-5.3	2.1		
HB (g/dL)	**2&3**	**296**	**11.1**	**7.8**	**14.3**	**7.8-14.3**	**6.5**	0.730	ρ = 0.466
	2	124	11.2	7.9	14.3	7.9-14.3	6.4		
	3	172	11.1	7.7	14.5	7.7-14.5	6.8		
PCV (%)	2&3	296	0.3	0.3	34.4	0.3-34.4	34.1	2.305	**ρ = 0.021**
	2	**124**	**0.3**	**0.3**	**71.2**	**0.3-71.2**	**70.9**		
	3	**172**	**0.3**	**0.3**	**45**	**0.3-45.0**	**44.7**		
MCH (pg)	**2&3**	**296**	**28.2**	**20.8**	**33.2**	**20.8-33.2**	**12.4**	0.428	ρ = 0.669
	2	124	28.4	22.5	33.5	22.5-33.5	11		
	3	172	28.1	19.6	33.7	19.6-33.7	14.1		
MCHC (g/dL)	**2&3**	**296**	**33.8**	**28**	**36.5**	**28-36.5**	**8.5**	1.337	ρ = 0.181
	2	124	33.6	23	36.6	23-36.6	13.6		
	3	172	33.7	29.2	36.5	29.2-36.5	7.3		
MCV (fL)	**2&3**	**296**	**82.9**	**61.9**	**94.8**	**61.9-94.8**	**32.9**	0.643	ρ = 0.52
	2	124	82.7	29.5	94.8	29.5-94.8	65.3		
	3	172	82.9	61.5	93.4	61.5-93.4	31.9		
RDW-SD (%)	**2&3**	**296**	**45.1**	**38**	**69.5**	**38-69.5**	**31.5**	1.851	ρ = 0.640
	2	124	46	39.2	72.6	39.2-72.6	33.4		
	3	172	45.2	37.8	65.5	37.8-65.5	27.7		
RDW-CV (%)	**2&3**	**296**	**12.8**	**11.27**	**20.85**	**11.3-20.9**	**9.6**	1.104	ρ = 0.269
	2	124	13.1	11.12	23	11.1-23	11.9		
	3	172	12.8	11.33	20.3	11.3-20.3	9		
WBC ($x10^9$/L)	**2&3**	**296**	**7.8**	**4.4**	**15.1**	**4.4-15.1**	**10.7**	0.581	ρ = 0.56
	2	124	7.7	4.4	15.3	4.4-15.3	10.9		
	3	172	7.8	4.5	14.6	4.5-14.6	10.1		
NEU ($x10^9$/L)	**2&3**	**296**	**5.2**	**2.3**	**12.5**	**2.3-12.5**	**10.2**	0.644	ρ = 0.52
	2	124	5.0	2.3	13	2.3-13	10.7		
	3	172	5.4	2.3	12.71	2.3-12.7	10.4		
NEU (%)	**2 & 3**	**296**	**67.4**	**39.1**	**85.9**	**39.1-85.9**	**46.8**	1.277	ρ = 0.201
	2	124	66.8	11.5	85.9	11.5-85.9	74.4		
	3	172	68.1	44.7	85.8	44.7-85.8	41.1		
LYM	**2&3**	**296**	**2.0**	**1.0**	**4.4**	**1-4.4**	**3.4**	0.180	ρ = 0.857

($x10^9$/L)	2	124	2.0	1.0	4.5	1-4.5	3.5		
	3	172	2.0	0.9	3.9	2-3.9	1.9		
LYM (%)	**2 & 3**	**296**	**25.8**	**10.8**	**44**	**10.8-44**	**33.2**	0.522	ρ = 0.602
	2	124	25.9	10.8	42.13	10.8-42.1	31.3		
	3	172	25.5	10.8	45	10.8-45	43.2		
MON ($x10^9$/L)	**2&3**	**296**	**0.3**	**0.12**	**1.03**	**0.12-1.03**	**0.91**	0.377	ρ = 0.880
	2	124	0.31	0.11	1.1	0.11-1.1	0.99		
	3	172	0.29	0.13	0.83	0.13-0.83	0.7		
MON (%)	**2 & 3**	**296**	**3.8**	**1.8**	**10.05**	**1.8-10.05**	**8.25**	0.147	ρ = 0.883
	2	124	3.6	1.81	10.41	1.81-10.41	8.6		
	3	172	3.8	1.83	9.34	1.83-9.34	7.51		
EOS ($x10^9$/L)	**2&3**	**296**	**0.14**	**0.07**	**0.63**	**0.07-0.63**	**0.56**	0.290	ρ = 0.770
	2	124	0.14	0.01	0.63	0.01-0.63	0.62		
	3	172	0.14	0.02	0.65	0.02-0.65	0.63		
EOS (%)	**2&3**	**296**	**1.8**	**0.3**	**6.21**	**0.3-6.21**	**5.91**	0.280	ρ = 0.88
	2	124	1.7	0.1	6	0.1-6	5.9		
	3	172	1.9	0.4	7.82	0.4-7.82	7.42		
BAS ($x10^9$/L)	2&3	296	0.03	0.01	0.08	0.01-0.08	0.07	2.966	**ρ = 0.030**
	2	**124**	**0.03**	**0.01**	**0.14**	**0.01-0.14**	**0.13**		
	3	**172**	**0.03**	**0**	**0.6**	**0.00-0.6**	**0.6**		
BAS (%)	2 & 3	296	0.4	0.1	0.94	0.1-0.94	0.84	2.676	**ρ = 0.0075**
	2	**124**	**0.5**	**0.2**	**0.99**	**0.2-0.99**	**0.79**		
	3	**172**	**0.4**	**0.1**	**0.9**	**0.1-0.90**	**0.80**		
PLT ($x10^9$/L)	**2&3**	**296**	**237**	**128**	**388.4**	**128-388**	**260**	1.646	ρ = 0.99
	2	124	228.5	103.1	387.9	103-388	285		
	3	172	239	128.3	396.8	128-397	269		
PCT (%)	**2&3**	**296**	**0.21**	**0.12**	**0.34**	**0.12-0.34**	**0.22**	1.403	ρ = 0.161
	2	124	0.2	0.10	0.33	0.10-0.33	0.23		
	3	172	0.21	0.12	0.35	0.12-0.35	0.23		
PDW (%)	**2&3**	**296**	**45.1**	**38**	**69.5**	**38-69.5**	**31.5**	3.145	ρ = 0.64
	2	124	46	39.2	72.6	39.2-72.6	33.4		
	3	172	45.2	37.8	65.6	37.8-65.6	27.8		
MPV (fL)	**2&3**	**296**	**8.9**	**7.5**	**11**	**7.5-11**	**3.5**	0.918	ρ = 0.354
	2	124	8.9	7.5	11.4	7.5-11.4	3.9		
	3	172	8.9	7.1	11	7.1-11	3.9		

Results are expressed as Median and range for the number of referent participants in the column labelled N. Statistical comparisons of the median values between the second and third trimesters were carried out using the Mann-Whitney U test. Differences were considered statistically significant at $\rho < 0.05$.

4.7.2 Comparison of developed trimester-specific reference intervals of hematological parameters for pregnant women of Taita-Taveta County, Kenya, with those reported in the literature

A comparison of developed reference intervals for hematological parameters for pregnant women in their second and third trimesters of Taita-Taveta County, Kenya population with those reported in medical literature are presented in Table 4.17. Results indicate that this study's trimester-independent lower reference interval limits for **RBC** ($x10^{12}$/L) for pregnant women in their second and third trimester is similar to that of the northwestern Moroccan (Bakrim *et al.,* 2018) population, higher than that of the western Kenya (Odhiambo *et al*., 2017) and African women (Mwinga et al., 2009) populations, but lower than that of western Indian (Purohit *et al*., 2015), northwest Ethiopia (Genetu *et al.,* 2017), and Australian (Balloch and Cauchi, 1993) populations. The upper limit is higher than that of the northwestern Moroccans (Bakrim *et al.,* 2018), western Kenya (Odhiambo *et al*., 2017), North West Ethiopia (Genetu *et al.,* 2017), and Australian (Balloch and Cauchi, 1993) populations, lower than that of the western Indian (Purohit *et al*., 2015), and lower and similar to that of African women (Mwinga *et al.,* 2009) population.

This study's trimester independent lower reference interval limits for HB (g/dL) for pregnant women in their second and third trimester is similar to that of the western Indian (Purohit *et al*., 2015) population, lower than that of the northwestern Moroccan (Bakrim *et al.,* 2018), African women (Mwinga *et al*.,

2009), and Australian (Balloch and Cauchi, 1993) and lower and similar to that of north West Ethiopia (Genetu *et al.,* 2017) populations, and higher than that of the western Kenya (Odhiambo *et al*., 2017), and Lagos Nigerian (Akinbami *et al.,* 2013) population. The upper limit was higher than that of the western Indian (Purohit *et al*., 2015), North West Ethiopia (Genetu *et al.,* 2017), African Women (Mwinga *et al.,* 2009), western Kenya (Odhiambo *et al*., 2017), northwestern Moroccan (Bakrim *et al.*, 2018), and Australian (Balloch and Cauchi, 1993), and similar and higher to that of Lagos Nigerian (Akinbami *et al.,* 2013) populations.

This study's trimester-dependent lower reference interval limit for PCV (%) for pregnant women in their second and third trimesters is lower than that of western Kenya (Odhiambo *et al*., 2017), western India (Purohit *et al*., 2015), northwestern Moroccan (Bakrim *et al.,* 2018), and Australian (Balloch and Cauchi, 1993) populations, while the upper limit is higher than that of western Kenya (Odhiambo *et al*., 2017), western Indian, Australian (Balloch and Cauchi, 1993), and northwestern Moroccan (Bakrim *et al.,* 2018) populations.

This study's trimester-dependent lower reference interval limit for MCH (pg) for pregnant women in their second and third trimesters is similar to that of the western Indian (Purohit *et al*., 2015) population, lower than that of Lagos Nigerian (Akinbami *et al.,* 2013), North West Ethiopia (Genetu et al., 2017), western Kenya (Odhiambo *et al*., 2017), northwestern Moroccan (Bakrim *et al.,* 2018), Australian (Balloch and Cauchi, 1993) populations. The upper limit is similar to that of the northwestern Moroccan (Bakrim *et al.*, 2018), western

Indian (Purohit *et al*., 2015) and Australian (Balloch and Cauchi, 1993) populations but lower than that of the North West Ethiopia (Genetu *et al*., 2017), western Kenya (Odhiambo *et al*., 2017), and lower and higher to that of Lagos Nigerian (Akinbami *et al.*, 2013) population.

For MCHC (g/dL), this study's lower reference interval limit for pregnant women in their second and third trimesters is lower than that of the Lagos Nigerian (Akinbami *et al.*, 2013), northwestern Moroccan (Bakrim *et al.*, 2018) and Australian (Balloch and Cauchi, 1993) populations, but higher than that of African women (Mwinga *et al.*, 2009), and western Indian (Purohit *et al*., 2015) population. The upper limit is similar to that of the northwestern Moroccan (Bakrim *et al.*, 2018), and Australian (Balloch and Cauchi, 1993) populations, but lower than that of the western Indian (Purohit *et al*., 2015), and African women (Mwinga *et al.*, 2009), and similar and higher than that of Lagos Nigerian (Akinbami *et al.*, 2009) population.

For MCV (fL), this study's trimester independent lower reference interval limit for pregnant women in their second and third trimesters is lower than that of the western Indian (Purohit *et al*., 2015), North West Ethiopia (Genetu *et al.*, 2017), northwestern Moroccan (Bakrim *et al.*, 2018), and Australian (Balloch and Cauchi, 1993), and lower and higher than that of Lagos Nigerian (Akinbami *et al.*, 2013) populations. The upper limit is lower than that of Australian women (Balloch and Cauchi, 1993), North West Moroccan women (Bakrim *et al.*, 2018), western Indian women (Purohit *et al*., 2015), and western Kenya women (Odhiambo *et al*., 2017), higher than that of Lagos

Nigerians (Akinbami *et al.,* 2013), and similar to that of North West Ethiopian (Genetu *et al.,* 2017) populations.

For RDW-CV (%), this study's trimester-independent lower reference interval limit for pregnant women in their second and third trimesters is higher and similar, respectively, to that of the western Indian (Purohit *et al*., 2015) population, while the upper limit is similar and higher, respectively, to this same Indian (Purohit *et al*., 2015) population.

Further, for **WBC** and differential white blood cells, this study's trimester independent lower reference interval limit for WBC ($x10^9$/L) for pregnant women in their second and third trimester is lower than that of the Israel women (Lurie *et al.,* 2008), north west Ethiopia (Genetu *et al.,* 2017), northwestern Moroccan (Bakrim *et al.,* 2018), western Indian (Purohit *et al*., 2015), and Australian (Balloch and Cauchi, 1993) populations, but higher than that of the African women (Mwinga *et al.,* 2009), Lagos Nigerian (Akinbami *et al.,* 2013), and western Kenya (Odhiambo *et al*., 2017) population, The upper limit is higher than that of the Lagos Nigerian (Akinbami *et al.,* 2013), Israel women (Lurie *et al.,* 2008), African women (Mwinga *et al.,* 2009), north west Ethiopians (Genetu *et al.,* 2017), northwestern Morocco (Bakrim *et al.,* 2018), western Kenya (Odhiambo *et al*., 2017), and western Indian (Purohit *et al*., 2015) populations; however, for the Australian (Balloch and Cauchi, 1993) population, this study's upper limit for WBC ($x10^9$/L) for pregnant women in their second trimester is lower while that in the third trimester is higher.

For NEU ($x10^9$/L), this study's trimester independent lower reference interval limit for pregnant women is similar to northwestern Moroccans (Bakrim *et al.,* 2018) in their second trimester but lower than that of Israel (Lurie *et al.,* 2008), northwestern Moroccans (Bakrim *et al.,* 2018) in their third trimester, higher than that of western Kenya (Odhiambo *et al*., 2017), and lower than that of Australian (Balloch and Cauchi, 1993) population. The upper limit is higher than that of Israel (Lurie *et al.,* 2008), northwestern Morocco (Bakrim *et al.,* 2018), and western Kenya (Odhiambo *et al*., 2017) populations but similar to that of Australians (Balloch and Cauchi, 1993) in their second trimester and lower than that of Australians (Balloch and Cauchi, 1993) in their third trimester. For NEU (%), this study's trimester independent lower reference interval limit for pregnant women in their second and third trimesters is lower than that of Israel (Lurie *et al.*, 2008) and west India (Purohit *et al*., 2015) populations, while the upper limit higher than that of Israel (Lurie *et al.,* 2008) and lower than that of west India (Purohit *et al*., 2015) populations.

For LYM ($x10^9$/L), this study's lower reference interval limit for pregnant women in their second and third trimesters is similar to that of Israel (Lurie *et al.,* 2008), northwestern Morocco (Bakrim *et al.,* 2018), western Kenya (Odhiambo *et al*., 2017), and Australian (Balloch and Cauchi, 1993) populations. The upper limit is higher than that of Israel (Lurie *et al.,* 2008), northwestern Morocco (Bakrim *et al.,* 2018), western Kenya (Odhiambo *et al*., 2017), and Australian (Balloch and Cauchi, 1993) populations. For LYM (%), this study's trimester independent lower reference interval limit for pregnant

women in their second and third trimesters is higher than that of west India (Purohit *et al*., 2015), and lower than that of Israel (Lurie *et al.,* 2008) and African (Mwinga *et al.,* 2009) populations, while the upper limit higher than that of Israel (Lurie *et al.,* 2008), and west India (Purohit *et al*., 2015), and lower than that of African women (Mwinga *et al.,* 2009) populations.

For MON ($x10^9$/L), this study's trimester independent lower reference interval limit for pregnant women is lower than that of Africans (Mwinga *et al.,* 2009), lower and similar to that of Israel (Lurie *et al.,* 2008), similar to that of northwestern Moroccan (Bakrim *et al.,* 2018), western Kenya (Odhiambo *et al*., 2017), and Australian (Balloch and Cauchi, 1993) population. The upper limit is similar to that of northwestern Moroccan (Bakrim *et al.,* 2018), higher than that of western Kenya (Odhiambo *et al*., 2017), and lower than that of the African (Mwinga *et al.,* 2009), Israel (Lurie *et al.,* 2008), and Australian (Balloch and Cauchi, 1993) population. For MON (%), this study's trimester independent lower reference interval limit in their second and third trimester is lower than that of Israel (Lurie *et al.,* 2008), and higher than that of west India (Purohit *et al*., 2015) population, while the upper limit is higher than that of Israel (Lurie *et al.,* 2008), and west India (Purohit *et al*., 2015) population.

For EOS ($x10^9$/L), this study's trimester independent lower reference interval limit for pregnant women is higher than that of Israel (Lurie *et al.,* 2008), northwestern Moroccan (Bakrim *et al.,* 2018), western Kenya (Odhiambo *et al*., 2017), and Australian (Balloch and Cauchi, 1993) populations, while the upper limit is similar to that of the Australian (Balloch and Cauchi, 1993),

higher than that of northwestern Morocco (Bakrim *et al.,* 2018), and lower than that of Israel (Lurie *et al.,* 2008), and western Kenya (Odhiambo *et al*., 2017) population. For EOS (%), this study's lower reference interval limit for pregnant women in their second and third trimesters is higher than that of Israel (Lurie *et al.,* 2008), and lower than that of west India (Purohit *et al*., 2015) population, while the upper limit is higher than that of Israel (Lurie *et al.,* 2008), and higher and similar to that of west India (Purohit *et al*., 2015) population.

For BAS ($x10^9$/L), this study's trimester-dependent lower reference interval limit for pregnant women is higher than that of (trimester independent) Israel (Lurie *et al.,* 2008), similar to that of northwestern Morocco (Bakrim *et al.,* 2018) and Australian (Balloch and Cauchi, 1993) populations. The upper limit is higher than that of Israel (Lurie *et al.,* 2008), northwestern Moroccans (Bakrim *et al.,* 2018) and Australians. For BAS (%), this study's trimester-dependent lower and upper reference interval limit for pregnant women in their second and third trimesters are higher than that of the trimester-independent Israel (Lurie *et al.,* 2008) population.

In addition, for PLT ($x10^9$/L), this study's trimester independent lower reference interval limit for pregnant women is higher than that of western Kenya (Odhiambo *et al*., 2017), [Lagos Nigerian (Akinbami *et al.,* 2013), African women (Mwinga *et al.,* 2009)], but lower than that of northwestern Morocco (Bakrim *et al.,* 2018), Australian (Balloch and Cauchi, 1993), [north west Ethiopia (Genetu *et al.,* 2017)] and western Indian populations. The **uppe**r

limit is lower than that of western Kenya (Odhiambo *et al*., 2017), and Australian (Balloch and Cauchi, 1993) populations, similar and h**igh**er for western India (Purohit *et al*., 2015), and higher than that of Lagos Nigerians (Akinbami *et al.,* 2013), northwest Ethiopians (Genetu *et al.,* 2017), and African women (Mwinga *et al.,* 2009) populations in their second and third trimester, respectively. For MPV (fL), this study's trimester-independent lower and upper reference interval limits for pregnant mothers is lower than that of the northwestern Moroccans (Bakrim *et al.,* 2018).

Table 4.17: Comparison of developed trimester-specific reference intervals of hematological parameters for pregnant women of Taita-Taveta County, Kenya, with those reported in the literature

Analyte (unit)	Gender	This study RI	Northwest Morocco	Western Kenya	Western India	Australian population	**La**gos Nigerians	**Nor**thwestern Ethiopians	**Af**rican women	**Is**rael women
RBC (x10^{12}/L)	*2&3*	*3.2-5.2*						*4.30-4.44*		
	2	3.2-5.2	*3.26-4.82*	*2.8-4.8*	*3.6-4.5*	3.43-4.49*			2.65-4.92*	
	3	3.2-5.3	*3.19-4.78*	*2.8-4.9*	*0.6-7.7*	3.38-4.43			2.87-5.34	
HB (g/dL)	*2&3*	*7.8-14.3*						*12.99-13.36*		
	2	7.9-14.3	*9.6-13.6*	6.1-13.0*	*8.0-12.1*	*10.6-13.3*	7.44-14.18*		8.2-13.2*	
	3	7.7-14.5	*9.1-13.4*	5.5-12.7	*7.6-12.5*	*10.4-13.5*	7.89-12.87		9.0-13.95	
PCV (%)	2 &3	0.3-34.4								
	2	0.3-71.2*	28.6-39.9*	21.4-38.1*	27.1-38.7	*31-39*	19.55-39.97*	39.63-41.14*	23.8-39.4*	
	3	0.3-45	27.3-39.3	17.7-38.6	27.3-40.1	*31-40*	25.44-40.64	41.17-42.75	25.9-41.85	
MCH (pg)	*2&3*	*20.8-33.2*						*28.88-34.81*		
	2	22.5-33.5	*24.0-33.3*		*20.5-33.5*	*27-33*	*23.73-33.53*			
	3	19.6-33.7	*23.0-33.4*		*22.4-32.3*	*28-33*	*24.42-31.94*			
MCHC (g/dL)	*2&3*	*28-36.5*						*31.91-33.37*		
	2	23-36.6	*31.2-36.6*		*29.1-36.9*	*33-36*	34.37-38.61*			
	3	29.2-36.5	*30.8-36.2*		*17.4-43.5*	*33-36*	29.87-32.81			
MCV (fL)	*2&3*	*61.9-94.8*						*93.33-94.63*		
	2	29.5-94.8	*74.7-97.7*	*58-101*	*66.5-99.3*	*83-96*	67.17-89.59*			
	3	61.5-93.4	*72.8-96.1*	*59-99*	*58.5-112.9*	*85-97*	59.44-80.60			
RDW-CV (%)	*2&3*	*11.3-20.9*								
	2	11.1-23			10.3-19.8*					
	3	11.3-20.3			11.4-26.5					
RDW-SD (%)	*2&3*	*38-69.5*								
	2	39.2-72.6								
	3	37.8-65.5								
WBC (x10^{9}/L)	*2&3*	*4.4-15.1*						*8.60-9.61*		
	2	4.4-15.3	4.6-12.6*	3.6-11.1*	4.9-14.5*	6.2-14.8*	3.33-12.43*		*3.3-11.1*	5.22-12.20*
	3	4.5-14.6	5.3-14.3	3.3-10.7	6.0-14.3	5.9-16.9	4.10-12.52		*3.8-11.2*	5.12-13.20
NEU (x10^{9}/L)	2&3	*2.3-12.5*								
	2	2.3-13	2.2-9.2*	1.9-7.1*		3.8-12.3*				3.37-9.05*
	3	2.3-12.7	3.0-11.0	2.0-5.7		3.9-13.1				3.26-9.76
NEU (%)	2&3	*39.1-85.9*								
	2	11.5-85.9			*59.6-87.9*					*62.9-79.5*
	3	44.7-85.8			*52.9-88.4*					*61.7-79.7*
LYM (x10^{9}/L)	2&3	*1-4.4*						*2.11-2.33*		

	2	1-4.5	1.2-3.6*	0.9-3.3*		0.9-3.9*				0.85-2.69*
	3	2-3.9	1.1-3.8	1.2-3.8		1.0-3.6				1.01-2.75
LYM (%)	2&3	*10.8-44*								
	2	10.8-42.1			*8.1-31.2*				*19-57*	*11.2-30.8*
	3	10.8-45			*6.5-33.2*				*18.1-53.6*	*12.5-30.1*
MON (x10^9/L)	2&3	*0.12-1.03*								
	2	0.11-1.1	*0.2-1.0*	*0.1-0.9*		0.1-1.1*			1.7-17*	0.163-0.705*
	3	0.13-0.83	*0.1-1.0*	*0.1-0.8*		0.1-1.4			1.1-23.1	0.120-0.874
MON (%)	2&3	*1.8-10.05*								
	2	1.81-10.41			*1.8-5.0*					2.2-7.8
	3	1.83-9.34			*1.2-5.6*					2-9
EOS (x10^9/L)	2&3	*0.07-0.63*								*0-0.326*
	2	0.01-0.63	*0-0.4*	*0-0.9*		*0-0.6*				
	3	0.02-0.65	*0-0.4*	*0-1.0*		*0-0.6*				
EOS (%)	2&3	*0.3-6.21*								*0-3.9*
	2	0.1-6			*1.4-5.8*					
	3	0.4-7.82			*0.9-6.3*					
BAS (x10^9/L)	2&3	0.01-0.08								
	2	0.01-0.14*	*0-0.1*	0.01-0.12*		*0-0.1*				*0.003-0.067*
	3	0-0.60	*0-0.1*	0.01-0.09		*0-0.1*				*0.006-0.068*
BAS (%)	2&3	0.1-0.94								
	2	0.2-0.99*								*0.04-0.80*
	3	0.1-0.90								*0.07-0.77*
PLT (x10^9/L)	**2&3**	*128-388*						*221-240*		
	2	103-388	140-364*	98-395*	234-390*	171-409*	104-351*		97-350*	
	3	128-397	139-398	105-425	170-338	155-429	15.8-385.8		86.5-344.5	
PCT (%)	**2&3**	*0.12-0.34*								
	2	0.10-0.33								
	3	0.12-0.35								
PDW (%)	2&3	*38-69.5*								
	2	39.2-72.6								
	3	37.8-65.6								
MPV (fL)	2&3	*7.5-11*								
	2	7.5-11.4	*8.9-13.5*							
	3	7.1-11	*8.9-13.2*							

Australian population by Balloch and Cauchi (1993), Israel population by Lurie et al. (2008), African women (Malawians, Tanzanians & Zambians) population by Mwinga et al. (2009), Lagos Nigerians by Akinbami et al. (2013), Western India population by Purohit et al. (2015), Northwest Ethiopians by Genetu et al. (2017), Western Kenya population by Odhiambo et al. (2017), and Northwest Morocco population by Bakrim et al. (2018). *Italicized* values represent trimester-independent reference intervals; *stared** values represent trimester-dependent reference intervals.

4.8. Reference intervals for serum biochemistry analytes for pregnant mothers of Taita-Taveta county, Kenya

4.8.1 Established reference intervals for liver and kidney tests and electrolytes for pregnant mothers of Taita-Taveta County, Kenya

The established reference intervals for liver and kidney tests and electrolytes for expectant mothers of Taita-Taveta County, Kenya, are presented in Table 4.18. Results indicate that the established reference intervals for liver and kidney tests and electrolytes for pregnant mothers of Taita-Taveta County, Kenya in their second trimester for TP, ALB, ALT, AST, GGT, BUN, K, CL, and CA were similar to those for pregnant mothers in their third trimester ($\rho > 0.05$); hence combined reference intervals for these parameters were established. The combined (second & third trimesters) established reference intervals for liver and kidney tests. Electrolytes for pregnant mothers of Taita-Taveta County, Kenya, for TP is 67.6 (55.6-72.2) g/L, ALB is 38.5 (29.1-46.7) g/L, ALT is 10.6 (3.8-42.4) U/L, AST is 18 (9.7-41.9) U/L, GGT is 9.1 (1.7-79.9) U/L, BUN is 1.8 (0.8-4.1) mmol/L, K is 4.3 (3.6-5.2) mmol/L, CL is 99.5 (89.9-105.7) mmol/L, and CA is 2 (0.1-2.1) mmol/L. Results also indicate that the established reference intervals for liver and kidney tests and electrolytes for pregnant mothers of Taita-Taveta County, Kenya in their second trimester for ALP, T-BIL, D-BIL, CREAT, UA, and NA significantly differed from those of pregnant mothers in their third trimester of the same County ($\rho < 0.05$). The established reference interval for liver and kidney tests and electrolytes for these pregnant mothers of Taita Taveta County, Kenya for ALP is 3.1 (0-

133.9) U/L in their second trimester and 1.9 (0-165.8) U/L in the third trimester, T-BIL is 2.4 (0-14.5) µmol/L in their second trimester and 3.5 (0.4-17) µmol/L in their third trimester, D-BIL is 0.9 (0-3.1) µmol/L in their second trimester and 0.8 (0.1-6.5) µmol/L in their third trimester, CREAT is 48 (29.6-113.4) µmol/L in their second trimester and 52.5 (36.3-89) µmol/L in their third trimester, UA is 0.22 (0.18-0.35) µmol/L in their second trimester and 0.21 (0.15-0.32) µmol/L in their third trimester, and NA is 133.7 (126.3-140.6) mmol/L in their second trimester and 132 (121.9-139.9) mmol/L in their third trimester (Table 4.18).

Table 4.18: Established reference intervals for liver and kidney tests, and electrolytes for expectant mothers population of Taita-Taveta County, Kenya

Analyte (unit)	Trimester	N		Percentile		Reference Interval	IV	Difference between second and third trimisters	
			Median	2.5^{th}	97.5^{th}			Z value	Sig
TP (g/L)	2&3	**296**	**67.6**	**55.6**	**72.2**	**55.6-72.2**	**16.6**	0.667	ρ = 0.5048
	2	124	67.3	48.2	76.5	48.2-76.5	28.3		
	3	172	67.7	58.2	76.2	58.2-76.2	18.0		
ALB (g/L)	2&3	**296**	**38.5**	**29.1**	**46.7**	**29.1-46.7**	**17.6**	1.493	ρ = 0.1353
	2	124	38.8	27.7	46.9	27.7-46.9	19.2		
	3	172	38.1	31.9	46	31.9-46.0	14.1		
ALT (U/L)	2&3	**296**	**10.6**	**3.8**	**42.4**	**3.8-42.4**	**37.8**	1.229	ρ = 0.219
	2	172	11	4.6	30.9	4.6-30.9	26.3		
	3	124	10	3.3	52	3.3-52	48.7		
AST (U/L)	2&3	**296**	**18**	**9.7**	**41.9**	**9.7-41.9**	**32.2**	0.886	ρ = 0.376
	2	172	18.3	10.3	42.6	10.3-42.6	32.3		
	3	125	21.9	9.31	91.2	9.3-91.2	81.9		
ALP (U/L)	2&3	296	2.1	0	126.3	0-126.3	126.3	2.148	**ρ = 0.031**
	2	**124**	**3.1**	**0**	**133.9**	**0-133.9**	**133.9**		
	3	**172**	**1.9**	**0**	**165.8**	**0-165.8**	**165.8**		
GGT (U/L)	2&3	**296**	**9.1**	**1.7**	**79.9**	**1.7-79.9**	**78.2**	0.750	ρ = 0.657
	2	124	9.1	1.6	115.9	1.6-115.9	114.3		
	3	172	10	1.7	68.5	1.7-68.5	66.8		
T-BIL (µmol/L)	2&3	**296**	2.6	0	14.3	0-14.3	14.3	2.322	**ρ = 0.0202**
	2	**172**	**2.4**	**0**	**14.5**	**0-14.5**	**14.5**		
	3	**124**	**3.5**	**0.4**	**17**	**0.4-17**	**16.6**		

D-BIL (µmol/L)	2&3	296	0.8	0	3.6	0-3.6	3.6	1.690	**ρ = 0.005**
	2	**172**	**0.9**	**0**	**3.1**	**0-3.1**	**3.1**		
	3	**124**	**0.8**	**0.1**	**6.5**	**0.1-6.5**	**6.4**		
CREAT (µmol/L)	2&3	**296**	51	34	95	34-95	61	2.802	**ρ = 0.05**
	2	**124**	**48**	**29.6**	**113.4**	**29.6-113.4**	**83.8**		
	3	**172**	**52.5**	**36.3**	**89**	**36.3-89**	**52.7**		
BUN (mmol/L))	2&3	**296**	**1.86**	**0.8**	**4.1**	**0.8-4.1**	**3.3**	1.341	ρ = 0.1798
	2	124	1.9	0.9	4.3	0.9-4.3	3.4		
	3	172	1.8	0.7	4.1	0.7-4.1	3.4		
UA (µmol/L)	2&3	**296**	0.21	0.16	0.35	0.16-0.35	0.19	3.142	**ρ = 0.0017**
	2	**124**	**0.22**	**0.18**	**0.35**	**0.18-0.35**	0.17		
	3	**172**	**0.21**	**0.15**	**0.32**	**0.15-0.32**	0.17		
K (mmol/L)	2&3	**296**	**4.3**	**3.6**	**5.2**	**3.6-5.2**	**1.6**	1.483	ρ = 0.138
	2	124	4.4	3.6	5.2	3.6-5.2	1.6		
	3	172	4.3	3.6	5.3	3.6-5.3	1.7		
NA (mmol/L)	2&3	296	132.8	126.2	140.5	126.2-140.5	14.3	4.021	**ρ = 0.001**
	2	**124**	**133.7**	**126.3**	**140.6**	**126.3-140.6**	**14.3**		
	3	**172**	**132.0**	**121.9**	**139.9**	**121.9-139.9**	**18.0**		
CL (mmol/L)	2&3	**296**	**99.5**	**89.9**	**105.7**	**89.9-105.7**	**15.8**	0.553	ρ = 0.580
	2	172	99	89.9	104.7	89.9-104.7	14.8		
	3	124	99	89.9	107.9	89.9-107.9	18.0		
CA (mmol/L)	2&3	**296**	**2**	**0.1**	**2.1**	**0.1-2.1**	**2.0**	0.851	ρ = 0.395
	2	124	2	0.1	2.1	0.1-2.1	2.0		
	3	172	2	0.1	2.1	0.1-2.1	2.0		

Results are expressed as Median and range for the number of referent participants in the column labelled N. Statistical comparisons of the median values between second and third trimesters referent participants were carried out using the Mann-Whitney U test. Differences were considered statistically significant at $\rho < 0.05$.

4.8.2 Comparison of developed reference intervals for liver and kidney tests, and electrolytes for pregnant women of Taita-Taveta County, Kenya with those reported in medical literature

A comparison of developed reference intervals for liver and kidney tests, and electrolytes for pregnant women of Taita-Taveta County, Kenya, with those reported in medical literature are presented in Table 4.19 For the liver function tests, this study's combined lower reference interval limit for TP for pregnant women is similar to that reported by Abbassi-Ghanavati *et al.* (2009) for Americans, and higher than that of the Denmark population, while the upper limit is higher than that reported for both populations. This study's trimester-independent lower and upper reference interval limit for ALB is higher than the trimester-dependent reference intervals reported by Abbassi-Ghanavati *et al.* (2009) for Americans.

This study's lower reference interval limit for ALT for pregnant women is higher than that of western Kenya (Odhiambo *et al.,* 2017) population and that reported by Abbassi-Ghanavati *et al.* (2009) for Americans, but lower than that for the Denmark population, while the upper limit is higher than the trimester dependent limits for western Kenya (Odhiambo *et al.,* 2017), and Denmark populations and that reported by Abbassi-Ghanavati *et al.*,(2009) for Americans. This study's lower reference interval limit for AST for pregnant women is higher than the trimester-dependent limits for western Kenya (Odhiambo *et al.,* 2017) and Denmark populations, while the upper limit is higher than that of the trimester-dependent Denmark population and lower and

higher than that of western Kenya (Odhiambo *et al.,* 2017) population in the second and third trimester, respectively. This study's trimester-dependent lower reference interval limit for ALP for pregnant women is lower than that of the trimester-dependent limits for the Denmark population and that reported by Abbassi-Ghanavati *et al.,* (2009) for Americans, while the upper limit is lower than that of the trimester dependent Denmark population, and higher and lower than that reported by Abbassi-Ghanavati *et al.,* (2009) for Americans for the second and third trimester, respectively. This study's trimester-independent lower reference limit for GGT for pregnant women is higher than the trimester-dependent limit reported by Abbassi-Ghanavati *et al.,* (2009) for Americans, while the upper trimester-dependent limits are lower.

For T-BIL, this study's trimester-dependent lower reference interval limit for pregnant women is lower than the trimester-dependent limit of western Kenya (Odhiambo *et al.,* 2017), and that reported by Abbassi-Ghanavati *et al,.* (2009) for Americans, and the trimester independent limit for the Denmark population, while the upper limit is lower than the trimester dependent limit for western Kenya (Odhiambo *et al.,* 2017) population, higher than the trimester independent limit for Denmark population, and higher and lower than that reported by Abbassi-Ghanavati *et al.,* (2009) for Americans for the second and third trimester, respectively. For D-BIL, this study's trimester-dependent lower reference interval limit is similar to the trimester-independent limit reported by Abbassi-Ghanavati *et al.,* (2009) for Americans, while the upper limit is higher.

For the kidney function tests, this study's trimester-dependent lower reference interval for CREAT for pregnant mothers is higher than that of western Kenya (Odhiambo *et al.,* 2017), the trimester-independent limit for the Denmark populations, and lower and higher than the trimester-dependent limits reported by Abbassi-Ghanavati *et al.,* (2009) for Americans for the second and third trimester, respectively, while the upper limit is higher than that of western Kenya (Odhiambo *et al.,* 2017) and Denmark populations and that reported by Abbassi-Ghanavati *et al.*, (2009) for Americans.

For BUN, this study's trimester independent lower reference interval limit is lower than the trimester independent limit for Denmark population and the trimester dependent limits reported by Abbassi-Ghanavati *et al.,* (2009) for Americans, while the upper limit is lower than that of the Denmark population, and lower and similar to that reported by Abbassi-Ghanavati *et al.,* (2009) for Americans for the second and third trimester, respectively. This study's trimester dependent lower reference interval limit for UA for pregnant mothers is lower than that of Denmark population, and that reported by Abbassi-Ghanavati et al., (2009) for Americans, while the upper limit is lower than the trimester dependent limit of the Denmark population, and that reported by Abbassi-Ghanavati *et al.*, (2009) for Americans.

For electrolytes, this study's trimester independent lower reference interval limit is higher than that of the Denmark population and that reported by Abbassi-Ghanavati *et al.,* (2009) for Americans, while the upper limit is higher

than that of the Denmark population but similar to those reported by Abbassi-Ghanavati *et al.,* (2009) for Americans. For NA, this study's trimester-dependent lower reference interval limit is lower than the trimester-independent limit of the Denmark population. That was reported by Abbassi-Ghanavati *et al.,* (2009) for Americans, while the upper limit is similar to that of the Denmark population, and lower than that reported by Abbassi-Ghanavati *et al.,* (2009). For CL, this study's trimester independent lower and upper reference interval limits are lower than that reported by Abbassi-Ghanavati *et al.,* (2009) for Americans. For CA, this study's trimester-independent lower reference interval limit is lower than that reported by Abbassi-Ghanavati *et al*., (2009) for Americans, while the upper limit is lower (Table 4.19).

Table4.19: Comparison of developed reference intervals for liver and kidney tests and electrolytes for pregnant women of Taita-Taveta County, Kenya with those reported in medical literature

Analyte	Trimester	This study RI	Western Kenya population	Denmark population	American population
TP (g/L)	2&3	**55.6-72.2**		30.2-40.5	56.0-59.0
	2	48.2-76.5			
	3	58.2-76.2			
ALB (g/L)	2&3	**29.1-46.7**			
	2	27.7-46.9			26.0-45.0
	3	31.9-46.0			23.0-42.0
ALT (U/L)	2 &3	**3.8-42.4**			
	2	4.6-30.9	0.0-26.0	9.0-32.4	2.0-33.0
	3	3.3-52	0.0-37.0	8.4-36.0	2.0-25.0
AST (U/L)	2&3	**9.7-41.9**			3.0-33.0
	2	10.3-42.6	0.0-39.0	16.2-34.2	
	3	9.3-91.2	0.0-44.0	0.0-40.2	
ALP (U/L)	2 &3	0-126.3			
	2	**0-133.9***		43.8-119.4	25.0-126.0
	3	**0-165.8**		56.4-159.6	38.0-229.0
GGT	2&3	**1.7-79.9**			

(U/L)	2	1.6-115.9			4.0-22.0
	3	1.7-68.5			3.0-26.0
T-BIL (µmol/L)	2 &3	0-14.3		**2-12**	
	2	**0-14.5***	1.4-26.3		1.7-13.7
	3	**0.4-17**	2.2-22.1		1.7-18.8
D-BIL (µmol/L)	2&3	0-3.6			**0-1.7**
	2	**0-3.1***			
	3	**0.1-6.5**			
CREAT (µmol/L)	2 &3	34-95		**42-74**	
	2	**29.6-113.4***	0-59		35-71
	3	**36.3-89**	0-67		35-80
BUN (mmol/L)	2&3	**0.8-4.1**		**1.7-4.3**	
	2	0.9-4.3			1.1-4.6
	3	0.7-4.1			1.1-3.9
UA (µmol/L)	2&3	0.16-0.35			
	2	**0.18-0.35***		**0.71-1.76**	0.85-1.73
	3	**0.15-0.32**		0.74-1.96	1.09-2.23
K (mmol/L)	2&3	**3.6-5.2**		**3.2-4.2**	**3.3-5.1**
	2	3.6-5.2			
	3	3.6-5.3			
NA (mmol/L)	2 &3	126.2-140.5		135-142	129-148
	2	**126.3-140.6***			
	3	**121.9-139.9**			
CL (mmol/L)	2 &3	**89.9-105.7**			**97-109**
	2	89.9-104.7			
	3	89.9-107.9			
CA (mmol/L)	2 &3	**0.1-2.1**			
	2	0.1-2.1			1.1-1.25
	3	0.1-2.1			1.1-1.33

Western Kenya population by Odhiambo *et al.*, (2017), the Denmark population by Klajnbard *et al.*,(2010), American population by Abbassi-Ghanavati *et al,*. (2009).

4.9 Reference intervals for hematological parameters for adults and geriatric population of Taita-Taveta county, Kenya

4.9.1 Results of the developed median reference interval limits for hematological parameters of adults and geriatrics of Taita-Taveta County, Kenya

The established median reference interval limits for hematological parameters for the adult and geriatric male population of Taita-Taveta County, Kenya for red blood cells (RBC $x10^{12}$/L), hemoglobin (HB g/dL), packed cell volume (PCV %), mean cell hemoglobin (MCH pg), mean cell hemoglobin concentration (MCHC g/dL), mean cell volume (MCV fL), red blood cell distribution width-standard deviation (RDW-SD %), red blood cell distribution width-coefficient variation (RDW-CV %), white blood cell (WBC $x10^9$/L), neutrophils (NEU $x10^9$/L), lymphocytes (LYM $x10^9$/L), monocytes (MON $x10^9$/L, %), absolute and percent eosinophils (EOS $x10^9$/L, %), absolute basophils (BAS $x10^9$/L, %), platelets (PLT $x10^9$/L), plateletcrit (PCT %), platelet distribution width (PDW %) and mean platelet volume (MPV fL) were similar to those of the female population of the same age range ($\rho > 0.05$). Therefore, combined reference interval limits of these parameters were developed for this population. The established combined median reference interval limits for adult and geriatric population of Taita-Taveta County, Kenya for red blood cell count (RBC) is 4 (3-6) x 10^9/L, hemoglobin (HB) is 13 (6.6-16.4) g/dL, packed cell volume (PCV) is 0 (0-45) %, mean corpuscular hemoglobin (MCH) is 29 (21-35) pg, mean corpuscular hemoglobin

concentration (MCHC) is 33 (27-36) g/dL, mean corpuscular volume (MCV) is 88 (70-106) fL, red cell distribution width-standard deviation (RDW-SD) is 49 (39-67) %, red cell distribution width-coefficient of variation (RDW-CV) is 13 (11-18.4) %, white blood cell count (WBC) is 6 (3-14.4) x 10^9/L, absolute neutrophils (NEU) is 3 (1-11) x 10^9/L), absolute lymphocytes (LYM) is 2 (1-5) x 10^9/L, absolute monocytes (MON) is 0 (0-1) x 10^9/L, percent monocytes (MON) is 4 (0-13.4) %, absolute basophils (BAS) is 0 (0-0.10) x 10^9/L, percent basophil (BAS) is 0 (0-5) %, eosinophils (EOS) is 0 (0-1) x 10^9/L, percent eosinophils (EOS) is 2 (0-12) %, platelets (PLT) is 238 (116.3-513) x 10^9/L, plateletcrit (PCT) is 0 (0-0.4) %, platelet distribution width (PDW) is 16 (7.6-16.9) fL, and mean platelet volume (MPV) is 9 (7-11) fL (Table 4.20).

The established median reference interval limits for the adult and geriatric male population of Taita-Taveta County, Kenya for percent neutrophils (NEU %), and percent lymphocytes (LYM %) significantly differ from that of the female population of the same County ($\rho < 0.05$). The established median reference interval limits for percent neutrophils (NEU %) for male adults and geriatrics (52 (28.55-88) %) with a mean rank of 166.41 is significantly higher than those of females (50.5 (24-92.25) %) with a mean rank of 138.95 ($U = 9463.5$, $z = -2.724$, $\rho = 0.006$, $r = 0.1562$) while the median reference interval limits for percent lymphocytes (LYM %) for male adults and geriatrics (37 (7-66.45) %) with a mean rank of 134.48 is significantly lower than those for females (41 (4.75-70.88) %) with mean rank of 170.05 ($U = 8847$, $z = -3.529$, $\rho = 0.000$, $r = 0.2024$) (Table 4.20).

Table 4.20:Established median reference interval limits for haematological parameters for the adult and geriatric population of Taita-Taveta County, Kenya

Analyte (Unit)	Sex	N	Median	Percentiles		Reference Interval	IV	Difference between M&F	
				2.5^{th}	97.5^{th}			Z value	Sig
RBC ($x10^{12}$/L)	M&F	304	4.47±0.82					-0.184	ρ = 0.854
			4	3	6	3-6	3		
	F	154	4.48±0.79						
			4	3	6	3-6	3		
	M	150	4.45±0.82						
			4	3	6	3-6	3		
HB (g/dL)	M&F	304	12.72±2.35					-1.773	ρ = 0.076
			13	6.63	16.38	6.6-16.4	9.8		
	F	154	12.66±2.09						
			13	8	17	8-17	9		
	M	150	12.79±2.58						
			14	6	17.23	6-17.2	11.2		
PCV (%)	M&F	304	3.12±11.09					-0.131	ρ = 0.896
			0	0	45	0-45	45		
	F	154	2.40±9.67						
			0	0	43	0-43	43		
	M	150							
			0	0	46.23	0-46.2	46.2		
MCH (pg)	M&F	304	28.53±3.46					-0.887	ρ = 0.375
			29	21	35	21-35	14		
	F	154	28.36±2.92						
			28	22	33	22-33	11		
	M	150							
			29	18	38	18-38	20		
MCHC (g/dL)	M&F	304	32.11±2.96					-0.946	ρ = 0.344
			33	27	36	27-36	9		
	F	154	32.32±2.14						
			33	28	36	28-36	8		
	M	150	32.18±2.90						
			33	26.55	36.23	26.6-36.2	9.6		
MCV (fL)	M&F	304	88.46±8.99					-0.289	ρ = 0.772
			88	70	106	70-106	36		
	F	154	87.75±8.15						

			87.5	69.75	104.25	69.8-104.3	34.5		
	M	150	88.61±9.29						
			89	69.33	106	69.3-106	36.7		
RDW-SD (%)	M&F	304	50.34±6.85					-0.137	ρ = 0.891
			49	39	67	39-67	28		
	F	154	49.77±7.07						
			49.5	39	71	39-71	32		
	M	150	50.22±6.24						
			49	40	64	40-64	24		
RDW-CV (%)	M&F	304	13.54±1.69					-0.213	ρ = 0.831
			13	11	18.38	11-18.4	7.4		
	F	154	13.51±1.42						
			13	11.88	16	11.9-16	4.1		
	M	150	13.63±1.89						
			13	11	19	11-19	8		
WBC ($x10^9$/L)	M&F	304	7.10±3.49					-0.765	ρ = 0.444
			6	3	14.38	3-14.4	11.4		
	F	154	7.28±4.59						
			6	3	23.38	3-23.4	20.4		
	M	150	7.05±2.80						
			6.5	2	14	2-14	12		
NEU ($x10^9$/L)	M&F	304	3.90±3.19					-0.984	ρ = 0.325
			3	1	11	1-11	10		
	F	154	4.19±4.54						
			3	1	21.38	1-21.4	20.4		
	M	150	3.89±2.34						
			3	**1**	**11**	**1-11**	**10**		
NEU (%)	M&F	304	52.63±14.59					-2.724	ρ = 0.006
			51	25.88	88	25.9-88	62.1		
	F	154	52.12±14.41						
			50.5	24	92.25	24-92.3	68.3		
	M	150	54.67±14.78						
			52	28.55	88	28.6-88	59.4		
LYM ($x10^9$/L)	M&F	304	2.53±1.14					-1.411	ρ = 0.158
			2	1	5	1-5	4		
	F	154	2.52±1.13						
			2	1	5	1-5	4		
	M	150	2.45±1.13						

			2	1	5	1-5	4		
LYM (%)	M&F	304	38.88±13.90					-3.529	ρ = 0.000
			39	7	70	7-70	63		
	F	154	40.12±14.09						
			41	4.75	70.88	4.8-70.9	66.1		
	M	150	36.30±13.75						
			37	7	66.45	7-66.5	59.5		
MON ($x10^9$/L)	M&F	304	0.17±0.45					-0.843	ρ = 0.399
			0	0	1	0-1	1		
	F	154	0.16±0.41						
			0	0	1	0-1	1		
	M	150	0.15±0.45						
			0	0	1.22	0-1.2	1.2		
MON (%)	M&F	304	4.73±3.79					-0.069	ρ = 0.945
			4	0	13.38	0-13.4	13.4		
	F	154	4.64±4.04						
			4	1	13.5	1-13.5	12.5		
	M	150							
			4	0	14.68	0-14.7	14.7		
EOS ($x10^9$/L)	M&F	304	0.13±0.33					-0.087	ρ = 0.931
			0	0	1	0-1	1		
	F	154	0.10±0.31						
			0	0	1	0-1	1		
	M	150	0.13±0.33						
			0	0	1	0-1	1		
EOS (%)	M&F	304	3.46±3.04					-1.346	ρ = 0.178
			2	0	12	0-12	12		
	F	154	2.92±2.35						
			2	0	10.13	0-10.1	10.1		
	M	150	3.81±3.45						
			2	0	12.45	0-12.5	12.5		
BAS ($x10^9$/L)	M&F	304						-1.013	ρ = 0.311
			0.03	0.01	0.10	0.01-0.10	0.09		
	F	154							
			0.03	0.01	0.09	0.01-0.09	0.08		
	M	150							
			0.02	0.04	0.98	0.04-0.98	0.94		
BAS	M&F	304	0.62±1.04					-0.380	ρ = 0.704

(%)			0	0	5	0-5	2.84		
	F	154	0.00±0.00						
			0	0	0	0-0	0		
	M	150	0.57±0.77						
			0.5	0	1.22	0-1.2	1.2		
PLT ($x10^9$/L)	M&F	304	254.09±96.71					-1.171	ρ = 0.241
			238	116.25	513	116.3-513	396.7		
	F	154	256.55±81.02						
			246	124	399	124-399	275		
	M	150	250.43±99.00						
			232	113.43	543.43	113.4-543.4	430		
PCT (%)	M&F	304						-0.602	ρ = 0.547
			0.22	0.1	0.42	0.1-0.4	0.3		
	F	154							
			0.22	0.12	0.4	0.1-0.4	0.3		
	M	150							
			0.204	0.1	0.4314	0.1-0.4	0.3		
PDW (%)	M&F	304						0.0	ρ = 1.000
			16.1	7.6	16.9	7.6-16.9	9.3		
	F	154							
			16	7.33	17	7.3-17	9.7		
	M	150							
			16.1	7.1	17	7.1-17	9.9		
MPV (fL)	M&F	304	8.95±1.02					-1.132	ρ = 0.258
			9	7	11	7-11	4		
	F	154	8.95±1.00						
			9	7	11	7-11	4		
	M	150	8.89±0.99						
			9	7	11	7-11	4		

Results are expressed as mean ± standard deviation (SD), and median and 95% range for the number of referent participants in the column labelled N. Statistical comparisons of the median values between male and female referent participants were carried out using the Mann-Whitney U test. Differences were considered statistically significant at $\rho < 0.05$.

4.9.2 Effects of age on the reference interval limits of hematological parameters for the adult and geriatric population of Taita-Taveta County, Kenya

The effects of age on the reference interval limits of hematological parameters for the adult and geriatric population of Taita-Taveta County, Kenya, are presented in Table 4.21. This was investigated by categorizing this population into three age categories as follows: (a) age ≥ 50-60 years), (b) age ≥ 60-70 years, and (c) age ≥ 70-95 years. Significant statistical differences for median reference interval limits between males and females were estimated within each age category by the Mann-Whitney U test, where ρ-values less than 0.05 were considered significant. Significant statistical differences within and between the three age categories were carried out using the Kruskal-Wallis H test followed by the Mann-Whitney U test with Bonferroni corrections where ρ-values less than 0.0167 were considered statistically significant.

Results of the Kruskal-Wallis H test indicate that red blood cell count (RBC $x10^{12}/L$) (χ^2 (2) = 2.630, ρ = 0.269), hemoglobin (HB g/dL) (χ^2 (2) = 4.959, ρ = 0.084), mean cell hemoglobin (MCH pg) (χ^2 (2) = 2.204, ρ = 0.332), mean cell hemoglobin concentration (MCHC g/dL) (χ^2 (2) = 3.508, ρ = 0.173), red cell distribution width-standard deviation (RDW-SD %) (χ^2 (2) = 1.794, ρ = 0.408), red cell distribution width-coefficient of variation (RDW-CV %) (χ^2 (2) = 0.199, ρ = 0.905), white blood cell count (WBC $x10^9/L$) (χ^2 (2) = 2.308, ρ =0.315), absolute monocytes (MON $x10^9/L$), (χ^2 (2) = 1.235, ρ = 0.539), percent monocytes (MON %) (χ^2 (2) = 0.935, ρ = 0.627), absolute eosinophils

(EOS x10^9/L) (χ^2 (2) = 1.339, ρ = 0.512), percent eosinophils (EOS %) (χ^2 (2) = 0.971, ρ = 0.615), absolute basophils (BAS x10^9/L) (χ^2 (2) = 1.923, ρ = 0.382), percent basophils (BAS %) (χ^2 (2) = 0.085, ρ = 0.958), plateletcrit (PCT %) (χ^2 (2) = 5.808, ρ = 0.055), and platelet distribution width (PDW %) (χ^2 (2) = 0.000, ρ = 1.000) were statistically not affected by advancement in age for combined male and female adults and geriatrics of Taita-Taveta County, Kenya (ρ > 0.05). There was, therefore, no need for pairwise comparisons using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167.

However, the Kruskal-Wallis H test indicates that the packed cell volume (PCV %) (χ^2 (2) = 15.085, ρ = 0.001), mean cell volume (MCV fL) (χ^2 (2) = 8.747, ρ = 0.013), absolute neutrophils (NEU x10^9/L) (χ^2 (2) = 10.120, ρ = 0.006), percent neutrophils (NEU %) (χ^2 (2) = 18.562, ρ = 0.000), absolute lymphocytes (LYM x10^9/L) (χ^2 (2) = 9.617, ρ = 0.008), percent lymphocytes (LYM %) (χ^2 (2) = 20.516, ρ = 0.000), platelets (PLT x10^9/L) (χ^2 (2) = 13.079, ρ = 0.001) and mean platelet volume (MPV fL) (χ^2 (2) = 7.759, ρ = 0.021) for combined male and female adults and geriatrics of Taita-Taveta County, Kenya were statistically significantly affected by advancement in age. Therefore, pairwise comparisons were needed using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167.

Further pairwise comparisons of these age-based significant hematological parameters using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167 indicates that the combined median reference interval limits for

absolute neutrophils (NEU $x10^9$/L) for adults and geriatrics of Taita-Taveta County, Kenya in their fifth decade (3 (1-9) $x10^9$/L) with a mean rank of 98.45 is significantly lower than that of their sixth decade (4 (1-5.65) $x10^9$/L) with a mean rank of 127.51 (U = 4575, z = -3.431, ρ = 0.001, r = 0.2298); absolute neutrophils (NEU $x10^9$/L) for adults and geriatrics in their fifth decade (3 (1-9) $x10^9$/L) with a mean rank of 90.31 is significantly lower than that of their seventh decade onwards (3 (2-5.43) $x10^9$/L) with a mean rank of 115.48 (U = 3606.5, z = -3.096, ρ = 0.002, r = 0.2189). Further, the combined reference interval limits for percent neutrophils (NEU %) for adults and geriatrics in their fifth decade (48 (19-84) %) with a mean rank of 100.95 is significantly lower than that of their sixth decade (52 (28.88-63) %) with a mean rank of 124.63 (U = 4874, z = -2.735, ρ = 0.006, r = 0.1831); percent neutrophils (NEU %) for adults and geriatrics in their fifth decade (48 (19-84) %) with a mean rank of 84.89 is significantly lower than that of their seventh decade onwards (55 (34.05-68.5) %) with a mean rank of 123.43 (U = 2962.5, z = -4.624, ρ = 0.000, r = 0.3270).

The combined median reference interval limits for absolute lymphocytes (LYM $x10^9$/L) for adults and geriatrics in their fifth decade (2 (1-4) $x10^9$/L) with a mean rank of 110.87 is significantly higher than that of their seventh decade onwards (2 (1-2) $x10^9$/L) with a mean rank of 85.26 (U = 3585, z = -3.273, ρ = 0.001, r = 0.2314), and for absolute lymphocytes (LYM $x10^9$/L) for adults and **geriatrics** in their sixth decade (2 (1-3) $x10^9$/L) with a mean rank of 101.56 is significantly higher than that in their seventh decade onwards (2 (1-2)

$x10^9$/L) with a mean rank of 82.01 (U = 3321.5, z = -2.582, ρ = 0.010, r = 0.1898). The combined median reference interval limits for percent lymphocytes (LYM %) for adults in their fifth decade (44 (13-78) %) with a mean rank of 124.76 is significantly higher than that of geriatrics in their sixth decade (37 (4.25-44.75) %) with a mean rank of 97.40 (U = 4670, z = -3.160, ρ = 0.002, r = 0.2116), and for percent lymphocytes (LYM %) for adults in their fifth decade (44 (13-78) %) with a mean rank of 117.22 is significantly higher than that of geriatrics in their seventh decade onwards (35 (5.05-40.5) %) with a mean rank of 75.93 (U = 2829.5, z = -4.955, ρ = 0.000, r = 0.3504).

The combined **refe**rence interval limits for PCV (%) for adults in their fifth decade, with a mean rank of 105.97, is significantly higher than that of geriatrics in their seventh decade onwards, with a mean rank of 92.46 (U = 4165.5, z = -2.925, ρ = 0.003, r = 0.2068). The combined reference interval limits for mean cell volume (MCV %) for adults in their fifth decade (90 (70-110) fL) with a mean rank of 109.82 is significantly higher than that of geriatrics in the seventh decade onwards (86 (64.15-103.9) fL) with a mean rank of 86.81 (U = 3710.5, z = -2.763, ρ = 0.006, r = 0.1954). **Th**e combined reference interval limits for platelets (PLT $x10^9$/L) for adults in their fifth decade (218 (110-536) $x10^9$/L) with a mean rank of 98.79 is significantly lower than that of geriatrics in the sixth decade (252.5 (129.25-526.75) $x10^9$/L) with a mean rank of 127.12 (U = 4616, z = -3.271, ρ = 0.001, r = 0.2190), and for platelets (PLT $x10^9$/L) for adults in their fifth decade (218 (110-536) $x10^9$/L) with a mean rank of 87.71 is significantly lower than that of geriatrics in their

seventh decade onwards (251 (115.1-399) $x10^9$/L) with a mean rank of 119.28 (U = 3298, z = -3.787, ρ = 0.000, r = 0.2678). The combined reference interval limits for mean platelet volume (MPV fL) for adults in their fifth decade (9 (7-11) fL) with a mean rank of 108.28 is significantly higher than that of geriatrics in their seventh decade onwards (9 (7-11) fL) with a mean rank of 89.07 (U = 3893.5, z = -2.402, ρ = 0.0163, r = 0.1698).

Results of the Kruskal-Wallis H test indicate that red blood cell count (RBC $x10^{12}$/L) (χ^2 (2) = 1.906, ρ = 0.386), packed cell volume (PCV %) (χ^2 (2) = 2.553, ρ = 0.279), mean cell volume (MCV fL) (χ^2 (2) = 5.503, ρ = 0.064), mean cell hemoglobin (MCH pg) (χ^2 (2) = 4.084, ρ = 0.130), mean cell hemoglobin concentration (MCHC g/dL) (χ^2 (2) = 1.692, ρ = 0.429), red cell distribution width-standard deviation (RDW-SD %) (χ^2 (2) = 0.270, ρ = 0.874), red cell distribution width-coefficient of variation (RDW-CV %) (χ^2 (2) = 2.165, ρ = 0.339), white blood cell count (WBC $x10^9$/L) (χ^2 (2) = 0.316, ρ = 0.854), percent lymphocytes (LYM %) (χ^2 (2) = 5.948, ρ = 0.051), absolute monocytes (MON $x10^9$/L), (χ^2 (2) = 0.015, ρ = 0.993), percent monocytes (MON %) (χ^2 (2) = 0.734, ρ = 0.693), absolute eosinophils (EOS $x10^9$/L) (χ^2 (2) = 1.132, ρ = 0.568), percent eosinophils (EOS %) (χ^2 (2) = 0.257, ρ = 0.882), absolute basophils (BAS $x10^9$/L) (χ^2 (2) = 1.586, ρ = 0.452), percent basophils (BAS %) (χ^2 (2) = 0.086, ρ = 0.958), platelets (PLT $x10^9$/L) (χ^2 (2) = 3.933, ρ = 0.140), plateletcrit (PCT %) (χ^2 (2) = 3.194, ρ = 0.203), platelet distribution width (PDW %) (χ^2 (2) = 0.000, ρ = 1.000) and mean platelet volume (MPV fL) (χ^2 (2) = 0.272, ρ = 0.873) for male adult and geriatric

population of Taita-Taveta County, Kenya were not statistically affected by advancement in age ($\rho > 0.05$). There was, therefore no need for pairwise comparisons using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167.

However, Kruskal-Wallis H test analysis indicates that hemoglobin (HB g/dL) (χ^2 (2) = 7.373, $\rho = 0.025$), and percent neutrophils (NEU %) (χ^2 (2) = 6.48, $\rho = 0.039$) for the male adults and geriatrics of Taita-Taveta County, Kenya were statistically significantly affected by advancement in age. Therefore, pairwise comparisons were needed using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167. Further pairwise comparison analysis using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167 indicates that the median reference interval limits for hemoglobin (HB g/dL) for male adults in their fifth decade (14 (6.4-17.8) g/dL)) with a mean rank of 53.7 are, significantly higher than that of male geriatrics in their seventh decade onwards (13 (6-17.7) g/dL) with a mean rank of 38.77 (U = 709.5, z = -2.760, $\rho = 0.006$, r = 0.2877); the median reference interval limits for percent neutrophils (NEU %) for male adults in the fifth decade (49 (19.4-84) %) with a mean rank of 39.72 is significantly lower than that of male geriatrics in the seventh decade (55 (34-88) %) with a mean rank of 53.58 (U = 739, z = -2.490, $\rho = 0.013$, r = 0.2596).

Further, results of the Kruskal-Wallis H test indicate that red blood cells (RBC $x10^{12}$/L) (χ^2 (2) = 0.460, $\rho = 0.795$), hemoglobin (HB g/dL) (χ^2 (2) = 2.494, $\rho = 0.287$), mean cell volume (MCV fL) (χ^2 (2) = 4.152, $\rho = 0.125$), mean cell

hemoglobin (MCH pg) (χ^2 (2) = 3.393, ρ = 0.183), mean cell hemoglobin concentration (MCHC g/dL) (χ^2 (2) = 2.275, ρ = 0.321), red cell distribution width-standard deviation (RDW-SD %) (χ^2 (2) = 3.185, ρ = 0.203), red celldistribution width-coefficient of variation (RDW-CV %) (χ^2 (2) = 3.919, ρ = 0.141), absolute lymphocytes (LYM x10^9/L) (χ^2 (2) = 4.757, ρ = 0.093), absolute monocytes (MON x10^9/L), (χ^2 (2) = 0.221, ρ = 0.895), percent monocytes (MON %) (χ^2 (2) = 0.513, ρ = 0.774), absolute eosinophils (EOS x10^9/L) (χ^2 (2) = 3.410, ρ = 0.182), percent eosinophils (EOS %) (χ^2 (2) = 5.246, ρ = 0.073), absolute basophils (BAS x10^9/L) (χ^2 (2) = 0.000, ρ = 1.000), percent basophils (BAS %) (χ^2 (2) = 0.207, ρ = 0.902), plateletcrit (PCT %) (χ^2 (2) = 1.139, ρ = 0.566), and platelet distribution width (PDW %) (χ^2 (2) = 0.000, ρ = 1.000) for female adults and geriatrics of Taita-Taveta County, Kenya were not statistically affected by advancement in age (ρ > 0.05). There was, therefore no need for pairwise comparisons using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167.

However, Kruskal-Wallis H test analysis indicates that packed cell volume (PCV %) (χ^2 (2) = 14.694, ρ = 0.001), white blood cells (WBC x10^9/L) (χ^2 (2) = 7.647, ρ = 0.022), absolute neutrophils (NEU x10^9/L) (χ^2 (2) = 12.337, ρ = 0.002), percent neutrophils (NEU %) (χ^2 (2) = 14.357, ρ = 0.001), percent lymphocytes (LYM %) (χ^2 (2) = 17.837, ρ = 0.000), platelets (PLT x10^9/L) (χ^2 (2) = 26.044, ρ = 0.000) and mean platelet volume (MPV fL) (χ^2 (2) = 15.801, ρ = 0.000) for the female adults and geriatrics of Taita-Taveta County, Kenya were statistically significantly affected by advancement in age (Table4.21).

Therefore, a pairwise follow-up analysis was needed using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167.

Further pairwise analysis using the Mann-Whitney U test with adjusted significant ρ-value of 0.0167 indicates that the median reference interval limits for white blood cells (WBC $x10^9$/L) for female adults in their fifth decade (6 (3-14.7) $x10^9$/L)) with a mean rank of 52.88 is significantly lower than that of female geriatrics in their seventh decade onwards (7 (3.2-10.5) $x10^9$/L) with a mean rank of 69.86 (U = 1179.55, z = -2.655, ρ = 0.008, r = 0.2444). The median reference interval limits for absolute neutrophils (NEU $x10^9$/L) for female adults in their fifth decade (3 (0-11) $x10^9$/L) with a mean rank of 52.36 is significantly lower than that of geriatrics in their sixth decade (3 (0-11) $x10^9$/L) with a mean rank of 70.67 (U = 1142, z = -2.914, ρ = 0.004, r = 0.2683), and the reference interval limits for absolute neutrophils (NEU $x10^9$/L) for female adults in their fifth decade (3 (0-11) $x10^9$/L) with a mean rank of 48.52 is significantly lower than that of geriatrics in their seventh decade onwards (3.5 (2-6.9) $x10^9$/L) with a mean rank of 66.46 (U = 865.5, z = -2.891, ρ = 0.004, r = 0.2782). The median reference interval limits for percent neutrophils (NEU %) for female adults in their fifth decade (47 (19-78.6 %) with a mean rank of 53.40 is significantly lower than that of female geriatrics in their sixth decade (48 (19-86) %) with a mean rank of 69.04 (U = 1156, z = -2.424, ρ = 0.015, r = 0.2231), and the reference interval limits for percent neutrophils (NEU %) for female adults in their fifth decade (47 (19-78.6 %) with a mean rank of 46.99 is significantly lower than that of geriatrics in their

seventh decade onwards (55 (35-68) %) with a mean rank of 69.5 (U = 755.5, z = -3.525, ρ = 0.000, r = 0.3392) (Table 4.21).

The median reference interval limit for percent lymphocytes (LYM %) for female adults in their fifth decade (45.5 (14.95-50) %) with a mean rank of 66.44 is significantly higher than that of female geriatrics in their sixth decade (37 (3-47.5) %) with a mean rank of 48.63 (U = 1156, z = -2.761, ρ = 0.006, r = 0.2542), and the reference interval limit for percent lymphocytes (LYM %) for female adults in their fifth decade (45.5 (14.95-50) %) with a mean rank of 62.83 is significantly higher than that of female geriatrics in their seventh decade onwards (33 (3-44.3) %) with a mean rank of 37.83 (U = 696, z = -3.913, ρ = 0.000, r = 0.3765) (Table 4.21).

The median reference interval limit for platelets (PLT $x10^9$/L) for female adults in their fifth decade (211 (122.4-444.4) $x10^9$/L) with a mean rank of 47.4 is significantly lower than that of female geriatrics in their sixth decade (2467 (125-625) $x10^9$/L) with a mean rank of 78.45 (U = 784.5, z = -4.809, ρ = 0.000, r = 0.4427); further the reference interval limit for platelets (PLT $x10^9$/L) for female adults in their fifth decade (211 (122.4-444.4) $x10^9$/L) with a mean rank of 47.30 is significantly lower than that of female geriatrics in their seventh decade onwards (274.5 (117-354) $x10^9$/L) with a mean rank of 68.90 (U = 777.5, z = -3.380, ρ = 0.001, r = 0.3252) (Table 4.21).

The median reference interval limit for mean platelet volume (MPV fL) for female adults in their fifth decade (9 (7-11) %) with a mean rank of 67.22 is significantly higher than that of geriatrics in their sixth decade (9 (8-11) fL)

with a mean rank of 47.41 (U = 784.5, z = -4.809, ρ = 0.000, r = 0.4427), and the reference interval limits for mean platelet volume (MPV fL) for female adults in their fifth decade (9 (7-11) fL) with a mean rank of 61.26 is significantly higher than that of female geriatrics in their seventh decade onwards (8.8 (7-8.98) fL) with a mean rank of 40.97 (U = 777.5, z = -3.380, ρ = 0.001, r = 0.3252) (Table 4.21).

An investigation on the effect of gender on the reference interval limits for hematological parameters in specific age stratifications indicates that for adults and geriatrics of Taita-Taveta County, Kenya, the reference interval limits for percent lymphocytes (LYM %) for male adults in their fifth decade (40 (13-77) %) with mean rank of 51.66 are significantly lower than those of their female counterparts (45.5 (14.95-50) %) with mean rank of 65.44 (U = 1300, z = -2.132, ρ = 0.033, r = 0.1954); the reference interval limits for hemoglobin (HB g/dL) for male adults in their fifth decade (14 (6.4-17.8) g/dL) with mean rank of 71.95 are significantly higher than those of their female counterparts (13 (8-18.5) g/dL) with mean rank of 52.20 (U = 1130.5, z = -3.103, ρ = 0.002, r = 0.2845); and the reference interval limits for mean platelet volume (MPV fL) for male adults in the fifth decade (9 (7-11) fL) with mean rank of 51.04 are significantly lower than those of their female counterparts (9 (7-11) fL) with mean rank of 65.85 (U = 1271, z = -2.393, ρ = 0.017, r = 0.2194). Further, the reference interval limits for platelets (PLT $x10^9$/L) for male geriatrics in their sixth decade (225.5 (94.4-673) $x10^9$/L) with a mean rank of 43.51 are significantly lower than those of their female counterparts (246 (125-625)

x10^9/L) with a mean rank of 63.84 (U = 812.5, z = -3.414, ρ = 0.001, r = 0.3348); and the reference interval limits for percent eosinophils (E**OS** %) for male geriatrics in their seventh decade onwards (2 (0-14) %) with mean rank 46.09 are significantly higher than those of their female counterparts (2 (0-3.75) %) with a mean rank of 34.64 (U = 581, z = -2.213, ρ = 0.027, r = 0.2459) (Table4.21).

Table 4.21: Effects of age on the median reference interval limits of hematological parameters for adults and geriatrics of Taita-Taveta County, Kenya

Analyte (unit)	Changes of hematological parameter levels with age						
	Gender	N	≥50-60 years	N	≥60-70 years	N	≥70-95 years
RBC ($x10^{12}$/L)	M&F	119	4.52±0.90	104	4.48±0.72	81	4.37±0.75
			5 (3-7)		**4.5 (3-6)**		**4 (3-6)**
	M	47	4.53±1.06	58	4.47±0.68	45	4.36±0.68
			5 (1.2-7)		**4.5 (3-6)**		**4 (3-5.9)**
	F	72	4.51±0.79	46	4.50±0.78	36	4.39±0.84
			4 (3-7)		**4.5 (3-6)**		**4 (3-5)**
HB (g/dL)	M&F	119	13.07±2.21	104	12.82±2.11	81	12.11±2.69
			13 (8-18)		**13 (8-16)**		**13 (6-17)**
	M	47	13.49±2.34	58	12.81±2.34	45	12.04±2.95
			14 (6.4-17.8)*↑		**14 (7.5-17.1)**		**13 (6-17.7)[b]↓**
	F	72	12.79±2.09	46	12.83±1.82	36	12.19±2.38
			13 (8-18.5)		**13 (8-15.8)**		**13 (8-14)**
MCH (pg)	M&F	119	29.02±3.48	104	28.49±2.67	81	27.81±3.71
			29 (22-39)		**29 (21-33)**		**28 (18-35)**
	M	47	29.85±3.96	58	28.33±2.54	45	27.89±4.34
			30 (23.2-42.2)		**29 (21-32.53)**		**29 (16.3-35)**
	F	72	28.47±3.04	46	29.11±3.23	36	27.72±2.76
			29 (22-34.9)		**30 (22-38)**		**28 (21-29.8)**
MCHC (g/dL)	M&F	119	32.24±2.61	104	32.20±2.75	81	32.35±2.16
			33 (27-37)		**33 (28-36)**		**33 (25.1-36)**
	M	47	32.57±2.73	58	31.81±3.31	45	32.24±2.41
			33 (27-37)		**32 (20.13-36)**		**33 (25-36.9)**
	F	72	32.01±2.53	46	32.83±2.56	36	32.47±1.67

			32 (28-37)		**33 (28-37)**		**33 (29-33.1)**
MCV (fL)	M&F	119	90.09±9.10	104	87.85±7.28	81	85.77±9.29
			90 (70-110)		**88 (70-100.4)**		**86 (64.2-103.9)[b]↓**
	M	47	91.77±10.12	58	88.00±6.88	45	86.09±10.31
			91 (71-123.6)		**87 (74.5-103.6)**		**86 (59.8-104)**
	F	72	89.00±8.27	46	88.65±8.72	36	85.36±7.95
			88 (69.7-110)		**89.5 (70.4-110)**		**84.5 (67-92)**
RDW-SD (%)	M&F	119	50.34±6.48	104	49.96±6.89	81	49.52±6.70
			50 (40-71)		**48.5 (38.6-65.1**)		**49 (39-67)**
	M	47	49.70±5.89	58	50.53±6.50	45	50.36±6.38
			49 (39.2-63)		**48.5 (39-64**)		**49 (41.2-67)**
	F	72	50.76±6.85	46	49.63±7.29	36	48.47±7.04
			50 (40-71)		**48 (39.4-71)**		**46 (38-54)**
RDW-CV (%)	M&F	119	13.45±1.34	104	13.50±1.57	81	13.84±2.14
			13 (11-16)		**13 (11-18)**		**13 (12-20)**
	M	47	13.17±1.26	58	13.72±1.65	45	13.98±2.55
			13 (12-16)		**13 (11-18)**		**13 (11.2-21.7**)
	F	72	13.63±1.37	46	13.24±1.40	36	13.67±1.47
			14 (11-16)		**13 (11-16)**		**13.5 (12-14.2)**
WBC ($x10^9$/L)	M&F	119	6.59±2.60	104	7.78±4.63	81	7.23±4.04
			6 (3-12)		**7 (2-8.8)**		**6 (4-8.3)**
	M	47	6.89±2.56	58	7.24±3.05	45	6.98±2.65
			7 (1.4-12)		**6.5 (2-15.1)**		**6 (4-15.7)**
	F	72	6.39±2.56	46	8.64±6.04	36	7.56±5.32
			6 (3-14.7)		**7 (3.2-10.5)[a]↑**		**6 (3-9.6)**
NEU	M&F	119	3.27±1.95	104	4.63±4.55	81	4.42±3.99

($x10^9$/L)			**3 (1-9)**		**4 (1-5.7)[a]↑**		**3 (2-5.4)[b]↑**
	M	47	3.32±2.01	58	4.17±2.54	45	4.11±2.43
			3 (1-8)		**3.5 (0.5-11.5)**		**3 (1.2-11.9)**
	F	72	3.24±2.01	46	3.46±2.29	36	4.81±5.35
			3 (0-11)		**3 (0-11)[a]↑**		**3.5 (2-6.9)[b]↑**
NEU (%)	M&F	119	49.25±13.92	104	54.17±14.77	81	58.42±13.84
			48 (19-84)		**52 (28.9-63)[a]↑**		**55 (34.1-68.5)[b]↑**
	M	47	51.34±15.19	58	54.19±14.71	45	58.78±13.75
			49 (19.4-84)		**52 (22.3-89.1)**		**55 (34-88)[b]↑**
	F	72	47.89±12.95	46	47.26±15.08	36	57.97±14.13
			47 (19-78.6)		**48 (19-86)[a]↑**		**55 (35-68)[b]↑**
LYM ($x10^9$/L)	M&F	119	2.59±1.05	104	2.59±1.19	81	2.21±1.15
			2(1-4)		**2 (1-3)[a]↓**		**2 (1-2)[bc]↓↓**
	M	47	2.57±1.06	58	2.55±1.19	45	2.20±1.12
			2 (0.2-4.8)		**2 (1-6.1)**		**2 (1-6.6)[b]**
	F	72	2.60±1.04	46	2.76±0.97	36	2.22±1.20
			2 (1-4.7)		**3 (1-4.8)**		**2 (1-3)↓[b]**
LYM (%)	M&F	119	42.73±13.90	104	37.09±13.79	81	33.11±12.56
			44 (13-78)		**37 (4.3-44.8)**		**33 (5.1-40.5)[b]↓**
	M	47	39.68±14.89	58	36.66±13.69	45	32.31±11.69
			40 (13-77.2)*↓		**37 (6.5-73.3)**		**35 (6.2-54)[b]↓**
	F	72	44.72±12.92	46	37.63±14.05	36	34.11±13.67
			45.5 (15-50)		**37 (3-47.5)[a]↓**		**33 (3-44.3)[b]↓**
MON ($x10^9$/L)	M&F	119	0.16±0.45	104	0.15±0.46	81	0.15±0.36
			0 (0-1)		**0 (0-0.3)**		**0 (0-1)**
	M	47	0.15±0.42	58	0.17±0.53	45	0.13±0.34

			0 (0-1.8)		**0 (0-2.5)**		**0(0-1)**
	F	72	0.17±0.48	46	0.11±0.32	36	0.17±0.38
			0 (0-1.4)		**0 (0-1)**		**0 (0-0.3)**
MON (%)	M&F	119	4.69±3.71	104	4.88±4.41	81	4.16±2.27
			4 (0-17)		**4 (0.6-6)**		**4 (1-6)**
	M	47	4.96±3.80	58	4.74±3.39	45	4.02±2.27
			4 (0-16.4)		**4 (0-17)**		**4 (1-10.55**)
	F	72	4.51±3.67	46	4.96±5.55	36	4.33±2.29
			4 (0-17.4)		**3.5 (1-32.8)**		**4 (1-6)**
EOS ($x10^9$/L)	M&F	119	0.07±0.25	104	0.71±1.30	81	0.15±0.36
			0 (0-1)		**0.5 (0-1**)		**0 (0-1)**
	M	47	0.09±0.28	58	0.53±0.54	45	0.16±0.37
			0 (0-1)		**1 (0-1.5)**		**0 (0-1)**
	F	72	0.06±0.23	46	0.67±1.49	36	0.14±0.35
			0 (0-1)		**0 (0-8.4)**		**0 (0-0.3)**
EOS (%)	M&F	119	3.47±2.88	104	3.23±2.78	81	3.36±3.37
			2 (1-11)		**2 (0-4)**		**2 (0-5)**
	M	47	3.68±3.40	58	3.62±3.08	45	4.20±3.98
			2 (0-14.2)		**3 (0-11.5)**		**2 (0-14)*↑**
	F	72	3.33±2.50	46	3.20±2.31	36	2.31±2.00
			3 (1-11)		**2 (0.2-10.8)**		**2 (0-3.8**)
BAS (%)	M&F	119	0.54±0.73	104	0.71±1.30	81	0.70±1.21
			0 (0-2)		**0.5 (0-1)**		**0 (0-1)**
	M	47	0.60±0.95	58	0.53±0.54	45	0.58±0.84
			0 (0-5)		**1 (0-1.5)**		**0 (0-4.4)**
	F	72	0.50±0.56	46	0.67±1.49	36	0.86±1.55

			0 (0-2)		**0 (0-8.4)**		**0 (0-1.5)**
PLT ($x10^9$/L)	M&F	119	232.96±90.08	104	264.63±93.82	81	269.52±80.57
			218 (110-536)		**252.5 (129.3-526.8)[a]↑**		**251 (115-399)[b]↑**
	M	47	242.02±100.69	58	247.64±110.63	45	262.82±80.57
			219 (92-562.4)		**225.5 (94.4-673)*↓**		**251 (115-445.8)**
	F	72	227.04±82.63	46	250.28±89.16	36	277.89±80.92
			211 (122.4-444.4)		**246 (125-625)[a]↑**		**274.5 (117-354)[b]↑**
MPV (fL)	M&F	119	9.11±1.00	104	8.83±0.90	81	**8.75±1.06**
			9 (7-11)		**9 (7-11)**		**9 (7-11)[b]**
	M	47	8.85±1.00	58	8.93±0.86	45	8.87±1.14
			9 (7-11)*↓		**9 (8-11)**		**9 (7-11)**
	F	72	9.28±0.97	46	9.30±0.89	36	8.61±0.93
			9 (7-11)		**9 (8-11)[a]↓**		**8.5 (7-8.98)[b]↓**

Results are expressed as Mean ± Standard deviation (SD), and Median and range for the number of subjects indicated in the column labeled N. $^{*}\rho < 0.05$ when male reference interval limits are significantly different when compared to female reference interval limits for each age category by Mann-Whitney U test. $^{a}\rho < 0.0167$ when reference interval limits in age range ≥ 50-60 years is significantly different when compared to reference interval limits in age range ≥ 60-70 years, $^{b}\rho < 0.0167$ when reference interval limits in age range ≥ 50-60 years is significantly different when compared to reference interval limits in age range ≥ 70-95 years, and $^{c}\rho < 0.0167$ when reference interval limits in age range ≥ 60-70 years is significantly different when compared to reference interval limits in age range ≥ 70-95 years by Kruskal-Wallis H test followed by Mann-Whitney U test with Bonferroni correction.

4.9.3 Comparison of developed reference interval limits for haematological parameters for the adult and geriatric population of Taita-Taveta County, Kenya, with those reported in the medical literature

A comparison of developed reference interval limits for haematological parameters for the adult and geriatric population of Taita-Taveta County, Kenya, with those reported in the medical literature is presented in Table 4.22. These comparisons were performed by comparing the lower and upper limits of this study's reference interval limits with those of other studies reported in the medical literature. Results indicate that for red blood cells (RBC $x10^{12}$/L), this study's lower limit is lower than that observed for American (Hale *et al.,* 1983,Yip *et al.,* 1984), Dutch (Swaanerburg *et al.,* 1987), Chinese (Woo *et al.,* 1989), Australian (Tsang *et al.,* 1998), Ugandan (Lugada *et al.,* 2004), Burkina Faso (Böhler *et al.,* 2008), Uganda(Mugisha *et al.,* 2016) and Oman (Al-Mawali *et al.,* 2018) populations, while the upper limit is similar to that of the Ducth (male and female) American (Yip *et al.,* 1984), (Swaanerburg *et al.,* 1987), Chinese (Woo *et al.,* 1989), Burkina Faso (Böhler *et al.,* 2008) and South-West Ugandan (Mugisha *et al.,* 2016), and female population, lower than the Oman (male and female) population, and higher than, American (Hale *et al.,* 1983), Chinese (Woo *et al.,* 1989), Australian (Tsang *et al.,* 1998) and Ugandan (Lugada *et al.,* 2004) and the Burkina Faso (Böhler *et al.,* 2008), for male populations.

For hemoglobin (HB g/dL), this study's lower limit is lower than that of the American (Hale *et al.,* 1983 ,Yip *et al.,* 1984), Dutch (Swaanerburg *et al.,*

1987), Chinese (Woo *et al.*, 1989), Australian (Tsang *et al.*, 1998) , Ugandan (Lugada *et al.,* 2004), Burkina Faso (Böhler *et al.,* 2008), Ugandan (Mugisha *et al.*, 2016), and Oman (Al-Mawali *et al.*, 2018), populations, while the upper limit is higher than those of the American (Hale *et al.,* 1983 ,Yip *et al.*, 1984), Dutch (Swaanerburg *et al.*, 1987), Chinese (Woo *et al.*, 1989), Australian (Tsang *et al.,* 1998) ,Ugandan(Luganda *et al.*,2004) Burkina Faso (Böhler *et al.,* 2008), Ugandan (Mugisha *et al.*, 2016), and Oman (Al-Mawali *et al.,* 2018) populations, and is lower than that of the American 1 (Yip *et al.*, 1984), Australian (Tsang *et al.,* 1998) and Ugandan (Lugada *et al.,* 2004 female populations.

For packed cell volume (PCV %), this study's lower limit is lower than that of the American (Hale *et al.,* 1983), American 2 (Yip *et al.,* 1984), Dutch (Swaanerburg *et al.,* 1987) , Chinese (Woo *et al.,* 1989), Australian (Tsang *et al.,* 1998), Ugandan (Lugada *et al.,* 2004), Burkina Faso (Böhler *et al.,* 2008), South-West Ugandan (Mugisha *et al.,* 2016), and Oman (Al-Mawali *et al.,* 2018) population, while the upper limit is lower than those of the American (Hale *et al.,* 1983,Yip *et al.,* 1984), Dutch (Swaanerburg *et al.,* 1987), Chinese (female) (Woo *et al.,* 1989), Australian (Tsang *et al.,* 1998), Ugandan (female) (Lugada *et al.,* 2004), Burkina Faso (female) (Böhler *et al.,* 2008), Ugandan (Mugisha *et al.,* 2016), and Oman (female) (Al-Mawali *et al.,* 2018) populations, similar to those of the Chinese (male) (Woo *et al.,* 1989) and South-West Ugandan (male) (Mugisha *et al.,* 2016), populations, and higher

than those of the Ugandan (male) (Lugada *et al.,* 2004), Burkina Faso (male), and Oman (male) (Al-Mawali *et al.,* 2018) populations.

For mean cell hemoglobin (MCH pg), this study's lower limit is lower than those of Australian (Tsang *et al.,* 1998), Burkina Faso (Böhler *et al.,* 2008), and southwest Ugandan (Mugisha *et al.,* 2016), populations, similar to those of American (Yip *et al.,* 1984) and Oman (Al-Mawali *et al.,* 2018) populations, while the upper limit is higher than those of the Australian (Tsang *et al.,* 1998) and Burkina Faso (Böhler *et al.,* 2008), southwest Ugandan (Mugisha *et al*., 2016), Oman (Al-Mawali *et al.,* 2018) populations.

For mean cell hemoglobin concentration (MCHC g/dL), this study's lower limit was higher than those of the American 2 (Yip *et al.,* 1984), Dutch (Swaanerburg *et al.,* 1987), Burkina Faso (Böhler *et al.,* 2008), and Oman (Al-Mawali *et al.,* 2018) populations,while the upper limit was higher than that of the American 2 (Yip *et al.,* 1984),Dutch (Swaanerburg *et al.,* 1987) population, similar to that of the American 2 (Yip *et al.,* 1984) and Burkina Faso (Böhler *et al.,* 2008) populations, and lower than that of the Oman (Al-Mawali *et al.,* 2018) population.

For mean cell volume (MCV fL), this study's lower limit was lower than those of the American 2 (Yip *et al.,* 1984), Dutch (Swaanerburg *et al.,* 1987), Australian (Tsang *et al.,* 1998) Burkina Faso (Böhler *et al.,* 2008), and southwest Ugandan (Mugisha *et al*., 2016 populations, similar to that of the American (Yip *et al.,* 1984) populations. Chinese (Woo *et al.,* 1989) population, and higher than that of the Oman (Al-Mawali *et al.,* 2018)

population, while the upper limit was higher than those of the Dutch (Swaanerburg et al., 1987), Chinese (Woo *et al.,* 1989), Australian (Tsang *et al.,* 1998), Burkina Faso (Böhler *et al.,* 2008), southwest Ugandan (Mugisha et al., 2016), and Oman (Al-Mawali *et al.,* 2018)

For red blood cell distribution width-coefficient of variation (RDW-CV %), this study's lower and upper reference interval limits are similar to those reported by Al-Mawali *et al.,* (2018) for Oman population.

For white blood cells (WBC $x10^9$/L), this study's gender-independent lower reference interval limit is lower than that of the gender-independent for American[1] (Hale *et al.,* 1983), Dutch (Swaanerburg *et al.,* 1987), Chinese (Woo et al., 1989), Eastern Ugnda (Lugada *et al.,* 2004), Burkina Faso (Böhler *et al.,* 2008), and similar to that of the Oman (Al-Mawali et al., 2018) populations, and gender dependent Australian (Tsang *et al.,* 1998) and south-west Uganda (Mugisha *et al.,* 2016) population, while the upper reference interval limit is higher than that of the American[1] (Hale *et al.,* 1983), Dutch (Swaanerburg *et al.,* 1987), Chinese (Woo *et al.,* 1989), Australian (Tsang *et al.,* 1998), eastern Uganda (Lugada *et al.,* 2004), Burkina Faso (Böhler *et al.,* 2008), south-west Uganda (Mugisha *et al.,* 2016) and Oman (Al-Mawali *et al.,* 2018) populations.

For absolute neutrophils (NEU 10^9/L), this study's gender-dependent lower reference interval limit for females and males is lower and higher, respectively, than the gender-dependent values reported for the Dutch population by Swaanerburg *et al.* (1987). Gender-independent values reported for the eastern

Uganda[L] population by Lugada *et al*. (2004), South-West Uganda[M] population by Mugisha *et al.* (2016), Oman population by Al-Mawali *et al.* (2018), while the upper reference interval limit is higher than those reported for the Dutch, South-West Uganda[M], and eastern Uganda[L] populations.

For percent neutrophils (NEU %), this study's gender-dependent lower reference interval limit is lower than the gender-dependent values reported for Burkina Faso population by Böhler *et al.,* (2008) and South-West Uganda[M] population by Mugisha *et al.,* (2016) and gender independent values reported for the Dutch population by Swaanerburg *et al.,* (1987), while the upper reference interval limit is higher than those reported for the Dutch, Burkina Faso, and South-West Uganda[M] populations.

For absolute lymphocytes (LYM 10^9/L), this study's gender-independent lower reference interval limit is lower than the gender-independent values reported for Dutch population by Swaanerburg *et al.,* (1987), eastern Uganda[L] population by Lugada et al. (2004), and South-West Uganda[M] population by Mugisha *et al*. (2016), and Oman population by Al-Mawali *et al.* (2018) and gender-dependent values reported for Burkina Faso population by Böhler *et al*. (2008), while the upper reference interval limit is higher than those reported for the Dutch, Burkina Faso, eastern Uganda[L], South-West Uganda[M] and Oman populations.

For percent lymphocytes (LYM %), this study's gender-dependent lower reference interval limit is lower than the gender-dependent values reported for Burkina Faso population by Böhler *et al.,* (2008), gender-independent values

reported for Dutch population by Swaanerburg *et al.,* (1987), and South-West Uganda^{M} population by Mugisha *et al.,* (2016), while the upper reference interval limit is higher than those reported for the Dutch, Burkina Faso and South-West Uganda populations.

For absolute monocytes (MON 10^9/L), this study's gender-independent lower reference interval limit is lower than the gender-dependent values reported for Dutch population by Swaanerburg *et al.,* (1987), and gender-independent values for the eastern Uganda^{L} population by Lugada *et al.,* (2004), South-West Uganda^{M} population by Mugisha *et al.,* (2016), and Oman population by Al-Mawali *et al.,* (2018), while the upper reference interval limit is higher than that reported for the Dutch, South-West Uganda^{M} population, eastern Uganda^{L} population and Oman population.

For percent monocytes (MON %), this study's lower reference interval limit is lower than that reported for Dutch population by Swaanerburg *et al.,* (1987), and South-West Uganda^{M} population by Mugisha *et al.,* (2016), while the upper reference interval limit is lower than that reported for the Dutch and South-West Uganda populations.

For absolute eosinophils (EOS 10^9/L), this study's gender-independent lower reference interval limit is lower than the gender-dependent values reported for the Dutch population by Swaanerburg *et al.,* (1987), eastern Uganda^{L} population by Lugada *et al.,* (2004), gender-independent values for South-West Uganda^{M} population by Mugisha *et al.,* (2016), and similar to that of the Oman population, while the upper reference interval limit is higher than that reported

for the Dutch and Oman population and lower than that reported for eastern Uganda[L] population by Lugada *et al.,* (2004) but similar to that reported for the South-West Uganda[M] population by Mugisha *et al.,* (2016)

For percent eosinophils (EOS %), this study's gender-dependent lower reference interval limit is lower than the gender-dependent values reported for Dutch population by Swaanerburg *et al.* (1987). Gender-independent values reported for South-West Uganda[M] population by Mugisha *et al.,* (2016), while the upper reference interval limit is higher than that reported for the Dutch population and lower than that reported for South-West Uganda populations.

For absolute basophils (BAS 10^9/L), this study's lower reference interval limit is similar to that reported for Dutch population by Swaanerburg *et al.,* (1987), eastern Uganda[L] population by Lugada *et al.,* (2004),South-West Uganda[M] population by Mugisha *et al.,* (2016),and Oman population by Al-Mawali *et al*. (2018), while the upper reference interval limit is similar to that reported for the Dutch and South-West Uganda populations but higher than that reported for eastern Uganda[L] population by Lugada *et al.,* (2004), and Oman population by Al-Mawali *et al.,* (2018).

For percent basophils (BAS %), this study's lower reference interval limit is lower than that reported for Dutch population by Swaanerburg *et al.,* (1987), and higher than that reported for South-West Uganda[M] population by Mugisha *et al.,* (2016), while the upper reference interval limit is higher than that reported for the Dutch and South-West Uganda populations, respectively.

For platelets (PLT), this study's gender-independent lower reference interval limit is higher than the gender-dependent reference interval limit reported for Chinese population by Woo *et al.,* (1989), eastern Uganda[L] population by Lugada *et al.,* (2004), South-West Uganda[M] population by Mugisha *et al.,* (2016), and lower than that reported for Dutch population by Swaanerburg *et al.,* (1987), Australian population by Tsang *et al.,* (1998),Burkina Faso population by Böhler *et al.,* (2008), Oman population by Al-Mawali *et al*., (2018), and Further, this study's upper reference interval limit is higher than that reported for Dutch population by Swaanerburg *et al.,* (1987), Chinese population by Woo *et al.,* (1989), Australian population by Tsang *et al.,* (1998), eastern Uganda[L] population by Lugada *et al.,* (2004), Burkina Faso population by Böhler *et al.,* (2008), South-West Uganda[M] population by Mugisha *et al.,* (2016) and Oman population by Al-Mawali *et al.,* (2018).

For plateletcrit (PCT), this study's lower and upper reference interval limit is lower than that reported by Al-Mawali *et al.,* (2018) for Oman population. For platelet distribution width (PDW), this study's lower reference interval limit is similar to that reported by Al-Mawali *et al.,* (2018) for Oman population and the upper reference interval limit is higher than that reported by Al-Mawali *et al.,* (2018) for the same Oman population. For mean platelet volume (MPV), this study's lower and upper reference limit is similar to that reported by Al-Mawali *et al.,* (2018) for Oman population.

Table 4.22: Comparison of developed reference interval limits for hematological parameters for adult and geriatric population of Taita-Taveta County, Kenya with those reported in medical literature

Analyte (Unit)	Sex	This study RI	Dutch population	Uganda[L] Population	Burkina Faso Population	Uganda[M] Population	American Population 1	American Population 2	Chinese population	Oman population	Australian population
RBC ($x10^{12}$/L)	**M&F**	**2.8-5.7**									
	F	2.83-5.63	4.0-**5.6**	3.7-5.3	3.5-4.9	3.7-**5.4**	4.2-**5.4**	3.8-5.2	3.8-**5.4**	4.07-6.17	4.0-**5.4**
	M	2.81-6.11	4.4-**5.8**	3.8-**6.0**	3.8-**5.6**	3.6-**5.7**	4.7-6.1	3.8-**5.8**	4.0-**5.7**	4.45-6.75	4.2-**5.9**
HB (g/dL)	**M&F**	**6.6-16.6**									
	F	7.7-16.6	7.5-10.2	10.1-14.3	9.8-13.5	11.0-15.0	12-**16**	11.7-**16.1**	10.7-15.0	11-15.1	12.2-**16.1**
	M	6.5-17.4	8.7-11.1	11.1-**16.8**	11.3-15.6	10.6-**16.4**	14-18	12.6-**17.4**	12.1-**16.5**	12.4-**16.4**	13.1-17.5
PCV (%)	**M&F**	**0.2-44.9**									
	F	0.2-44.1	38-50	29.6-41.4	30-39	32.7-**45.4**	37-**47**	35-**47**	32-**45**	33-**43**	36-**47**
	M	0.2-47.1	42-54	32.2-47.8	34-**46**	32.4-49.1	42-52	37-51	36-49	36-**47**	39-51
MCH (pg)	**M&F**	**20.6-35.1**			***24.5-32.5***	25-33.4		***27-34.5***		20.81-31.2	***27-33.5***
	F	21.6-34.6			***24-32***			***27-35***			***27-33***
	M	17.7-37.6			***25-33***			***27-34***			***27-34***
MCHC (g/dL)	**M&F**	**27.3-36.1**	19-21		***31.6-36.2***	32-35.2		***31.5-36***		31-37.2	
	F	27.7-36.3			***31.4-36.1***			***32-36***			
	M	25.4-36.6			***31.7-36.3***			***31-36***			
MCV (fL)	**M&F**	**70.-106.2**	87-98		***74-93***	75-99		***81-102.5***	***70.5-96***	62.5-88.5	***80-98***
	F	69.2-107.1		67.7-92.6	***75-92***		81-99	***81-102***	***70-96***		***80-97***
	M	67.3-106.1		69.9-95.2	***73-94***		80-94	***81-103***	***71-96***		***80-99***
RDW-CV (%)	**M&F**	**11.4-18.6**								11.1-17.8	
	F	11.5-16.9									
	M	11.1-19.1									
RDW-SD (%)	M&F	**39.4-66.8**									
	F	39.1-70.9									
	M	39.8-64.2									
WBC ($x10^{9}$/L)	**M&F**	**2.8-14.6**		↑3.4-8.7↓	***3.3-8.3***		↑4.8-10.8↓		***4.6-11***	2.77-8.09↓	
	F	3.1-16.5	↑4-10↓		↑***3.4-7.4***↓	↑3.0-7.2↓			↑**4.5-11.5**↓		↑3.6-9.4↓
	M	2.5-14.0	↑4-11↓		↑***3.2-9.2***↓	↑3.2-7.8↓			↑**4.6-10.5**↓		↑3.9-9.5↓

NEU ($x10^9$/L)	M&F	0.9-11.4		↑0.84-3.37↓		↑1.05-3.78↓				↑0.9-4.64↓	
	F	**0.6-7.7**	↑1.7-6.0↓								
	M	**2.3-6.9**	↓1.7-6.7								
NEU (%)	M&F	26-88.22	↑45-75↓		***28.5-63***						
	F	**22.3-86**			↑***30-62***↓	↑28.1-58.5↓					
	M	**22.6-86**			↑***27-64***↓	↑26-62.4↓					
LYM ($x10^9$/L)	**M&F**	**0.8-5.0**	↑1.1-3.3↓	↑1.4-4.2↓	***1.4-3.6***	↑1.15-3.51↓				↑1.21-3.15↓	
	F	0.8-5.1			↑***1.4-3.2***↓						
	M	0.8-5.0			↑***1.3-4.0***↓						
LYM (%)	M&F	6.8-70	↑16-50↓		***23.5-58.5***	↑28.6-59.8↓					
	F	**10.2-72.7**			↑***27-57***↓						
	M	**10.2-63.6**			↑***20-60***↓						
MON ($x10^9$/L)	**M&F**	**0.01-0.98**		↑0.17-0.59↓		↑0.17-0.57↓				↑0.23-0.74↓	
	F	0.01-1.10	↑0.15-0.58↓								
	M	0.01-1.40	↑0.18-0.65↓								
MON (%)	**M&F**	**0.4-13.3**				↑4.1-10.7↓					
	F	0.4-14.47	↑7.8-8.5↓								
	M	0.24-13.4	↑3.4-8.3↓								
EOS ($x10^9$/L)	**M&F**	**0.02-0.77**		↑0.13-2.11↑		↑0.50-0.81				0.03-0.37↓	
	F	0.02-0.65	↑0.04-0.32↓								
	M	0.01-0.92	↑0.04-0.40↓								
EOS (%)	M&F	0.22-12.2				↑1.0-15.3↑					
	F	**0.27-10.8**	↑0.6-4.3↓								
	M	**0.20-11.92**	↑0.7-5.2↓								
BAS ($x10^9$/L)	**M&F**	**0.01-0.10**	0.02-0.11	0.01-0.07↓		0.02-0.08				0.002-0.058↓	
	F	0.01-0.09									
	M	0.04-0.98									
BAS (%)	**M&F**	**0.2-3.04**	↑0.3-1.5↓			↓0-2↓					
	F	0.17-4.75									
	M	0.12-1.44									

PLT ($x10^9$/L)	**M&F**	**116.3-513**									
	F	122.2-513	↑170-375↓	↓100-297↓	↑159-356↓	↓98-359↓			↓93-267↓	↑164-368↓	↑163-414↓
	M	115-562	↑160-350↓	↓80-288↓	↑127-365↓	↓60-336↓			↓95-224↓	↑146-347↓	↑153-382↓
PCT (%)	**M&F**	**0.1-0.4**									
	F	0.1-0.4								↑15-30↑	
	M	0.1-0.4								↑13-28↑	
PDW (fL)	**M&F**	**7.6-16.9**								7.7-7.4↓	
	F	7.3-17.0									
	M	7.1-17.0									
MPV (fL)	**M&F**	**6.9-10.9**								6.61-10.3	
	F	7.3-11.3									
	M	6.8-11.0									

American p**opu**lation 1 by Hale *et al.*, 1983; American po**pul**ation 2 by Yip *et al.*, 1984; Du**tch** population by Swaanerburg *et al.*, 1987; C**hin**ese population by Woo *et al.*, 1989; Au**stra**lian population by Tsang *et al.*, 1998; **rura**l parish in eastern Uganda[L] population by Lugada *et al.*, 2004; B**ur**kina Faso population by Böhler *et al.*, 2008; rural S**out**h-West Uganda[M] population by Mugisha *et al.*, 2016; O**ma**n population by Al-Mawali *et al.*, 2018.

4.10 Reference interval limits for serum biochemistry analytes for adult and geriatric population of Taita-Taveta County, Kenya.

4.10.1 Reference interval limits for liver and kidney function tests and electrolytes for adults and geriatrics of Taita-Taveta County, Kenya

The established median reference interval limits for liver and kidney function tests, and electrolytes for the adult and geriatric male population of Taita-Taveta County, Kenya for random blood glucose (RBS), total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (T-BIL), blood urea nitrogen (BUN), sodium (NA), potassium (K), and chloride (CL) was statistically similar to that of the female population of the same age range ($\rho > 0.05$). Therefore, combined male and female reference interval limits for these liver and kidney function tests, and electrolytes for this population were established. The established combined reference interval limits for liver and kidney function tests, and electrolytes for the adult and geriatric population of Taita-Taveta County, Kenya for RBS is 5.5 (4.2-10.8) mmol/L, TP is 74.15 (57.4-88.5) g/L, ALB is 44.1 (33.1-49.7) g/L, AST is 20.9 (8.3-66.5) U/L, ALP is 21 (0-147.6) U/L, T-BIL is 4.55 (1-19.7) µmol/L, BUN is 3.68 (1.8-8.6) mmol/L, NA is 138 (122.2-148.9) mmol/L, K is 4.28 (3.2-5.7) mmol/L, and CL is 97 (81.8-112.3) mmol/L.

The established median reference interval limit for liver and kidney function tests for adult and geriatric male population of Taita-Taveta County, Kenya for alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), direct bilirubin (D-BIL), and creatinine (CREAT) significantly differed from that of

adult and geriatric female population of the same age range ($\rho < 0.05$). The established reference interval limits for liver and kidney function tests for this adult and geriatric population of Taita Taveta County, Kenya, for ALT is 15 (4.8-52) U/L for males and 13.5 (5.9-37) U/L for females ($U = 9617.5$, $z = -2.522$, $\rho = 0.012$, $r = 0.1446$), GGT is 27 (9-186.3) U/L for males and 21.5 (7.6-160.5) U/L for females ($U = 9396$, $z = -2.811$, $\rho = 0.005$, $r = 0.1612$), D-BIL is 2 (0-6) µmol/L for males and 4 (0-6) µmol/L for females ($U = 9869$, $z = -2.196$, $\rho = 0.028$, $r = 0.1259$), and CREAT is 77.5 (44.3-163.5) µmol/L for males and 72 (34.1-134.8) µmol/L for females ($U = 9599.5$, $z = -2.546$, $\rho = 0.011$, $r = 0.1460$) (Table 4.23).

Table 4.23: Results of the normality statistics of serum biochemistry analytes for adults and geriatrics of Taita-Taveta County, Kenya

Analyte (unit)	Sex	N	Mean±SD (Median)	Percentile		Reference Interval	IV	Difference between M & F	
				2.5^{th}	97.5^{th}			Z value	Sig
RBS (mmol/L)	M&F	310	6.05±1.94					-1.191	ρ = 0.234
			5.5	**4.2**	**10.82**	**4.2-10.8**	**6.6**		
	F	178	6.05±2.11						
			6.05	4.2	10.90	4.2-10.9	6.7		
	M	132	6.05±1.70						
			5.7	3.93	10.31	3.9-10.3	6.4		
TP (g/L)	M&F	304	73.87±7.01					-1.863	ρ = 0.062
			74.15	**57.41**	**88.50**	**57.4-88.5**	**31.1**		
	F	154	74.31±7.61						
			75	56.88	88.25	56.9-88.3	31.4		
	M	150	73.46±6.41						
			73	61.33	89	61.3-89	27.7		
ALB (U/L)	M&F	304	43.23±4.33					-0.555	ρ = 0.576
			44.1	**30.07**	**49.65**	**33.1-49.7**	**16.6**		
	F	154	43.23±3.93						
			44	30.88	49	30.9-49.0	18.1		
	M	150	43.23±4.79						
			44	29.78	51.23	29.9-51.2	21.3		
ALT (U/L)	M&F	304	17.73±19.91					-2.522	ρ = 0.012
			14.2	5.13	44.4	5.1-44.4	39.3		
	F	154	15.39±8.21						
			13.5	**5.88**	**37**	**5.9-37**	**31.1**		
	M	150	20.14±26.91						
			15	**4.78**	**52**	**4.8-52**	**47.2**		
AST (U/L)	M&F	304	24.32±15.35					-1.728	ρ = 0.084
			20.9	**8.3**	**66.54**	**8.3-66.5**	**58.2**		
	F	154	22.68±12.35						

			20	8	50	8-50	42		
	M	150	26.00±17.81						
			22	9	93.8	9-93.8	84.8		
ALP (U/L)	M&F	304	42.12±50.97					-0.277	ρ = 0.781
			21	**0**	**147.56**	**0-147.6**	**147.6**		
	F	154	43.66±52.88						
			19	0	160.5	0.0-160.5	160.5		
	M	150	40.21±49.01						
			19.5	0	138.58	0.0-138.6	138.6		
GGT (U/L)	M&F	304	36.36±38.16					-2.811	ρ = 0.005
			23.85	8.8	174.25	8.8-174.3	165.5		
	F	154	31.97±32.84						
			21.5	**7.63**	**160.5**	**7.6-160.5**	**152.9**		
	M	150	40.98±42.57						
			27	**9**	**186.25**	**9-186.3**	**177.3**		
T-BIL (μmol/L)	M&F	304	6.50±10.91					-1.491	ρ = 0.136
			4.55	**1.0**	**19.7**	**1-19.7**	**18.7**		
	F	154	6.77±14.63						
			4	1	20	1-20	19		
	M	150	6.25±4.77						
			5	1	20	1-20	19.3		
D-BIL (μmol/L)	M&F	304	1.91±1.62					-2.196	ρ = 0.028
			1.6	0.26	5.81	0.3-5.8	5.5		
	F	154	1.76±1.54						
			4	**0**	**6**	**0-6**	**6**		
	M	150	2.05±1.74						
			2	**0**	**6**	**0-6**	**6**		
BUN (mmol/L)	M&F	304	4.30±4.68					-0.317	ρ = 0.751
			3.68	**1.84**	**8.59**	**1.8-8.6**	**6.8**		
	F	154	4.33±5.35						

			4	2	8.38	2-8.4	6.4		
	M	150	4.25±3.90						
			4	2	11.02	2-11	9		
CREAT (µmol/L)	M&F	304	80.68±37.34					-2.546	ρ = 0.011
			75	41	144.5	41-144.5	103.5		
	F	154	77.51±41.55						
			72	**34.13**	**134.75**	**34.1-134.8**	**100.7**		
	M	150	83.93±32.28						
			77.5	**44.33**	**163.45**	**44.3-163.5**	**119.2**		
NA (mmol/L)	M&F	304	137.63±6.61					-0.160	ρ = 0.873
			138	**122.19**	**148.9**	**122.2-148.9**	**26.7**		
	F	154	137.69±5.83						
			138	123.4	148.9	123.4-148.9	25.2		
	M	150	137.66±7.35						
			138	119.3	155	119.3-155	35.7		
K (mmol/L)	M&F	304	4.40±1.64					-0.927	ρ = 0.354
			4.28	**3.2**	**5.68**	**3.2-5.7**	**2.5**		
	F	154	4.52±2.26						
			4	3	6	3-6	3		
	M	150	4.25±0.63						
			4	3	5.22	3-5.2	2.2		
CL (mmol/L)	M&F	304	97.33±8.54					-0.014	ρ = 0.989
			97	**81.8**	**112.31**	**81.8-112.3**	**30.5**		
	F	154	97.55±8.24						
			97	82.88	114.34	82.9-114.3	31.4		
	M	150	97.11±8.91						
			97	79.88	113.15	79.9-113.2	33.3		

Results are expressed as mean ± standard deviation (SD), and median and 95% range for the number of referents in the column labeled N. Statistical comparisons of the median reference interval limits between male and female referents were carried out using the Mann-Whitney U test. Differences were considered statistically significant at $\rho < 0.05$.

4.10.2 Effect of age and gender on the developed reference interval limits for liver and kidney function tests, and electrolytes for adult and geriatric population of Taita-Taveta County, Kenya

The effects of age on the established reference interval limits for liver and kidney function tests, and electrolytes for the adult and geriatric population of Taita-Taveta County, Kenya, was assessed by categorizing the results into three age categories as follows: (a) age ≥ 50-60 years, (b) age ≥ 60-70 years, and (c) age ≥ 70-95 years. Significant reference interval limits differences between males and females for the measured analytes were estimated by the Mann-Whitney U test within each age category. Significant reference interval limits differences for the measured analytes within and between the three age categories were estimated using the Kruskal-Wallis H test followed by the Mann-Whitney U test with Bonferroni correction where ρ-values less than 0.0167 was considered statistically significant. The Mean ± SD, Median and 95 % range, sex and age categories are presented in Table 4.24.

Among the measured analytes, total protein (TP), serum albumin (ALB), γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), total bilirubin (T-BIL), direct bilirubin (D-BIL), blood urea nitrogen (BUN), potassium (K), and chloride (CL) are not significantly affected by advancement in age for the studied adults and geriatrics of Taita-Taveta County, Kenya ($\rho > 0.05$). However, the other measured analytes, including random blood glucose (RBS), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine

(CREAT), and sodium (NA) are significantly affected by advancement in age for adults and geriatrics of Taita-Taveta County, Kenya ($\rho > 0.05$).

A Kruskal-Wallis H test for random blood glucose (RBS) for the study male and female referent participants was significantly affected by advancement in age (χ^2 (2) = 12.113, ρ = 0.002). Pairwise comparisons using the Mann-Whitney U test with an adjusted significant ρ-value of less than 0.0167 indicates that there was a significant increase in median random blood glucose (RBS) levels between the fifth decade (5.2 (3.82-10.94) mmol/L) with a mean rank of 116.41 and the sixth decade (5.6 (4.4-15.73) mmol/L) with a mean rank of 143.91 (U = 6604, z = -2.955, ρ = 0.003, r = 0.1836), and between the fifth decade (5.2 (3.82-10.94) mmol/L) with a mean rank of 84.62 and the seventh decade onwards (6 (4.4-10.35) mmol/L) with a mean rank of 109.91 (U = 2439.5, z = -2.825, ρ = 0.005, r = 0.2094). However, there was no significant difference in the median random blood glucose (RBS) levels between the sixth decade and the seventh decade onwards.

A Kruskal-Wallis H test for random blood glucose (RBS) for all the study female referent participants was significantly affected by advancement in age (χ^2 (2) = 9.057, ρ = 0.011). Pairwise comparisons using the Mann-Whitney U test with adjusted significant ρ-value of less than 0.0167 indicates that there was a significant increase in median random blood glucose (RBS) levels between the fifth decade (5 (4-11) mmol/L) with mean rank 66.66 and the sixth decade (6 (4-16) mmol/L) with a mean rank of 84.65 (U = 1971, z = -2.613, ρ = 0.009, r = 0.2155). However, there was no statistically significant difference

in the female median random blood glucose (RBS) levels between the fifth decade with a mean rank of 55.36 and the seventh decade onwards (z = -2.281, ρ = 0.023), and between the sixth decade and the seventh decade onwards (z = -0.065, ρ = 0.948). A Kruskal-Wallis H test for random blood glucose (RBS) for the study male referent participants was not statistically significantly affected by advancement in age (χ^2 (2) = 1.167, ρ = 0.558). There was, therefore, no need for a follow-up pairwise comparison using the Mann-Whitney U test with adjusted ρ-value.

A Kruskal-Wallis H test for alanine aminotransferase (ALT) activity for the study combined male and female referent participants was significantly affected by advancement in age (χ^2 (2) = 9.949, ρ = 0.007). Pairwise comparisons using the Mann-Whitney U test with adjusted significant ρ-value of less than 0.0167 indicates that there was a significant decrease in median alanine aminotransferase (ALT) activity between the fifth decade (15.6 (5.3-45.8) U/L) with a mean rank of 110.83 and the seventh decade onwards (12.9 (4.21-36.88) U/L) with a mean rank of 85.33 (U = 3590.5, z = -3.059, ρ = 0.002, r = 0.2163). However, there was no significant difference in the median alanine aminotransferase (ALT) activity between the fifth decade, with a mean rank of 118.03 and the sixth decade with a mean rank of 105.10 (z = -1.494, ρ = 0.135), and between the sixth decade with a mean rank of 99.54 and the seventh decade onwards with a mean rank of 84.60 (z = -1.882, ρ = 0.060).

A Kruskal-Wallis H test for alanine aminotransferase (ALT) activity for the study male referent participants was significantly affected by advancement in

age (χ^2 (2) = 13.379, ρ = 0.001). Pairwise comparisons using the Mann-Whitney U test with an adjusted significant ρ-value of less than 0.0167 indicates that there was a significant decrease in median alanine aminotransferase (ALT) levels between the fifth decade (18 (5-52 U/L) with a mean rank of 61.65 and the sixth decade (14 (5.48-194.7) U/L) with a mean rank of 45.99 (U = 956.5, z = -2.625, ρ = 0.009, r = 0.2562) and between the fifth decade (18 (5-52 U/L) with a mean rank of 55.84 and the seventh decade onwards (13 (4-18.49) U/L) with a mean rank of 36.74 (U = 618.5, z = -3.437, ρ = 0.001, r = 0.3583). However, there was no significant difference in the median alanine aminotransferase (ALT) levels between the sixth decade, with a mean rank of 55.55 and the seventh decade onwards with a mean rank of 47.42 (z = -1.374, ρ = 0.384). A Kruskal-Wallis H test for alanine aminotransferase (ALT) for the study female referent participants was not significantly affected by advancement in age (χ^2 (2) = 2.436, ρ = 0.296). There was, therefore no need for a follow-up pairwise comparison using the Mann-Whitney U test with adjusted ρ-value.

A Kruskal-Wallis H test for aspartate aminotransferase (AST) activity for the study combined male and female referent participants was significantly affected by advancement in age (χ^2 (2) = 7.047, ρ = 0.029). Pairwise comparisons using Mann-Whitney U test with adjusted significant ρ-value of less than 0.0167 indicates that there was a significant decrease in median aspartate aminotransferase (AST) activity between the fifth decade (23.6 (8.6-51.5) U/L) with a mean rank of 122.51 and the seventh decade onwards (19.3

(8.3-101.9) U/L) with a mean rank of 99.97 (U = 4937, z = -2.603, ρ = 0.009, r = 0.1743). However, there was no significant difference in the median aspartate aminotransferase (ALT) levels between the fifth decade with a mean rank of 105.93 and the sixth decade with a mean rank of 92.52 (z = -1.608, ρ = 0.108), and between the sixth decade with a mean rank of 90.30 and the seventh decade onwards with a mean rank of 96.460 (z = -0.776, ρ = 0.438).

A Kruskal-Wallis H test for aspartate aminotransferase (AST) activity for the study female referent participants was not significantly affected by advancement in age (χ^2 (2) = 4.968, ρ = 0.083). Further, a Kruskal-Wallis H test for aspartate aminotransferase (AST activity for the study male referent participants was not significantly affected by advancement in age (χ^2 (2) = 3.799, ρ = 0.150). There was therefore no need for a follow-up of separate male and female aspartate aminotransferase (AST) activity pairwise comparison using the Mann-Whitney U test with adjusted ρ-value.

A Kruskal-Wallis H test for creatinine (CREAT) level for the study female referent participants was significantly affected by advancement in age (χ^2 (2) = 6.417, ρ = 0.040). Pairwise comparisons using the Mann-Whitney U test with adjusted significant ρ-value of less than 0.0167 indicates that there was a significant decrease in median creatinine (CREAT) levels between the fifth decade (76 (32.09-209.65) μmol/L) with a mean rank of 59.72 and the seventh decade onwards (65.5 (49-78.75) μmol/L) with a mean rank of 44.07 (U = 920.5, z = -2.488, ρ = 0.014, r = 0.2394). However, there was no significant difference in the median creatinine (CREAT) levels between the fifth decade

with a mean rank of 62.48 and sixth decade with a mean rank of 54.84 (z = -1.184, ρ = 0.236) and between the sixth decade with a mean rank of 44.89 and the seventh decade onwards with a mean rank of 37.17 (z = -1.458, ρ = 0.145).

A Kruskal-Wallis H test for creatinine (CREAT) level for the study combined male and female referent participants was not significantly affected by advancement in age (χ^2 (2) = 5.600, ρ = 0.061). Further, a Kruskal-Wallis H test for creatinine (CREAT) for the study of male referent participants was not significantly affected by advancement in age (χ^2 (2) = 2.406, ρ = 0.300). There was, therefore no need for a follow-up pairwise comparison for combined male and female referents, and male-only referents using the Mann-Whitney U test with adjusted ρ-value.

A Kruskal-Wallis H test for sodium (NA) level for the study combined male and female referent participants was significantly affected by advancement in age (χ^2 (2) = 13.386, ρ = 0.001). Pairwise comparisons using Mann-Whitney U test with adjusted significant ρ-value of less than 0.0167 indicates that there was a significant decrease in median sodium (NA) levels between the fifth decade (139.7 (127.7-148.9) mmol/L) with mean rank of 112.40 and the seventh decade onwards (136.8 (114.2-154.26) mmol/L) with mean rank of 83.01 (U = 3403, z = -3.526, ρ = 0.000, r = 0.2493), and between the sixth decade (138.1 (117.69-151.04) mmol/L) with mean rank of 102.32 and the seventh decade onwards (136.8 (114.2-154.26) mmol/L) with mean rank of 81.03 (U = 3242.5, z = -2.683, ρ = 0.007, r = 0.1973. However, there was no significant difference in the median sodium (NA) levels between the fifth

decade with mean rank of 116.25 and the sixth decade with mean rank of 107.13 ($z = -1.053$, $\rho = 0.292$).

A Kruskal-Wallis H test for sodium (NA) level for the study female referent participants was significantly affected by advancement in age (χ^2 (2) = 16.865, $\rho = 0.000$). Pairwise comparisons using the Mann-Whitney U test with n adjusted significant ρ-value of less than 0.0167 indicates that there was a significant decrease in median sodium (NA) levels for female referents between the fifth decade (139.75 (126.31-149.08) mmol/L) with mean rank of 63.14 and the seventh decade onwards (135 (114-138.75) mmol/L) with mean rank of 37.22 ($U = 674$, $z = -4.064$, $\rho = 0.000$, $r = 0.3911$), and between the sixth decade (138 (123.05-149) mmol/L) with mean rank of 55.26 and the seventh decade onwards (135 (114-138.75) mmol/L) with mean rank of 47.80 ($U = 533.5$, $z = -2.759$, $\rho = 0.006$, $r = 0.3947$). However, there was no significant difference in the median sodium (NA) levels for female referents between the fifth decade with mean rank of 62.61 and the sixth decade with mean rank of 54.63 ($z = -1.240$, $\rho = 0.215$).

A Kruskal-Wallis H test for sodium (NA) level for the study male referent participants was not significantly affected by advancement in age (χ^2 (2) = 1.888, $\rho = 0.389$). There was therefore no need for a follow-up for sodium (NA) pairwise comparison using the Mann-Whitney U test with adjusted ρ-value of less than 0.0167 (Table 4.24).

Results on the effect of gender on the developed reference interval limits indicate that in the fifth decade, the median reference interval limits for alanine aminotransferase (ALT) activity for males (18 (5-52) U/L) with mean rank of 71.71 is significantly higher than that of females (14.1 (5.63-41.1) U/L) with mean rank of 52.35 of Taita-Taveta County, Kenya ($U = 1141.5$, $z = -2.996$, $\rho = 0.003$, $r = 0.2747$). Further, in the fifth decade, the median reference interval limits for direct bilirubin (D-BIL) levels for males (2 (1-7.2) mmol/L) with mean rank of 69.34 is significantly higher than that of females (1.4 (0.1-4.31) mmol/L) with mean rank of 53.90 of Taita-Taveta County, Kenya ($U = 1253$, $z = -2.535$, $\rho = 0.011$, $r = 0.2324$). In the seventh decade onwards, the median reference interval limits for creatinine (CREAT) levels for males (76 (30.55-103.65) μmol/L) with mean rank of 45.96 is significantly higher than that of females (65.5 (49-78.75) μmol/L) with mean rank of 34.81 of Taita-Taveta County, Kenya ($U = 587$, $z = -2.120$, $\rho = 0.034$, $r = 0.2356$) (Table 4.24).

Table 4.24: Effect of age and gender on the developed median reference interval limits for liver and kidney function tests and electrolytes for adults and geriatrics of Taita-Taveta County, Kenya

Analyte (units)	Gender	N	≥ 50-60 years	N	≥ 60-70 years	N	≥ 70-95 years
RBS (mmol/L)	M&F	131	5.75±1.96	128	6.33±2.15	51	6.13±1.31
			5.2 (3.9-10.9)		**5.6 (4.4-15.7)[a]**		**6 (4.4-10.4)[b]**
	M	44	5.82±1.45	68	6.22±2.39	20	6.05±1.10
			6 (4-10.8)		**6 (4-16)**		**6 (5-7)**
	F	87	5.71±2.19	60	6.50±2.39	31	6.19±1.54
			5 (4-11)		**6 (4-16)[a]**		**6 (4-7)**
TP (g/L)	M&F	119	74.12±8.05	104	74.02±5.91	81	73.31±6.73
			74.3 (56.6-89.9)		**74.9 (60.4-87.1)**		**73.2 (51.5-91.3)**
	M	47	74.02±5.99	58	73.41±6.35	45	72.93±6.98
			74 (58.6-85.6)		**73 (54.2-89)**		**73 (52.7-76)**
	F	72	74.20±9.17	46	74.83±5.34	36	73.81±6.66
			74.9 (51.0-92.2)		**76 (60.4-85.48)**		**74.5 (51-77.5)**
ALB (U/L)	M&F	119	43.33±4.48	104	43.30±4.53	81	42.98±3.86
			44.3 (29.9-51.68)		**44.1 (28.75-49.91**)		**44 (30.8-48.2)**
	M	47	43.30±5.37	58	42.97±5.05	45	43.49±3.78
			44 (29.2-52.8)		**44 (28-50.5)**		**44 (30.3-49)**
	F	72	43.41±3.86	46	43.78±3.92	36	42.25±3.95
			44.3 (29.8-49.2)		**44 (30.1-50.7**)		**44 (31-45)**
ALT (U/L)	M&F	119	18.61±10.60	104	19.00±31.17	81	14.84±8.56
			15.6 (5.3-45.8)		**14.2 (5.6-45.6)**		**12.9 (4.2-36.9)↓[b]**
	M	47	22.38±12.26	58	21.95±41.08	45	15.47±8.73
			18 (5-52)*		**14 (5.5-194.7)↓[a]**		**13 (4-18.5)↓[b]**

	F	72	16.16±8.65	46	15.17±7.41	36	14.11±8.34
			14.1 (5.6-41.1)		**14 (4.35-35.83**)		**12.5 (4-17.3)**
AST (U/L)	M&F	119	25.14±13.10	104	23.44±17.24	81	24.25±15.97
			23.6 (8.6-51.5)		**19.3 (8.3-101.9)[a]**		**20.9 (8.3-89.1)**
	M	47	27.28±17.13	58	24.74±17.38	45	26.29±19.29
			24 (6.6-108.8)		**21.5 (12-106.2)**		**22 (9.2-33)**
	F	72	23.73±9.34	46	21.74±17.10	36	21.69±9.97
			22.8 (8.6-50.4)		**18 (8-106.9)**		**20 (8-25.4)**
ALP (U/L)	M&F	119	36.30±45.64	104	42.37±49.82	81	49.85±58.74
			3.9 (0-143.8)		**16.9 (0-160.5)**		**43.9 (0-265.6)**
	M	47	36.66±43.49	58	38.43±44.64	45	46.22±59.33
			16 (0-144)		**15 (0-129.9)**		**44 (0-69)**
	F	72	36.52±47.47	46	47.24±55.79	36	54.44±58.43
			1.4 (0-155.4)		**34 (0-202.9)**		**54 (0-74.8)**
GGT (U/L)	M&F	119	38.13±42.06	104	33.60±28.34	81	37.30±43.08
			23.8 (8.2-178)		26.25 (8.3-135.3)		21.5 (8.9-214.4)
	M	47	47.96±51.94	58	35.12±27.93	45	41.24±47
			27 (8.2-241.6)		**27.5 (9.43-147.73)**		**23 (10.3-57.5)**
	F	72	31.73±32.96	46	31.80±29.01	36	32.58±37.8
			22.4 (7.62-178)		**23 (5.7-150)**		**21 (5-49.3)**
T-BIL (µmol/L)	M&F	119	6.25±12.53	104	5.97±4.97	81	7.57±13.64
			4.6 (2.9-17.9)		**4.25 (1-20.4)**		**4.9 (1-19.6)**
	M	47	6.02±3.96	59	6.43±5.63	45	6.24±4.42
			5 (2-18)		**4 (1-22.6)**		**5 (1-8.5)**
	F	72	6.41±15.84	46	5.39±4.03	36	9.25±19.89

			4.1 (1.1-33.6)		**4 (1-19.3)**		**5 (1-18.7)**
D-BIL (µmol/L)	M&F	119	1.69±1.13	104	2.07±1.89	81	2.02±1.83
			1.5 (0.3-4.4)		**1.6 (0.3-5.8)**		**1.8 (0.2-6.3)**
	M	47	2.00±1.29	59	2.28±2.15	45	1.80±1.55
			2 (1-7.2)*		**2 (0-10.7)**		**2 (0-2.5)**
	F	72	1.49±1.00	46	1.91±1.47	36	2.19±2.20
			1.4 (0.1-4.3)		**2 (0-6)**		**2 (0-3.1)**
BUN (mmol/L)	M&F	119	4.74±6.66	104	4.03±2.98	81	3.97±2.38
			3.9 (1.9-11.7)		**3.6 (1.9-7.9)**		**3.4 (1.7-10.8**)
	M	47	4.60±4.96	58	4.10±3.75	45	4.07±2.72
			4 (2-31)		**3 (1.5-19.6)**		**4 (1.2-5)**
	F	72	4.79±7.58	46	3.98±1.54	36	3.89±1.92
			3.9 (1.6-21.3)		**4 (2-8)**		**3 (2-4.8)**
CREAT (µmol/L)	M&F	119	84.84±46.72	104	76.89±22.39	81	79.42±37.02
			80 (39-147)		**74 (28.1-134.8**)		**70 (45.2-193.9)**
	M	47	85.68±27.41	58	79.59±21.02	45	87.69±46.14
			88 (43.2-199.2)		**76 (43.4-145.5)**		**76 (30.6-103.7)***
	F	72	84.30±56.04	46	73.50±23.79	36	69.08±16.08
			76 (32.1-209.7)		**70 (4.5-131.6**)		**65.5 (49-78.8)[b]**
NA (mmol/L)	M&F	119	138.67±5.05	104	138.09±7.18	81	135.52±7.43
			139.7 (127.7-148.9)		**138.1 (117.7-151)**		**136.8 (114.2-154.3)[bc]**
	M	47	137.89±4.95	58	138.24±8.48	45	136.7±7.92
			139 (128-145)		**139 (110-164)**		**138 (111.8-141)**
	F	72	139.23±5.01	46	138.00±5.29	36	134.14±6.58
			139.8 (126.3-149.1)		**138 (123.1-149)**		**135 (114-138.8)[bc]**

K (mmol/L)	M&F	119	4.34±0.57	104	4.50±2.69	81	4.38±0.56
			4.3 (3.2-5.5)		**4.21 (3.2-6**)		**4.3 (3.2-5.7)**
	M	47	4.38±0.61	104	4.19±0.66	81	4.21±0.60
			4 (3-5.8)		**4 (3-5.5)**		**4 (3-5.9)**
	F	72	4.34±0.61	58	4.83±4.00	45	4.20±0.59
			4.4 (3.1-5.6)		**4 (3-26.6)**		**4 (3-5.9)**
CL (mmol/L)	M&F	119	96.89±7.93	104	97.53±8.05	81	97.70±9.96
			96.4 (82.5-111.7)		**97.8 (81-110.8)**		**97.5 (78.5-124.2**)
	M	47	95.49±7.46	58	97.74±9.06	45	98±10
			96 (81.2-108.8)		**97.5 (76-125.2)**		**100 (76.7-104)**
	F	72	97.82±8.14	46	97.26±6.79	36	97.31±10.02
			96.6 (85-114.9)		**98 (84.2-111.7)**		**96.5 (78-102.8)**

Results are expressed as mean ± standard deviation (SD), and median and 95% range of the number of subjects indicated in the column labeled N. $^{*}\rho < 0.05$ when male reference interval limits are significantly different when compared to female reference interval limits per each age category by Mann-Whitney U test; $^{a}\rho < 0.0167$ when reference interval limits in age range ≥ 55-60 years is significantly different when compared to reference interval limits in age range ≥ 60-70 years, $^{b}\rho < 0.0167$ when reference interval limits in age range ≥ 55-60 years is significantly different when compared to reference interval limits in age range ≥70-95 years, and $^{c}\rho < 0.0167$ when reference interval limits in age range ≥ 60-70 years is significantly different when compared to reference values in age range ≥ 70-95 years by Kruskal-Wallis H test followed by Mann-Whitney U test with Bonferroni correction

4:10.3 Comparison of developed reference interval limits for liver and kidney function tests and electrolytes for the adult and geriatric population of Taita-Taveta County, Kenya, with those reported in the medical literature

A comparison of the developed reference interval limits for liver and kidney function tests and electrolytes for the adult and geriatric population of Taita-Taveta County, Kenya with those reported in the medical literature is presented in Table 4.25. For the liver function tests, results indicate that: the combined male and female lower reference interval limit for random blood glucose (RBS) of the Taita-Taveta population is similar to that of the Canadian separate male and female population while the upper reference interval limit is higher than that of the Canadian population. The combined male and female lower reference interval limit for total protein (TP) of the Taita-Taveta population is lower than that of combined males and females of the Chinese and Canadian population while the upper reference interval limit is similar to that of the Chinese and higher than that of the Canadian **po**pulation. The combined male and female lower reference interval limit for serum albumin (ALB) of the Taita-Taveta population is lower than that of combined males and females of the Chinese and Canadian population while the upper reference interval limit is similar to that of the Chinese and Cana**dia**n population. However, the separate male and female lower reference interval limits for albumin (ALB) reported for Asmara Eritreans, while the upper reference interval limit is similar for males

and higher for females than that of the combined male and female reference interval limit for Taita-Taveta county po**pu**lation.

The separate male and female lower reference interval limit for alanine aminotransferase (ALT) of the Taita-Taveta population is lower than that of separate male and female of the Chinese and Canadian population while the upper reference interval limit is similar to that of the Chinese male population but lower than that of the Chinese female population and lower than that of the separate Canadian male and female population. The combined male and female lower reference interval limit for aspartate aminotransferase (AST) of the Taita-Taveta population is lower than that of the Canadian and Asmara Eritrean combined male and female population, while the upper reference interval limit is higher than that of the Canadian and Asmara Eritrean population.

The combined male and female lower reference interval limit for alkaline phosphatase (ALP) of the Taita-Taveta population is lower than that of Chinese and Asmara Eritrean combined male and female population and Canadian separate male and female population while the upper reference interval limit is lower than that of the Chinese combined and Canadian separate male and female population, respectively, but similar to that of As**ma**ra Eritreans. The combined male and female lower reference interval limit for gamma-glutamyltransferase (GGT) of the Taita-Taveta population is lower than that of the Canadian separate male and female population, lower than **that of the male** and lower than that of the female Chinese population, respectively, while the

upper reference interval limit is higher than that of the Chinese and Canadian separate male and female populations.

The combined male and female lower reference interval limit for total bilirubin (T-BIL) of the Taita-Taveta population is lower than that of the Chinese, Canadian and Asmara Eritrean separate male and female populations while the upper reference interval limit is lower than that of the separate Chinese male and female population, similar to the male and higher than the female Canadian populations, respectively, and lower than the male and similar to the female Asmara Eritreans, respectively. The combined male and female lower reference interval limit for direct bilirubin (D-BIL) of the Taita-Taveta population is similar to that of Asmara Eritreans separate males and females. In contrast, the upper reference interval limits are lower.

For the kidney function tests, results indicate that: the combined male and female lower reference interval limit for blood urea nitrogen (BUN) of the Taita-Taveta population is similar to that of the Canadian combined male and female population but lower than that of Asmara Eritreans while the upper reference interval limit is higher than that of the Canadian population but similar to that of Asmara Eritreans. The separate male and female lower reference interval limit for creatinine (CREAT) for the Taita-Taveta population is lower than that of the Canadian and Asmara Eritrea male and female population while the upper reference interval limit is higher than that for Canadian male and female population and higher than that of the male and lower than that of the female Asmara Eritreans.

The combined male and female lower reference interval limit for sodium (NA) for the Taita-Taveta population is lower than that of the Canadian, Chinese Han, and Asmara Eritrea separate male and female population, respectively. In contrast, the upper reference interval limit is similar to those of Canadian, Chinese Han and Asmara Eritrea separate male and female population. The combined male and female lower reference interval limit for potassium (K) for the Taita-Taveta population is lower than that of the Canadian, Chinese Han and Asmara Eritrea population. In contrast, the upper reference interval limit is higher than that of the Canadian and Chinese Han populations but similar to the Asmara Eritrea population. The combined male and female lower reference interval limit for chloride (CL) for the Taita-Taveta population is higher than that of the Canadian population, but lower than that of Chinese Han and Asmara Eritrea populations while the upper reference interval limit is higher than that of the Canadian population but similar to those of Chines Han and Asmara Eritrea populations.

Table 4.25: Comparison of developed reference interval limits for liver and kidney function tests and electrolytes for adult and geriatric population of Taita-Taveta County, Kenya with those reported in medical literature

Analyte (unit)	Gender	This study RI	Chinese population	Canadian population	Han population	Eritrea population
RBS (mmol/L)	**M&F**	**4.2-10 8**				
	F			4.4-5.9↓		
	M			4.2-6.4↓		
TP (g/L)	M&F	**57.4-88.5**	↑61.9-85.1	↑65-78↓		
	F					
	M					
ALB (U/L)	M&F	**33.1-49.7**	↑43.4-54.6	↑42-50		
	F					↑41-55↑

	M					↑39-52
ALT (U/L)	M&F					↑9-35
	F	**5.9-37**	↑9-60↑	↑16-44↑		
	M	**4.8-52**	↑9-50	↑20-62↑		
AST (U/L)	M&F	**8.3-66.5**		↑18-39↓		↑13.8-33.2↓
	F					
	M					
ALP (U/L)	**M&F**	**0-147.6**	↑49-134↓			↑55-156
	F			↑40-122↓		
	M			↑50-116↓		
GGT (U/L)	M&F					
	F	**7.6-160.5**	↓7-42↓	↑10-54↓		
	M	**9-186.3**	↑10-58↓	↑13-109↓		
T-BIL (μmol/L)	M&F	**1-19.7**				
	F		↑5-23.8↑	↑1.7-17↓		↑6.84-20.52
	M		↑5.9-30.4↑	↑1.7-20.5		↑6.84-28.91↑
D-BIL (μmol/L)	M&F	**0-6**				
	F	0-6				0-4.28↓
	M	0-6				0-5.13↓
BUN (mmol/L)	M&F	**1.8-8.6**		1.7-4.3↓		↑2.54-7.86
	F					
	M					
CREAT (μmol/L)	M&F					
	F	**34.1-134.8**		↑53-88↓		↑61.9-167.9↑
	M	**44.3-163.5**		↑62-106↓		↑53.0-132.6↓
NA (mmol/L)	M&F	**122.2-148.9**		↑136-143	↑136-146	
	F					↑134-148
	M					↑135-145
K (mmol/L)	M&F	**3.2-5.7**		↑3.8-4.9↓	↑3.6-5.2↓	↑3.6-5.3
	F					
	M					
CL (mmol/L)	M&F	**81.8-112.3**		↓28.8-30.5↓	↑99-110	↑101-113
	F					
	M					

Chinese population by Mu *et al.*, 2013; Canadian population by Adeli *et al.*, 2015; Chinese Han population by Jia et al., 2015; Asmara, Eritrea population by Achila *et al.* (2017).

4.11 Reference intervals for fasting lipid profiles for the adult and geriatric population of Taita-Taveta county, Kenya

4.11.1 Reference interval limits for fasting lipid profile for adults and geriatrics of Taita Taveta County, Kenya

The established median reference interval for triacylglycerols (TG), and the ratio of low-density lipoprotein cholesterol (LDL-Chol): high-density lipoprotein cholesterol (HDL-Chol) for male adult and geriatric population of Taita-Taveta County, Kenya were statistically similar to those of the female population of the similar age range ($\rho > 0.05$). Therefore, a combined reference interval of these parameters for this population was established. The established median reference interval limits for adults and geriatrics of Taita-Taveta County, Kenya for low-density lipoprotein cholesterol (LDL-Chol) is 3 (1-6) mmol/L, triacylglycerols (TG) is 2 (1-4.2) mmol/L, and low-density lipoprotein cholesterol (LDL-Chol): high-density lipoprotein cholesterol (HDL-Chol) ratio is 3 (1-6).

However, the established median reference interval for the adult and geriatric male population of Taita-Taveta County, Kenya for total cholesterol (T-Chol), high-density lipoprotein cholesterol (HDL-Chol), total cholesterol (T-Chol): high-density lipoprotein cholesterol (HDL-Chol) ratio, and triacylglycerol (TG): high-density lipoprotein cholesterol (HDL-Chol) ratio was statistically significantly different from that of the female population of the similar age range ($\rho < 0.05$). The established median reference interval limits for the adult and geriatric population of Taita-Taveta County, Kenya for total cholesterol

(T-Chol) for males (5 (2-7.9) mmol/L) with mean rank of 125.57 are significantly lower than those for females (5 (2-8) mmol/L) with mean rank of 144.67 (U = 7819, z = -2.061, ρ = 0.039, r = 0.1252), high density lipoprotein cholesterol (HDL-Chol) for males (1 (0-2) mmol/L) with mean rank of 121.81 are significantly lower than those for females (1 (0-2.28) mmol/L) with mean rank of 147.79 (U = 7356.5, z = -3.579, ρ = 0.000, r = 0.2174), total cholesterol (T-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio for males (4 (2-10.9)) with mean rank of 146.76 are significantly higher than those for females (4 (1.73-8)) with mean rank of 127.06 (U = 7778.5, z = -2.099, ρ = 0.036, r = 0.1275), and triacylglycerols (TG): high density lipoprotein cholesterol (HDL-Chol) ratio for males (1 (0-6.9)) with mean rank of 145.96 are significantly higher than those for females (1 (0-6.28)) with mean rank of 127.72 (U = 7876.5, z = -2.065, ρ = 0.039, r = 0.1254) (Table 4.26).

Table 4.26: Reference interval limits for fasting lipid profile for adult and geriatric population of Taita Taveta County, Kenya

Analyte (unit)	Sex	N	Median	Percentile		Reference Interval	IV	Difference between M&F	
				2.5^{th}	97.5^{th}			z-value	Sig
T-Chol (mmol/L)	M&F	271	5.02±1.34					-2.061	ρ = 0.039
			5	2	8	2-8	6		
	F	148	5.16±1.39						
			5	2	8	**2-8***	6		
	M	123	4.85±1.25						
			5	2	7.9	**2-7.9**	5.9		
HDL-Chol (mmol/L)	M&F	271	1.20±0.50					-3.579	ρ = 0.000
			1	0	2	0-2	2		
	F	148	1.30±0.58						
			1	0	2.28	**0-2.28***	2.28		
	M	123	1.08±0.38						
			1	0	2	**0-2**	2		
LDL-Chol (mmol/L)	M&F	271	3.04±1.20					-0.492	ρ = 0.623
			3	1	6	1-6	5		
	F	148	3.09±1.27						
			3	1	6	1-6	5		
	M	123	2.98±1.12						
			3	1	6	1-6	5		
TG (mmol/L)	M&F	271	1.78±1.01					-0.671	ρ = 0.502
			2	1	4.2	1-4.2	3.2		
	F	148	1.74±0.93						

			2	1	4.28	1-4.28	3.28		
	M	123	1.84±1.10						
			2	1	4.9	1-4.9	3.9		
Total Chol:HDL-Chol	M&F	271	4.46±2.02					-2.099	ρ = 0.036
			4	2	8.4	2-8.4	6.4		
	F	148	4.23±1.86						
			4	1.73	8	1.73-8*	6.27		
	M	123	4.73±2.17						
			4	2	10.9	2-10.9	8.9		
TG:HDL-Chol	M&F	271	1.80±1.56					-2.065	ρ = 0.039
			1	0	6.2	0-6.2	6.2		
	F	148	1.66±1.43						
			1	0	6.28	0-6.28*	6.28		
	M	123	1.98±1.69						
			1	0	6.9	0-6.9	6.9		
LDL-Chol:HDL-Chol	M&F	271	2.75±1.55					-1.298	ρ = 0.194
			3	1	6	1-6	5		
	F	148	2.64±1.40						
			2	1	6	1-6	5		
	M	123	2.89±1.72						
			3	1	8.8	1-8.8	7.8		

Results are expressed as median and range for the number of referent participants in the column labeled N. Statistical comparisons of the median values between male and female referent participants were carried out using the Mann-Whitney U test. Differences were considered statistically significant at $\rho < 0.05$

4.11.2 Effect of age and gender on the reference intervals for fasting lipid profiles for adults and geriatrics of Taita Taveta County, Kenya

The effect of age and gender on the reference intervals limits for fasting lipid profiles for the adult and geriatric population of Taita Taveta County, Kenya, was investigated by categorizing the results into two age groups: (a) age group 1 (18-55 years) and (b) age group 2 (56-95 years). The statistical difference between the reference intervals for fasting lipid profiles for adult and geriatric males and females was estimated using the Mann-Whitney U test, and ρ-values less than 0.05 was considered statistically significant. The Median and 95% range for males and females for the two age groups are presented in Table 4.27. Results indicate that all the measured and calculated fasting lipid profile analytes, including total cholesterol (Chol), high-density lipoprotein cholesterol (HDL-Chol) and the ratios of total cholesterol (Chol): high-density lipoprotein cholesterol (HDL-Chol) ratio, low-density lipoprotein cholesterol (LDL-Chol): high-density lipoprotein cholesterol (HDL-Chol) ratio, and triacylglycerol (TG): high-density lipoprotein cholesterol (HDL-Chol) ratio were statistically unaffected by advancement in age ($\rho > 0.05$; Table 4.27).

However, in the age category 18-55 years, males (1 (0.45-2 mmol/L)) with a mean rank of 61.39 had a statistically significantly lower levels of high-density lipoprotein cholesterol (HDL-Chol) compared to females (1 (0-2) mmol/L) with mean rank of 75.21 ($U = 1846$, $z = -2.666$, $\rho = 0.008$, $r = 0.2269$). Further, in age category 56-95 years, males (1 (0-2) mmol/L) with mean rank of 60.75 had a statistically significantly lower levels of high-density lipoprotein

cholesterol than females (1 (0-3) mmol/L) with mean rank of 73.16 (U = 1798.5, z = -2.416, $\rho = 0.016$, r = 0.2095), and males (5 (2-16)) with a mean rank of 75.10 had a statistically significantly higher levels of total cholesterol (T-Chol): high-density lipoprotein cholesterol (HDL-Chol) ratio than females (4 (0.7-7.9)) with a mean rank of 59.02 (U = 1676.5, z = -2.445, $\rho = 0.014$, r = 0.2120).

Table 4.27: Effect of age and gender on the reference intervals of the fasting lipid profile for adults and geriatrics of Taita Taveta County, Kenya

Analyte (unit)	Median reference intervals for fasting lipid profiles						
	Sex	N	18-55 years	N	56-95 years	N	18-95 years
T-Chol (mmol/L)	M&F	138	5.14±1.35	133	4.89±1.31	271	5.02±1.34
			5 (3-8.53)		**5 (2-7)**		**5 (2-8)**
	M	57	4.91±1.21	66	4.79±1.28	123	4.85±1.25
			5 (2-8.1)		**5 (2-8)**		**5 (2-7.9)***
	F	81	5.31±1.43	67	4.99±1.33	148	5.16±1.39
			5 (3-8.95)		**5 (2-7)**		**5 (2-8)**
HDL-Chol (mmol/L)	M&F	138	1.20±0.48	133	1.20±0.52	271	1.22±0.46
			1 (0-2)		1 (0-2)		**1 (0-2)**
	M	57	1.07±0.32	66	1.09±0.42	123	1.08±0.38
			1 (0.45-2)*		**1 (0-2)***		**1 (0-2)***
	F	81	1.28±0.55	67	1.31±0.58	148	1.30±0.57
			1 (0-2)		**1 (0-3)**		**1 (0-2.28)**
LDL-Chol (mmol/L)	M&F	138	3.19±1.27	133	2.89±1.12	271	3.04±1.19
			3 (1-6)		**3 (1-5)**		**3 (1-6)**
	M	57	3.09±1.17	66	2.86±1.08	123	2.98±1.12
			3 (0.46-6)		**3 (1-6)**		**3 (1-6)**
	F	81	3.26±1.33	67	2.88±1.16	148	3.09±1.27
			3 (1-6)		**3 (1-5)**		**3 (1-6)**
TG (mmol/L)	M&F	138	1.78±1.00	133	1.79±1.02	271	1.79±1.00
			2 (1-4)		**2 (1-5)**		**2 (1-4.2)**
	M	57	1.81±1.01	66	1.86±1.19	123	1.84±1.10
			1 (1-4)		**2 (0.68-6**)		**2 (1-4.9)***
	F	81	1.75±1.01	67	1.72±0.83	148	1.74±0.93
			2 (0.05-4.95)		**2 (1-4.3**)		**2 (1-4.28)**
T-Chol:HDL-Chol	M&F	138	4.50±1.84	133	4.41±2.19	271	4.48±2.00
			5 (2-9.05)		**4 (2-9.30)**		**4 (2-8.4)**

	M	57	4.56±1.68	66	4.88±2.52	123	4.73±2.17
			4 (1.9-10.55)		**5 (2-16)***		**5 (2-10.9)***
	F	81	4.46±1.96	67	3.96±1.72	148	4.23±1.86
			4 (2-8)		**4 (0.7-7.9)**		**4 (1.73-8**)
TG:HDL-Chol	M&F	138	1.79±1.40	133	1.81±1.71	271	1.80±1.56
			1 (0-6)		**1 (0-7)**		**1 (0-6.2)**
	M	57	1.84±1.22	66	2.09±2.01	123	1.98±1.69
			1 (1-6)		**1 (0-9.3)**		**1 (0-6.9)***
	F	81	1.75±1.52	67	1.54±1.30	148	1.66±1.43
			1 (0-6.95)		**1 (0-5.6)**		**1 (0-6.28)**
LDL-Chol:HDL-Chol	M&F	138	2.77±1.47	133	2.74±1.64	271	2.75±1.55
			3 (1-6.53)		**3 (1-5.65)**		**3 (1-6)**
	M	57	2.81±1.45	66	2.97±1.93	123	2.89±1.72
			3 (0.45-8.1)		**3 (0.68-10.63)**		**3 (1-8.8)**
	F	81	2.74±1.50	67	2.51±1.26	148	2.64±1.40
			3 (1-6)		**2 (0.7-5.3)**		**2 (1-6)**

Results are expressed as Mean ± standard deviation (SD), and Median and range of the number of subjects indicated in the column labeled N. $^{*}\rho < 0.05$ when male median reference interval limits are significantly different when compared to the female median reference interval limits within each age category (down the columns) by Mann-Whitney U test, $^{a}\rho < 0.0167$ when median reference interval limits in age category 18-55 years are significantly different when compared to the reference intervals in age category 56-95 year (along the rows) by Mann-Whitney U test for each gender**.**

4.11.3 Comparison of the developed reference interval limits for fasting lipid profile for adults and geriatrics of Taita-Taveta County, Kenya, with those reported in the medical literature

A comparison of the developed reference intervals for fasting lipid profile for adults and geriatrics of Taita-Taveta County, Kenya, with those reported in medical literature are presented in Table 4.28. For this study, the lower limit of the reference interval for fasting total cholesterol (T-Chol) levels is similar to that of the Haryana population (Agrawal *et al.*,2014) but ,the upper limit is greater than that of the Haryana population. However, the reference intervals for fasting total cholesterol were similar for males and females of both populations. The lower limit for the reference interval of fasting total cholesterol for Punjab population (Kaur *et al.*, 2012, is higher than that generated in this study for the Taita-Taveta county population while the upper limit are lower than that generated in this study. Further, the reference interval for fasting total cholesterol for the Punjab population differs by sex, while those generated in this study are similar. The lower reference interval limit for fasting total cholesterol for the Assamese population (Das and Saikia 2009) of India was similar to those generated for the Taita-Taveta County population while the upper reference interval limit is lower than that generated in this study. However, the Assamese population reference interval for fasting total cholesterol differs by sex. For the Western Maharashtra population of India (Durgawale *et al.*,2009), the lower and upper reference interval limits for

fasting total cholesterol are lower than those developed for the Taita-Taveta County population of this study.

Further, the Western Maharashtra population had different reference interval for fasting total cholesterol for males and females. For the Venezuelan Maracaibo population (Bermudez *et al.*,2012), the lower reference interval for fasting total cholesterol is higher than that established for Taita-Taveta County population in this study, while the upper limit for the same population is lower than that of the Taita-Taveta County population. However, the Venezuelan Maracaibo population has different reference intervals for fasting total cholesterol for males and females (Table 4.28).

The Punjab, Western Maharashtra, Assamese, and Taita-Taveta populations had different reference interval limits for fasting lipid profiles for males and females. For the Punjab population, the lower reference interval limit for fasting high density lipoprotein cholesterol (HDL-Chol) is higher than that established for the Taita-Taveta County population, while the upper reference interval limit is lower. For the Western Maharashtra and Assamese populations, the lower reference interval limit for fasting high density lipoprotein cholesterol is higher than those established for the Taita-Taveta County population. In contrast, the upper reference interval limits were lower. For the Haryana, and Venezuelan Maracaibo populations, the combined (male and female) lower reference interval limits for fasting high-density lipoprotein cholesterol are higher than the established (separate for males and females) for the Taita-Taveta County population, while the upper reference interval limit is

lower (Table 4.28). Punjab, Western Maharashtra, and Haryana population reported combined reference intervals for TG for adult and geriatric males and females just like that established for the adult and geriatric male and female population of Taita-Taveta County, Kenya. However, the lower combined established reference interval limits for triacylglycerol for the adult and geriatric male and female population of Taita-Taveta County, Kenya is lower than that reported for Punjab population, and the upper reference interval limit is higher; the lower combined established reference interval limits for triacylglycerol for adult and geriatric male and female population of Taita-Taveta County, Kenya is higher than that reported for Western Maharashtra population, and upper reference interval limit is lower; the lower combined established reference intervals for TG for adult and geriatric male and female population of Taita-Taveta County, Kenya is similar to that reported for the Haryana population. The upper reference interval limit is lower. Further, the lower combined established reference interval limits for triacylglycerol for adult and geriatric male and female population of Taita-Taveta County, Kenya is higher than the reported separate (male and female) reference interval limit for Venezuelan Maracaibo, and Assamese population, and the upper separate reference intervals for the two populations are lower. In addition, the established lower separate (male and female) reference interval limit for total cholesterol: high density lipoprotein cholesterol ratio for the adult and geriatric population of Taita-Taveta County, Kenya is lower than that reported for the

combined reference interval for the Punjab population, and the upper separate reference interval limit is lower (Table 4.28).

Table 4.28: Comparison of the developed reference intervals for fasting lipid profile for the adult and geriatric population of Taita-Taveta County, Kenya, with those reported in the literature

Analyte	Sex	This study RI	Durgawale et al., 2009.	Das and Saikia, 2009	Kaur et al., 2012	Bermudez et al., 2012	Agrawal et al., 2014
T-Chol (mmol/L)	M&F	**2-8**				**2.98-6.99**	2.21-5.49
	F	2-8*	0.48-3.95	2.40-6.80	4.70-4.84	3.14-7.02	
	M	2-7.9	0.00-5.54	2.51-6.05	4.57-4.73	2.79-6.99	
HDL-Chol (mmol/L)	M&F	0-2				0.78-1.98	0.52-1.63
	F	**0-2.28***	0.65-1.74	0.62-1.89	1.15-1.18	**0.79-1.96***	
	M	**0-2**	0.58-1.63	0.59-1.53	1.10-1.13	**0.78-1.98**	
LDL-Chol (mmol/L)	M&F	**1-6**			2.9-3.0	**1.39-4.80**	**1.30-3.81**
	F	1-6					
	M	1-6					
TG (mmol/L)	M&F	**1-4.2**	0.31-1.69		1.36-1.41	0.35-2.25	0.69-1.76
	F	1-4.28		0.46-2.54		**0.35-1.93***	
	M	1-4.9		0.45-2.89		**0.37-2.35**	
T-Chol: HDL-Chol	M&F	2-8.4			4.15-4.26		
	F	**1.73-8***					
	M	**2-10.9**					
TG: HDL-Chol	M&F	0-6.2					
	F	**0-6.28***					
	M	**0-6.9**					
LDL-Chol: HDL-Chol	M&F	**1-6**			2.58-2.69		
	F	1-6					
	M	1-8.8					

Assamese Population by Das and Saikia (2009).,Western Maharashtra population by Durgawale *et al.*, (2009), Punjab population by Kaur *et al.*, (2012, Venezuelan Maracaibo Population by Bermudez *et al.*, (2012), Haryana population by Agrawal *et al.*, (2014),

4.12 Reference intervals for thyroid stimulating hormone and thyroid hormones for adult and geriatric population of Taita-Taveta county, Kenya

4.12.1 Reference interval limits for thyroid hormones for adults and geriatrics of Taita-Taveta County, Kenya

The established reference intervals for stimulating thyroid hormone (TSH), total triiodothyronine (T3), and total thyroxine (T4) for male population of Taita-Taveta County, Kenya, was statistically similar to that of the female population of the same age range ($\rho > 0.05$). Therefore, combined reference interval limits of these parameters for this population were established. The established reference interval limits for thyroid hormones for the adult and geriatric population of Taita-Taveta County, Kenya is 2 (0-6) mU/L for TSH, 2 (0-4) mU/L for T3, and 4 (2-15) nmol/L for T4 (Table 4.29).

Table 4.29: Reference interval limits for thyroid hormones for adults and geriatrics of Taita-Taveta County, Kenya

Analyte (unit)	Sex	N	Median	Percentile		Reference Interval	IV	Difference between M&F	
				2.5th	97.5th			Z value	Sig
TSH (mU/L)	M&F	244	2.16±1.72					-0.519	
			2	0	6	0-6	6		
	M	120	2.08±1.54						ρ = 0.604
			2	0	5.97	0-5.97	5.97		
	F	124	2.24±1.87						
			2	0	6	0-6	6		
T3 (mU/L)	M&F	244	1.73±0.87					-0.174	
			2	0	4	0-4	4		
	M	120	1.81±0.96						ρ = 0.862
			2	0	5.95	0-5.95	5.95		
	F	124	1.66±0.79						
			2	0	3	0-3	3		
T4 (nmol/L)	M&F	244	5.19±3.64					-0.835	
			4	2	15	2-15	13		
	M	120	5.94±3.84						ρ = 0.403
			4	1	15.98	1-15.98	14.98		
	F	124	5.87±3.49						
			4	3	15	3-15	12		

Results are expressed as Mean ± standard deviation (SD) and Median and 95% range for the number of referent participants in the column labeled N. Statistical comparisons of the median values between male and female referent participants were carried out using the Mann-Whitney U test. Differences were considered significant at $\rho < 0.05$

4.12.2 Effects of age on the reference interval for thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya

The effects of age on the reference interval limits for thyroid hormone for the adult and geriatric population of Taita-Taveta County, Kenya, are presented in Table 4.30. This effect was investigated by dividing the study population into three age categories as follows: (a) age category 1 (≥ 50-60 years), (b) age category 2 (≥ 60-70 years), (c) age category 3 (≥ 70-95 years). Reference interval limits differences between males and females were estimated within each age category using the Mann-Whitney U test. Reference interval limits differences within and between the three age categories were carried out using the Kruskal-Wallis H test followed by the Mann-Whitney U test with Bonferroni correction where ρ-values less than 0.0167 were considered statistically significant.

Results indicate that using the Kruskal-Wallis H test, the established reference interval limits for thyroid stimulating hormone (TSH) ($\chi^2(2) = 0.390$, $\rho = 0.823$), total thyroxine ($\chi^2(2) = 1.955$, $\rho = 0.376$) and total triiodothyronine (T3) ($\chi^2(2) = 449$, $\rho = 0.799$) for combined male and female adults and geriatrics of Taita-Taveta County, Kenya, were not significantly altered by advancement in age ($\rho > 0.05$) (Table 4). There was, therefore no need for a follow-up pairwise comparison using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167.

In addition, results indicate that using the Kruskal-Wallis H test, the established reference interval limits for thyroid stimulating hormone (TSH)

($\chi^2(2) = 1.137$, $\rho = 0.567$), and total triiodothyronine (T3) ($\chi^2(2) = 2.018$, $\rho = 0.365$) for female adults and geriatrics of Taita-Taveta County, Kenya were not significantly altered by advancement in age (Table 4.30). However, the reference interval limits for total thyroxine (T4) for female adults and geriatrics of Taita-Taveta County, Kenya, were statistically significantly altered with advancement in age ($\chi^2(2) = 12.409$; $\rho = 0.002$). Therefore, a follow-up pairwise comparison was needed using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167.

A follow-up pairwise comparison using the Mann-Whitney U test indicates that the median reference interval limits for total thyroxine (T4) for female adults (5 (2-9.75) nmol/L) in the fifth decade with a mean rank of 38.31 was statistically significantly higher than that of female geriatrics (4 (3-5.4) nmol/L) in the seventh decade onwards with a mean rank of 24.23 ($U = 262$, $z = -3.220$, $\rho = 0.001$, $r = 0.4089$). However, this reference interval limit for total thyroxine (T4) for female adults in the fifth decade was statistically similar to that of female geriatrics in sixth decade. Further, the median reference interval limits for total thyroxine (T4) for female geriatrics (4 (2.15-15.43) nmol/L) in the sixth decade with a mean rank of 51.60 was statistically significantly higher than that of female geriatrics (4 (3-5.4) nmol/L) in the seventh decade onwards with mean rank of 35.97 ($U = 614$, $z = -2.864$, $\rho = 0.004$, $r = 0.2986$). Thus, the median reference interval limits for total thyroxine (T4) for female adults and geriatrics appear similar in the fifth and sixth decade but significantly drops from the seven decades onwards.

Results indicate that a Kruskal-Wallis H test for reference interval limits for thyroid stimulating hormone (TSH) ($\chi^2(2) = 0.161$; $\rho = 0.923$), total thyroxine (T4) ($\chi^2(2) = 1.352$; $\rho = 0.509$), and total triiodothyronine (T3) ($\chi^2(2) = 2.798$; $\rho = 0.247$) for male adults and geriatrics of Taita-Taveta County, Kenya were not significantly affected by advancement in age ($\rho > 0.05$). There was, therefore no need for a follow-up pairwise comparison using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167.

Further, an investigation on the effect of gender on the reference interval limits for thyroid hormones in specific age categories using the Mann-Whitney U test indicates that the total thyroxine (T4) for male geriatrics (4 (1.1-16.8) nmol/L) in the seventh decade onwards with a mean rank of 42.42 was statistically significantly higher than that for their female counterparts (4 (3-5.4) nmol/L) in the seventh decade onwards with a mean rank of 29.23 ($U = 412$, $z = -2.779$, $\rho = 0.005$, $r = 0.3253$).

Table 4.30: Effects of age on the median reference interval for thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya

Analyte (Units)	Changes in thyroid hormone concentration with age								
	Sex	N	≥ 50-60 years	N	≥ 60-70 years	N	≥ 70-95 years	N	≥ 50-95 years
TSH (mU/L)	M&F	**68**	**2.09±1.69**	**103**	**2.17±1.82**	**73**	**2.22±1.60**	**244**	**2.16±1.72**
			2 (0-6.55)		**2 (0-6)**		**2 (0-6)**		**2 (0-6)**
	M	36	2.25±1.84	41	1.95±1.41	43	2.07±1.40	120	2.08±1.54
			2 (0-3)		**2 (0-5)**		**2 (0-5.90)**		**2 (0-5.97)**
	F	32	1.91±1.51	62	2.32±2.05	30	2.43±1.85	124	2.24±1.87
			2 (0-3)		**2 (0-8.55)**		**2.5 (0-4)**		**2 (0-6)**
T4 (nmol/L)	**M&F**	**68**	**6.47±4.04**	**103**	**5.83±3.60**	**73**	**5.49±3.28**	244	5.91±3.64
			4 (1.73-16.28)		**4 (1-15.40)**		**4 (1.85-15.30)**		**4 (2-15)**
	M	36	6.06±4.09	41	5.54±3.81	43	6.25±3.63	120	5.94±3.84
			4 (1-9.50)		**4 (0.05-15.95)**		**4 (1.1-16.80)↑***		**4 (1-15.98)**
	F	32	6.94±4.00	62	6.02±3.47	30	4.43±2.37	124	5.87±3.49
			5 (2-9.75)		**4 (2.15-15.43)**		**4 (3-5.4)↓[bc]**		**4 (3-15)**
T3 (mU/L)	M&F	**68**	**1.69±0.78**	**103**	**1.69±0.78**	**73**	**1.75±1.06**	**244**	**1.73±0.87**
			2 (0-3.82)		**2 (0-4.40)**		**2 (0-6)**		**2 (0-4)**
	M	36	1.67±0.93	41	1.78±0.65	43	1.95±1.19	120	1.81±0.96
			2 (0-2.03)		**2 (0.05-3.95)**		**2 (0-6)**		**2 (0-5.95)**
	F	32	1.72±0.58	62	1.73±0.87	30	1.47±0.78	124	1.66±0.79
			2 (0-2)		**2 (0-5)**		**2 (0-2.2)**		**2 (0-3)**

Results are expressed as mean ± standard deviation (SD), and median and 95% range of the number of subjects indicated in the column labeled N. $^{*}\rho < 0.05$ when male median reference interval limits are significantly different when compared to female median reference interval limits per each age category by Mann-Whitney U test; $^{a}\rho < 0.0167$ when median reference interval limits in age range ≥ 50-60 years is significantly different when compared to median reference interval limits in age range ≥ 60-70 years, $^{b}\rho < 0.0167$ when median reference interval limits in age range ≥ 50-60 years is significantly different when compared to median reference interval limits in age range ≥ 70-95 years, and $^{c}\rho < 0.0167$ when median reference interval limits in age range ≥ 60-70 years is significantly different when compared to median reference interval limits in age range ≥ 70-95 years by Kruskal-Wallis H test followed by Mann-Whitney U test with Bonferroni corrections.Bracketed values are the median 2.5 and 97.5 percentiles for combined and separate gender in each of the three specific age categories.

4.12.3 Comparison of developed reference intervals for thyroid hormones for the adult and geriatric population of Taita-Taveta County, Kenya with those reported in the literature

A comparison of developed reference intervals for thyroid hormones for the adult and geriatric population of Taita-Taveta County, Kenya with those reported in the literature are presented in Table 4.31. These comparisons were performed by comparing the lower and upper reference interval limits of this study's reference intervals with those of other studies previously reported in the medical literature.

Results indicate that for TSH, this study's gender-independent lower reference interval limit is lower than that of American (Hollowell *et al.,* 2002), British (Kratzsch *et al.*, 2005), Australian (Hickman *et al.,* 2017), Srpska Republic (Mirjani-Azaric *et al.*, 2017) and Darfur (Sudan) (Ali *et al.*, 2018), populations, and gender-dependent upper reference interval limit for Khartoum (Sudan) (Musa *et al.,* 2018) population. However, the gender-independent upper reference interval limit for TSH in this study is higher than that of gender-independent upper reference interval limits for American (Hollowell *et al.,* (2002), British (Kratzsch *et al.*, 2005), Australian (Hickman *et al.*, 2017), and Srpska Republic (Mirjani-Azaric *et al.,* 2017) and Darfur (Sudan) (Ali *et al.,* 2018), populations, and gender-dependent upper reference interval limit for Khartoum (Sudan) (Musa *et al.,* 2018) population.

For T3, this study's gender-independent lower reference interval limit is lower than that of gender-dependent British (Kratzsch *et al.*, 2005) population, and

similar to that of Srpska Republic (Mirjani-Azaric *et al.,* 2017) population, while the upper reference interval limit is higher than that of British (Kratzsch *et al.,* 2005) population, and lower than that of the Srpska Republic (Mirjani-Azaric *et al.,* 2017) population.

For T4, this study' gender independent lower and upper reference interval limits are lower than that of gender-independent reference limits for American (Hollowell *et al.,* 2002), Srpska Republic (Mirjani-Azaric *et al.,* 2017) ,Darfur (Sudan) (Ali *et al.,* 2018), andpopulations, and gender-dependent lower and upper reference interval limits for British (Kratzsch *et al.,* 2005) and Khartoum (Sudan) (Musa *et al.,* 2018) populations. Age-dependent reference interval limits for TSH were reported by Hollowell *et al.,* (2002) for the American population, and Ali *et al.,* (2018) for the western Sudan population (Table 4.31). Further, Ali *et al.,* (2018) reported age-dependent reference interval limits for T4 for the western Sudan population (Table 4.32). These age-dependent reference interval limits for TSH and T4 differ from those reported in this study.

Table 4.31: Comparision of developed reference interval for thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya

Analyte (Unit)	Gender	This study RI	Hollowell et al., 2002	Kratzsch et al., 2005	Hickman et al., 2017	Mirjani-Azaric et al., 2017	Musa et al., 2018	Ali et al., 2018
TSH (mU/L)	**M&F**	**0-6**	0.45-4.12	0.40-3.77	0.43-3.28	0.65-5.39		0.05-3
	M	0-5.97					0.5-3	
	F	0-6					0.5-3.4	
T3 (mU/L)	M&F	**0-4**					0.8-2.7	0.8-2.8
	M	0-5.95		1.23-2.97		0-4.61		
	F	0-3		1.28-2.33		0-4.97		
T4 (nmol/L)	**M&F**	**2-15**	66.9-165.9			73.01-127.7		72-161.1
	M	1-15.98		71.4-166			63-165	
	F	3-15		68.4-125			62-148.2	

American population by Hollowell *et al.,* (2002), British population by Kratzsch *et al.,* (2005), Srpska Republic population by Mirjani-Azaric *et al., (*2017), Australian population by Hickman *et al.,* (2017), Khartoum (Sudan) population by Musa *et al.,* (2018), Darfur (Sudan) population by Ali *et al.,* (2018).

Table 4.32: Comparison of age-related developed reference interval limits for thyroid hormones for adults and geriatrics of Taita-Taveta County, Kenya with those reported in medical literature

Analyte							
	This study RI		≥ 50-60 years	≥ 60-70 years	≥ 70-95 years		50-95 years
TSH		M&F					**0-6**
(mU/L)		M					
		F					
	Americans[H]		≥ 50-60 years	≥ 60-70 years	70-80 years	≥ 80 years	

		M&F	0.52-4.03	0.49-4.33	0.45-5.90	0.33-7.50	
		M	0.50-4.04	0.56-4.27	0.47-6.39	0.36-6.82	
		F	0.53-4.02	0.45-4.48	0.44-5.77	0.17-7.87	
	Germany[V]		50-79 years				
		M&F	0.19-2.09				
	Sudan[A]		51-60 years	≥ 60 years			
		M&F	0.5-2.6	0.2-2.5			
	Srpska		51-60 years	61-70 years			
		M&F	0-5.82	0-5.82			
T4	This study RI	M&F	**2.38-15.63**	**2.38-15.63**			**2-15**
(nmol/L)		M				**1.1-16.8***	
		F				**3.0-5.4[bc]**	
	Sudan[A]		51-60 years	≥ 60 years			
		M&F	137-162	165-190.2			
	Srpska		51-60 years	61-70 years			
		M&F	0-286.04	0-286.04			
T3	This study RI	M&F					**0-4**
(mU/L)		M					
		F					
	Sudan[A]		51-60 years	≥ 60 years			
		M&F	1-3	1-3.1			
	Srpska		51-60 years	61-70 years			
		M&F	0-1.49	0-1.49			
		M					
		F					

American population by Hollowell *et al.,* (2002), Western Pomerania, northeast of Germany population by Völzke *et al.,* (2005), Srpska Republic population by Mirjani-Azaric *et al.,* (2017, Nyala, Darfur region, western Sudan population by Ali *et al.,* (2018

4.13 Reference interval limits for cancer biomarkers for adults and geriatrics of Taita-Taveta County, Kenya

4.13.1 Reference interval limits for serum cancer biomarkers for adults and geriatrics of Taita-Taveta County, Kenya

The median reference interval limits for the five serum cancer biomarkers for adults and geriatrics of Taita-Taveta County, Kenya, are presented in Table 4.33. Results indicate that the reference intervals for CA 19-9 and CEA are significantly different between males and females, with females having higher and lower interval limits than males. Therefore, separate reference interval limits were established for these two cancer biomarkers. The established median reference interval limits for CA 19-9 for males is 9 (0-42.8) U/mL with mean rank of 111.93 is significantly lower than that for females of 11 (0-58) U/mL with mean rank of 132.73 ($U = 6172$, $z = -2.324$, $\rho = 0.020$, $r = 0.1488$), while that for CEA for males is 2 (0-7) ng/mL with mean rank of 112.5 is significantly lower than those for females of 1 (0-6.9) ng/mL with mean rank of 132.67 ($U = 6211$, $z = -2.287$, $\rho = 0.022$, $r = 0.1464$). The median reference interval limits for CA 15-3 for males is 22 (0-57) U/mL with mean rank of 120.3 and females is 23 (0-65.5) U/mL with mean rank of 124.63 were statistically similar and therefore combined reference interval limits were established. The established combined median reference interval limits for CA 15-3 for adults and geriatrics of Taita-Taveta County, Kenya is 22 (0-56.9) U/mL ($U = 7176$, $z = -0.479$, $\rho = 0.632$, $r = 0.0307$). The established median

reference interval limits for CA 125 for female adults and geriatrics of Taita-Taveta County, Kenya is 9 (0-25) U/mL, while that for PSA is 1 (0-6.8) ng/mL

Table 4.33: Reference interval limits for serum cancer biomarkers for adults and geriatrics of Taita-Taveta County, Kenya

Analyte (unit)	Sex	N	Median	Percentile		Reference Interval	IV	Difference between M&F	
				2.5^{th}	97.5^{th}			z-value	Sig
CA-19-9 (U/mL)	M&F	244	12.80±14.47					-2.324	ρ = 0.020
			11	0	56.38	0-56.4	56.3		
	F	124	15.50±16.55						
			11	**0**	**58**	**0-58**	**58**		
	M	120	10.00±11.35						
			9	**0**	**42.77**	**0-42.8**	**42.8**		
CEA (ng/mL)	M&F	244	1.85±1.92					-2.287	ρ = 0.022
			2	0	7	0-7	7		
	F	121	1.59±1.60						
			1	**0**	**6.9**	**0-6.9**	**6.9**		
	M	123	2.11±2.17						
			2	**0**	**7**	**0-7**			
CA 15-3 (U/mL)	M&F	244	23.55±14.25					-0.479	ρ = 0.632
			22	**0**	**56.9**	**0-56.9**	**56.9**		
	F	124	23.86±15.50						
			23	0	65.5	0-65.5	65.5		
	M	120	23.23±14.68						
			22	0	56.98	0-57	57		
CA 125 (U/mL	F	126	15.39±19.09						
			9	0	25	**0-25**	25		
PSA (ng/mL)	M	139	4.60±11.65						
			1	0	6.84	**0-6.8**	6.8		

Results are expressed as Mean ± standard deviation (SD), and Median and range for the number of referent participants in the column labeled N. Statistical comparisons of the median values between male and female referent participants were carried out using the Mann-Whitney U test. Differences were considered significant at $\rho < 0.05$.

4.13.2 Effect of age on the reference interval limits for the five cancer biomarkers for adult and geriatric male and female population of Taita Taveta County, Kenya

The effect of age on the reference interval limits for cancer biomarkers CA 19-9, CEA, CA 15-3, CA 125 and PSA for adults and geriatrics of Taita Taveta County, Kenya, are reported in Table 4.34. Results for Kruskal-Wallis H test analysis indicate that the reference interval limits for cancer biomarkers, carbohydrate antigen 19-9 (CA 19-9) (χ^2 (2) = 5.744, ρ = 0.057 for combined males and females, and χ^2 (2) = 0.710, ρ = 0.701) for females), carcinoembryonic antigen (CEA) (χ^2 (2) = 2.270, ρ = 0.321 for combined males and females, χ^2 (2) = 0.727, ρ = 0.695 for males, and χ^2 (2) = 1.353, ρ = 0.508 for females), CA 15-3 (χ^2 (2) = 1.084, ρ = 0.582 for combined males and females, χ^2 (2) = 2.417, ρ = 0.325 for males, and χ^2 (2) = 4.952, ρ = 0.084 for females, carbohydrate antigen 125 (CA 125) (χ^2 (2) = 2.248, ρ = 0.299) and PSA (χ^2 (2) = 3.282, ρ = 0.194) for adults and geriatrics of Taita Taveta County, Kenya are not significantly affected by advancement in age (ρ > 0.05; Table 4.34). However, the reference interval limits for CA 19-9 for male adults and geriatrics of Taita-Taveta County, Kenya are significantly affected by advancement in age (χ^2 (2) = 8.932, ρ = 0.011). Further pairwise comparisons using Mann-Whitney U test with adjusted significant ρ-value of 0.0167 indicates that the reference interval limits for CA 19-9 for male adults in their sixth decade (1 (0-23.75) U/mL) with mean rank of 31.25 are significantly lower than those of their seventh decade onwards (15 (0-26) U/mL) with mean

rank of 45.72 (U = 3855, z = -2.900, ρ = 0.004, r = 0.3394). Reference interval limits for CA 19-9 for male adults and geriatrics in their fifth decade were similar to those of the sixth decade and the seventh decade onwards. However, an investigation on the effect of gender on the reference interval limits for biomarkers of cancer in specific age categories using Mann-Whitney U test indicates that for CA 19-9, male adults in the fifth decade (10 (0-63.08) U/mL) with mean rank of 40.85 are significantly lower than those of adult females (12 (0-82.5) U/mL) with mean rank of 52.40 (U = 792, z = -2.088, ρ = 0.037, r = 0.2177), and geriatric males in the sixth decade (1 (0-23.75) U/mL) with mean rank of 40.42 are significantly lower than those of geriatric females (11 (0-75.2) U/mL) with mean rank of 52.07 (U = 788.5, z = -2.134, ρ = 0.033, r = 0.2225).

Further, for CEA male geriatrics reference interval limits in their sixth decade (2 (0-11.95) ng/mL) with mean rank of 57.33 are significantly higher than those of their female counterparts (1 (0-6.8) ng/mL) with mean rank of 45.65 (U = 1017, z = -1.987, ρ = 0.047, r = 0.1948). In addition, for CA 15-3, adult male reference interval limits in their fifth decade (20 (0-56.65) U/mL) with mean rank of 34.72 are significantly lower than those of their female counterparts (25.5 (0-32.65) U/mL) with mean rank of 45.70 (U = 562.5, z = -2.127, ρ = 0.033, r = 0.2393).

Table 4.34: Effect of age on the reference interval limits for the five cancer biomarkers for adults and geriatrics of Taita Taveta County, Kenya

Analyte (unit)	Gender	Changes in the concentration of cancer biomarker by age					
		N	≥50-60 years	N	≥60-70 years	N	≥70-95 years
CA 19-9 (U/mL)	M&F	92	13.09±14.52	92	10.42±13.30	60	15.98±15.63
			11 (0-63.08)		8 (0-37.68)		12.5 (0-58)
	M	47	9.87±11.33	44	6.50±7.47	29	15.52±14.14
			10 (0-63.08)*		**1 (0-23.75)***		**15 (0-26)^{bc}**
	F	45	16.44±16.72	48	14.02±16.25	31	16.42±17.13
			12 (0-82.5)		**11 (0-75.2)**		11 (0-24)
CEA (ng/mL)	M&F	67	1.76±1.63	104	2.05±2.23	73	1.64±1.69
			2 (0-5.8)		2 (0-7)		1 (0-7.15)
	M	33	1.73±1.31	61	2.36±2.56	29	2.00±2.07
			2 (0-3)		**2 (0-11.95)***		2 (0-3)
	F	34	1.79±1.90	43	1.60±1.59	44	1.41±1.35
			1.5 (0-2.46)		**1 (0-6.8)**		1 (0-6.63)
CA 15-3 (U/mL)	M&F	79	23.48±15.50	96	24.80±14.00	69	21.88±13.09
			23 (0-57)		23.5 (1-62.33)		21 (0-59.5)
	M	41	20.49±14.89	43	25.42±13.37	36	23.72±15.79
			20 (0-56.65)*		23 (1-55.90)		21.5(0-33.75)
	F	38	26.71±15.68	53	24.30±14.59	33	19.88±9.13
			25.5 (0-32.65)		24 (0.35-77.40)		21 (0-26)
CA 125 (U/mL)	F	38	14.53±23.73	54	14.87±17.94	34	17.18±15.09
			4.5 (0-24.25)		11 (0-20.5)		15 (0-31)
PSA (ng/mL)	M	27	4.44±10.33	63	4.35±12.07	49	5.00±12.01
			1 (0-9.18)		0 (0-7.84)		1 (0-8.97)

Results are expressed as Mean ± standard deviation (SD), and Median and range for the number of subjects indicated in the column labeled N. $^{*}\rho < 0.05$ when male reference interval limits are significantly different when compared to female reference interval limits for each age category, $^{a}\rho < 0.05$ when the reference interval limits for age range ≥ 50-60 years is significantly different when compared to the reference interval limits for age range ≥ 60-70 years, $^{b}\rho < 0.05$ when the reference interval limits for age range 50-60 years is significantly different when compared to the reference interval limits for age range ≥ 70-95 years, and $^{c}\rho < 0.05$ when the reference interval limits for age range ≥ 60-70 years is compared to the reference interval limit for age range ≥ 70-95 years.

4.13.3 Comparison of developed reference interval limits of the five selected cancer biomarkers for adults and geriatrics of Taita-Taveta County, Kenya, with those reported in the medical literature

A comparison of developed reference intervals of the five selected cancer biomarkers for adult and geriatric population of Taita-Taveta County, Kenya with those reported in medical literature are presented in Table 4.35. Results indicate that the established reference interval limits for CA 19-9 lower limit for male and female population of Taita-Taveta County, Kenya are lower than those of the Caucasian population. In comparison, the upper reference interval limit is higher than that of the Caucasian population. Further, the established reference interval limits for CEA lower limit for male and female population of Taita-Taveta County, Kenya are similar to that of the Scandinavian and Han populations while the upper reference interval limit for the male and female population of Taita-Taveta County, Kenya is higher than that of the Scandinavian population but similar to that of the Han population. Further, the established lower reference interval limit for CEA for the adult and geriatric male and female population of Taita-Taveta County, Kenya was lower than that of the Shuyang population, but the upper reference interval limit was higher (Table 4.35).

Table 4.35: Comparison of developed reference interval limits of the five selected cancer biomarkers for the adult and geriatric population of Taita-Taveta County, Kenya with those reported in the medical literature

Analyte (unit)	Sex	This study RI	Vestergaard et al., 1999	Bjerner et al., 2008	Qin et al., 2011	Zhang et al., 2016.
CA-19-9	M&F	0-56.4				

(U/mL)	F	**0-58***	5.2-30.2			
	M	**0-42.8**	3.6-23.0			
CEA (ng/mL)	M&F	0-7		0-4.12	0-6.94	
	F	**0-6.9***				0.43-4.26
	M	**0-7**				0.60-5.43
CA 15-3 (U/mL)	M&F	**0-56.9**				
	F					
	M					
CA 125 (U/mL	F	0-25				
PSA (ng/mL)	M	0-6.8				

Caucasian population by Vestergaard et al. (1999), Scandinavian population by Bjerner *et al.,* (2008), Han population by Qin *et al.,* (2011), Shuyang population by Zhang *et al.,* (2016).CA 125 reference interval is 0-35 U/mL. The only tumor marker recommended for clinical use in diagnosing and managing ovarian cancer.

4.13.4 Effect of age on the reference intervals for PSA for adult and geriatric male population of Taita-Taveta County, Kenya with those reported in the medical literature

The effect of age on the reference intervals for PSA for adult and geriatric male population of Taita-Taveta County, Kenya with those reported in the medical literature, is presented in Table 4.36. Results show that this study's lower reference interval limit for PSA for the adult and geriatric male population of Taita-Taveta County, Kenya for all the compared age categories were similar to those of European, Beijing, Caucasian, African-American, Japanese, Chinese and Taiwanese populations, but lower than the lower reference interval limit reported for Bangladesh population. Further, this study's upper reference interval limit for PSA for adult and geriatric male population of Taita-Taveta County, Kenya for all the compared age categories are higher than those reported for European, Beijing, Bangladeshi, Caucasian, African-American, Japanese, Chinese and Taiwanese (Table 4.36).

Table 4.36: Effect of age on the reference intervals for PSA for adult and geriatric male population of Taita-Taveta County, Kenya with those reported in medical literature

	Population/Age	Changes in PSA concentration with age (years)			
PSA (ng/mL)		**50-59**	**60-69**	**70-79**	**80-95**
	The study RI	**0-6.8**			
	European population	0-2.27	0-3.46	0-4.26	
	Beijing	0-2.92	0.0-4.11	**0-5.59**	0-7.29
	Bangladeshi 1	2.1-3.7	2.6-3.4	**3.6-6.0**	**Up to 7.29**
	Bangladeshi 2	2.1-3.7	2.6-3.4	**3.6-6.0**	**0.9-9.5**
	Caucasians	0-3.5	0-4.5	**0-6.5**	
	African Americans	0-4.0	0-4.5	0-5.5	
	Japanese	0-3.65	0-4.06	0-5.09	0-5.66
	Chinese	0-2.35	0-3.20	0-3.39	0-3.39
	Taiwanese	0-3.31	0-5.05	0-5.73	
	Singapore	0-2.3	0-4.0	**0-6.3**	**0-6.6**
	Korean	0-2.5	0-3.9	0-5.8	
	Iranian	0-2.61	0-3.59	0-4.83	

African-American population by Hyeon, 2005; European population by Luboldt *et al.,* 2007; Iranian population by Khezri *et al.,* 2009; Chinese population by Qin et al., 2011; Taiwanese, Singapore and Korea by Park *et al*., 2012; Beijing population by Liu *et al.,* 2013; Bangladeshi 1 and 2 by Rahman *et al.,* 2014.

CHAPTER FIVE

5. DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1:1 Body Mass Index

Results of this study indicate a significantly increased established reference interval for body mass index (BMI) for females (22.0 [14.1-39.9] kg/m^2) compared to that of males (19.7 [14.5-31.8] kg/m^2) of Taita-Taveta population indicates that BMI is gender dependent for this population. This difference could be attributed to the greater body fat content in females compared to males of similar age. These established BMI reference intervals for the Taita-Taveta population differ from those reported in the literature (WHO BMI) of 18.5-24.9 kg/$m^{2,}$ indicating that the use of international WHO BMI reference interval for the Taita-Taveta population is inappropriate.

This difference of the established BMI reference interval for the Taita-Taveta County, Kenya population from the WHO BMI reference interval may be attributed to genetic factors, ethnicity, lifestyle, the influence of altitude adaptation, or other environmental factors. However, while this BMI reference interval gender difference was also reported by Foppa *et al.,* (2016), the males (20.5-36.5 kg/m^2) in their study demonstrated a higher BMI reference interval than the females (16.7-37.9 kg/m^2) suggesting that their male population had a higher body fat content than females, an observation contradicting expectation. Results indicating significantly increasing body mass index (BMI) reference interval for both the males and females of Taita-Taveta County, Kenya population with age indicates that this parameter is age dependent for this

population. This finding agrees with that reported by Peter *et al.,* (2015) indicating increasing BMI with age in an Austrian population. This increase in BMI with age could be attributed to the increase in body fat content caused by lifestyle changes. Merrill *et al.,* (2017) also demonstrated increasing BMI with age. Buffa *et al.,* (2011) reported that body fat mass increases as one ages during adulthood for both genders because of reduced overall energy expenditure. Shimokata *et al.*,(1989) demonstrated increasing upper and central body fat deposition with advancing age. Silawat *et al.* (2009) demonstrated increasing body fat content in males with advancing age. Roberts & Dallal (1998) demonstrated increasing body fat content in males and females with advancing age.

5.1.2 Temperature

Results of this study indicating similar reference interval limits for **temperature** (32.0-35.9 ^{o}C) for both females and males of the Taita-Taveta population suggests that temperature is gender independent for this population. This observation is in contrast to previous earlier reports indicating that females have a higher body temperature than males because of their thicker layer of subcutaneous fat, which insulates their bodies from faster heat loss resulting in higher body temperatures (Sund-Levander *et al.,* 2004). Normally, females have a higher body temperature than males when they are ovulating due to raised progesterone levels. This observation may therefore suggest that the females who participated in this reference interval limits development study were not in the ovulation stage of their menstrual cycle.

This established reference interval limits for temperature for the Taita-Taveta County, Kenya population is lower than the previously reported literature normal body temperature of 36.6-37.2 ^{o}C. It also contrasts with the axillary (armpit) temperature reference interval limits reported by Geneva et al. (2019) of 35.01-36.93^{o}C for males and females, Marui *et al.*, (2017) of 35.54-37.40^{o}C for Japanese males and females of mean age 20.7 years, Hasan *et al.,* (2010) of 35.61-37.50^{o}C for males and females of mean age 34 years, Gunes and Zaybak (2008) of 34.50-36.50^{o}C for males and females of 65-90 years, and Thomas *et al.* (2004) of 34.62-37.40^{o}C and 33.11-35.67^{o}C for females of 21-36 years and 39-59 years, respectively.

The difference in the established reference interval limits for temperature for this study relative to the previously reported literature temperature reference interval limits may be due to genetic and environmental factors of the population used in the development of the reference intervals. The established reference interval limits for temperature of the Taita-Taveta population was not affected by the age of the referent individual indicating that temperature is age independent for this population. This age independence of the established reference interval limits for temperature is in contrast with what is reported in literature; infants and children have a higher body temperature than adults because their hearts are immature and have faster and irregular heart beats per minute which generate more heat and therefore raises the body temperature. Adults have mature hearts with slower but regular heartbeats per minute and therefore generate less heat resulting in lowered temperature.

5.1.3 Systolic and diastolic blood pressure (mmHg)

The results of this study indicating significantly higher **systolic** and lower **diastolic** blood pressure reference interval limits in males (88-157 mmHg [69 mmHg]/46-100.8 mmHg [54.8 mmHg]) compared to females (84-178.2 mmHg [94.2 mmHg]/49-108 mmHg [59 mmHg]) of Taita-Taveta County population implies that these parameters are gender dependentes having a lower systolic and a higher diastolic reference interval limit than males. These findings are in agreement with those previously reported by King et al. (2006) on the Chinese population who demonstrated gender differences in systolic and diastolic pressure of males (91.5-138.2 mmHg [46.7 mmHg]/55.5-83.4 mmHg [27.5 mmHg]) and females (80.7-116.0 mmHg [35.3 mmHg]/53-74.6mmHg [21.6 mmHg]), respectively, with males having significantly higher systolic and diastolic reference interval limits than females. Foppa *et al.,* (2016) also demonstrated a higher reference interval limit for both systolic and diastolic blood pressure for males (94.6-157.4 mmHg/58.4-93.6 mmHg) compared to females (88.7-159.3mmHg/54.4-89.6mmHg).

However, while diastolic reference interval gender difference was demonstrated in this study, females had a significantly higher diastolic reference interval limits than males. The difference in the reference interval limits of the results of this study and those reported by King *et al.,* (2006) and Foppa *et al.,* (2016) could be due to ethnicity, genetic and environmental factors. Ethnic differences in diastolic blood pressure (mmHg), and the right ventricular measurements such as end-diastolic volume (mL) and ejection

fraction (%) are higher in nonwhite's compared to white's populations as demonstrated by Kawut *et al.,* (2011). The observed higher systolic reference interval limits in males compared to females could be due to the higher body mass index [kg/m^2], body surface area [m^2], and right ventricular measurements such as end-systolic volume [mL], end-diastolic volume [mL], stroke volume [mL], and cardiac output [L/min] in males compared to females as demonstrated by Foppa *et al.,* (2016). Results indicating increasing systolic, and diastolic (age category 1-12 < 13-17 < 18-55 ≈ 56-100 years) blood pressure (mmHg) and pulse rate (beats per minute) (age category 1-12 < 13-17 < 18-55 ≈ 56-100 years) with advancement of age in this Taita-Taveta County, Kenyan population could be due to the decrease in the right ventricular measurements (end-systolic volume [mL], end-diastolic volume [mL], stroke volume [mL], cardiac output [L/min]) with advancement in age as demonstrated by Foppa *et al.,* (2016). Kawut *et al.* (2011) also reported higher right ventricular measurements (end-systolic volume [mL], end-diastolic volume [mL], stroke volume [mL], and cardiac output [L/min]) and mass, and a lower ejection fraction (%) in males compared to females.

This could also be due to the reduction of expansion and contraction of blood vessels with advancing age resulting in increasing systolic and diastolic blood pressure. Further this could relate to the size of the cavity or space (lumen) within the blood vessels and the amount/volume of blood flowing through it. As a person advances in age, body fat increases (Silawat *et al.*, 2009) resulting in the deposition of the increased cholesterol in the lumen of blood vessels

making their diameter narrower thus increasing resistance to blood flow resulting in increasing systolic and diastolic blood pressure. In contrast, Silawat *et al.,* (2009) demonstrated a nonsignificant increase of males' systolic and diastolic blood pressure with advancing age. Results indicating significantly higher right-hand arm-side systolic reference interval limits in males compared to the left-hand arm-side reference interval limits indicate that this parameter is dependent on the hand arm-side. This could be attributed to the higher right hand arm-side heart rate variability measurements (that is fluctuations in cardiac rate or rhythms) (time domain: maximum beat-to-beat interval (ms), minimum beat-to-beat interval (ms), mean beat-to-beat interval (ms), standard deviation of beat to beat interval calculated in the entire recording session (ms), standard deviation of beat to beat intervals calculated on 5 minute segment (ms), square root of the mean squared difference of successive beat to beat intervals (ms), percentage of adjacent beat to beat intervals that differ by more than 50 ms (%), frequency domain: very low-frequency range (0.003-0.04Hz), low-frequency range (0.04-0.15Hz), and high-frequency range (0.15-0.40Hz) (representing parasympathetic and sympathetic control, respectively) compared to the left hand arm-side heart rate variability measurements as **demonstrated** by Yüksel *et al.,* (2014). Results indicating statistically similar diastolic blood pressure reference interval limits for males and females right and left-hand arm side suggest that this parameter is independent of the hand arm-side used.

5.1.4 Peripheral oxygen saturation ($S\rho O_2$) (%)

The results of this study indicating a similar peripheral oxygen saturation ($S\rho O_2$) reference interval limit for Taita-Taveta County, Kenya population of 86.8-100 % implies that this parameter is gender independent. This is similar to the normal peripheral oxygen saturation ($S\rho O_2$) of 95-100 % reported in medical literature. For this population, bronchodilation and oxygen treatment may be initiated in patients with peripheral oxygen saturation ($S\rho O_2$) below 86.8 %.

5.1.5 Pulse Rate

The results of this study indicating a significantly higher **pulse rate** reference interval in females (87 [60-129] beats per minute) compared to males (84 [62-117.6] beats per minute) of the Taita-Taveta population implies that this parameter is gender dependent. These results are in agreement with those reported by Foppa *et al.,* (2016), demonstrating females (46.4-85.6 beats per minute) having a higher pulse rate compared to males (39.5-86.5 beats per minute) of similar age. However, they contrast results reported by King *et al.* (2006) who demonstrated a statistically similar **pulse rate** for both males (**48.2-88.6** beats per minute) and females (**52.6-90.6** beats per minute) of Chinese origin.

This reduced pulse rate in males compared to females of the Taita-Taveta population may imply that the males of this coastal region have a more efficient circulatory system similar to that of athletes and physically fit persons who have lower pulse rates than females who may be having a poorer

circulatory system. It may also be possible that females are involved in more intense activity requiring more oxygen for muscles which fastens their heart beats per minute than males with reduced activity which requires less oxygen resulting in slower heart beats per minute (Taylor *et al.,* 2011). This study did not investigate these two possible causes of the observed results. In addition, results of this study demonstrating a significantly increasing pulse rate for this Taita-Taveta population with advancing age suggesting that pulse rate is age dependent. This observation contrasts reports in literature indicating that as age increases, pulse rate decreases since infants and children have a faster pulse rate than adults. They also contrast the findings Silawat *et al.,* (2009) reported, demonstrating similar pulse rates of males with advancing age. The decrease of pulse rate with age is based on the fact that for a normal person pulse rate is proportionate to body size. Infants and children have small bodies whose temperature homeostasis is maintained by their heartbeats per minute faster than adults. This results in a greater loss of the generated heat in infants and children than adults who have bigger bodies with lower heart beats per minute resulting with less heat generation but a longer retention time to compensate for their size (Taylor *et al.,* 2011).

Results indicating a statistically similar pulse rate regardless of whether it is measured from the left or the right-hand arm side suggests that the measurement of pulse rate is independent of the hand arm side used. These results may indicate that the Taita-Taveta population have no gender-based left-right hand dominant arm side normally contributed by the greater muscle

mass of the individual. The predominant left- or right-hand arm side of either gender would have produced a greater pulse rate. The observed similarity in pulse rate regardless of the hand arm side used may suggest that the Taita-Taveta population has similar blood vessels hardness/elasticity for both the right and the left-hand arm side. The right-hand arm side has normally harder blood vessels than the left-hand arm side for males and the left-hand arm side has harder blood vessels for females than the right-hand arm side. These observations agree with those reported by King *et al.,* (2006) who demonstrated similar pulse rates for both genders.

This study had several limitations including first, this study generated BMI and vital signs reference interval limits for the male and female population of Taita-Taveta County, Kenya which may not be generalized for other populations of Kenya. Secondly, the recommended sample size for each age subclass was below 120 referent individuals recommended by EP28 A3c guideline (CLSI, 2010). Thirdly, the pulse rate was only taken for the awake population but not for the sleeping population. Sleeping and awakeful state may interfere with the pulse rate value. Fourthly, the axillary (armpit) temperature taking time, which impacts temperature measurement accuracy, was not fixed to 6-7 minutes. Fifth, the reference intervals for respiratory rate, and oral, tympanic (ear), and rectal temperature which differ from axillary (armpit) temperature for the Taita-Taveta County, Kenya population were not developed.

5.2.1 Hematlogical of children and adolescents

Results indicating statistically similar hematological parameters reference intervals for red blood cells (RBC (x 10^{12}/L)), PCV (%), mean cell hemoglobin (MCH (pg)), mean cell hemoglobin concentration (MCHC (g/dL)), red blood cell distribution width-standard deviation (RDW-SD (%)), red blood cell distribution width-coefficient of variation (RDW-CV (%)), white blood cells (WBC (x10^9/L)), percent neutrophils (NEU (%)), absolute lymphocytes (LYM (x10^9/L)), percent lymphocytes (LYM (%)), absolute monocytes (MON (x10^9/L)), percent monocytes (MON (%)), absolute eosinophils (EOS (x10^9/L)), percent basophils (BAS (%)), plateletcrit (PCT (%)), platelet distribution width (PDW (%)), and mean platelet volume (MPV (fL)) for male and female children and adolescents population of Taita-Taveta County, Kenya imply that these parameters are gender independent.

The developed reference intervals for this population of Taita-Taveta County, Kenya for RBC is 3.2-8.3 x 10^{12}/L, PCV is 0.3-44.6 %, MCH is 20.4-33.9 pg, MCHC is 25.1-36.0 g/dL, RDW-SD is 38.8-68.8 %, RDW-CV is 11.4-19.2 %, WBC is 3.4-15.1 x 10^9/L, LYM is 0.5-7.3 x 10^9/L, LYM is 7.7-61.7 %, MON is 0-1.3 x 10^9/L, MON is 0.04-12.06 %, EOS is 0.0-0.2 x 10^9/L, BAS is 0.2-1.5 %, PCT is 0.05-0.8 %, PDW is 8.1-16.8 %, and MPV is 7.4-11.3 fL. The absence of significant gender differences for these hematological parameters may indicate the absence of the influence of sex hormones, androgen and oestrogen on erythropoiesis for these Taita-Taveta children and adolescents which have previously been reported by other researchers from different parts

of the world to differ (Bain 1993). The gender independence of RBC, PCV, MCH, MCHC, WBC, LYM, LYM (%), MON, MON (%), EOS, EOS (%), and BAS (%) of children and adolescents observed in this study agree with those reported by Humberg *et al*., (2011).]

Results indicating statistically different hematological parameters reference intervals for HB (g/dL), MCV (fL), NEU (x 10^9/L), BAS (x 10^9/L), EOS (%), and PLT (x 10^9/L) for male and female children and adolescents of Taita-Taveta County in the age range 15-18 years imply that these parameters are gender dependent. The developed reference intervals for this population for HB is 6.5-20 g/dL for males and 8.3-17.1 g/dL for females, EOS is 0.3-12.6 % for males and 0.2-9.1 % for females, **BA**S is **0.0-1.7** x 10^9/L for males and **0.0-0.8** x 10^9/L for females, and PLT is 22-886.2 x 10^9/L for males and 50.4-584.7 x 10^9/L for females with males having higher values than females, and MCV is 56.3-103 fL for males and 66.9-104.4 fL for females, and NEU is 1.4-17.2 x 10^9/L for males and 1.6-19.1 x 10^9/L for females with females having higher values than males.

These results could be explained by the influence of sex hormones in adolescents caused by stimulation of erythropoietin by androgens and its inhibition by estrogens in addition to the start of menstruation in adolescent girls. The higher red blood cells and hemoglobin observed in adolescent males compared to females in age 15-18 years in this study was also observed by Lugada *et al*. (2004),), Buseri *et al.* (2010), Bleyere *et al.*, (2013) and Dosoo *et al.* (2014 in adolescents. The gender dependence of mean cell volume (MCV)

values with adolescent males having lower values than females reported in this study for children and adolescents agrees with those reported by Lugada et al., (2004), Soldin *et al.,* (2011) and Bogner *et al.*,(2019), but contrast the gender independent mean cell volume reported by Bleyere *et al.,* (2013). Dosoo *et al.,* (2014) and Onwurah *et al.,* (2018) and Bimerew *et al.,* (2018), While the gender independence of RDW-SD observed in this study has not been reported elsewhere, the gender independence of RDW-CV reported here agrees with that reported by Dosoo *et al.,* (2014), and Bimerew *et al.,* (2018).

The lower white blood cells values in adolescent males compared to females observed in this study contrasts that gender independence of white blood cells reported by Lugada *et al., (*2004), Buseri *et al.,* (2010), Soldin *et al.,* (2011), Dosoo *et al.,* (2014), Bimerew *et al.,* (2018), Onwurah *et al.,* (2018), and Bogner *et al.,* (2019). Further the gender dependence of absolute neutrophils with males having lower values than females observed in this study agree with those reported by Soldin *et al.,* (2011) and Bogner *et al.,* (2019) but contrasts the gender independence values reported by Lugada *et al.,* (2004), Buseri *et al.,* (2010). and Bimerew *et al.,* (2018). In addition, the higher percent neutrophils in adolescent males compared to females observed in this study agree with that reported by Soldin *et al.,* (2011) but the contrast with the gender independence reported by Bogner *et al.,* (2019). Interestingly, the gender independence levels of absolute lymphocytes in adolescents observed in this study agree with those reported by Lugada *et al.,* (2004), Buseri *et al.,* (2010), Soldin *et al,* (2011), Dosoo *et al.,* (2014), and Bimerew *et al.,* (2018), but contrast its gender

dependence reported by Bogner *et al.,* (2019) with female adolescents having higher values than males.

Further, the gender dependence of percent lymphocytes with adolescent males having higher values than females observed here agree with that observed by Soldin *et al.,* (2011) and Dosoo *et al.,* (2014) with the Dosoo *et al.,* (2014) study reporting higher values for adolescent females compared to that for males. However, it contrasts the gender independence percent lymphocytes reported by Bogner *et al.,* (2019). Sex independent values of absolute monocytes observed in this study for adolescents agree with those reported by Lugada *et al.,* (2004), Buseri *et al.,* (2010), Dosoo *et al.,* (2014), Bimerew *et al.,* (2018), and Bogner *et al.,* (2019), but contrast its gender dependence reported by Soldin *et al.,* (2011) who observed that adolescent males have higher values than females. Sex dependent values of percent monocytes reported here with adolescent males having a higher value than females agree with those reported by Soldin *et al.,* (2011) but contrasts those reported by Dosoo *et al.,* (2014) and Bogner *et al.,* (2019). The sex independent values for absolute eosinophils observed in this study for adolescents agree with those reported by Lugada *et al.,* (2004), Buseri *et al.,* (2010), Bimerew *et al.,* (2018), and Bogner *et al.,* (2019), but contrast the gender dependent adolescent values reported by Soldin *et al.,* (2011) and Bogner *et al.,* (2019) both of whom reported higher adolescent male values compared to those of females. The sex-independent values for percent eosinophils observed in this study for adolescents' contrast those reported by Soldin *et al.,* (2011) and Bogner *et al.*

(2019) who reported higher adolescent male values compared to those of females. The sex-independent values for percent basophils observed in adolescents of this study agree with those reported by Soldin *et al.*, (2011) and Bogner *et al.*, (2019).

Platelet levels for adolescents in this study had higher values in males compared to females. This gender dependent observation agree with that reported by Lugada *et al.*, (2004), Buseri *et al.*, (2010), Soldin *et al.*, (2011), and Dosoo *et al.*, (2014); however, the four authors reported higher platelet levels in females than in males. The sex-dependent platelet results for adolescents reported in this study contrast the gender-independent platelets results for adolescents reported by Onwurah *et al.*, (2018), Bimerew *et al.*, (2018), and Bogner *et al.*, (2019). The gender-independent platelet distribution width (PDW) results for adolescents reported here contrast the gender-dependent results reported by Dosoo *et al.*, (2014) who observed higher PDW values for adolescent females compared to males. Interestingly, the sex-dependent results for mean platelet volume (MPV) reported here for children and adolescents in age 10-15 years had higher values for males compared to females while that for adolescents aged 15-18 years had higher values for females compared to males. The cause of this inversion is unclear.

Results indicating statistically different hematological parameters reference intervals with advancement of age for RBC (x 10^{12}/L), HB (g/dL), MCHC (g/L), MCV (fL), RDW-SD (%), RDW-CV (%), WBC (x 10^{9}/L), NEU (x10^{9}/L), NEU (%), LYM (x 10^{9}/L), LYM (%), BAS (%), and MPV (fL) for

male and female children and adolescents of Taita-Taveta County, Kenya imply that these parameters are age dependent. [Median values for red blood cells (RBC) were low in age 1-5 years among children and adolescents, and then increased and plateaued among males in age 5-18 years; an observation agreeing with that reported by other researchers (Bleyere *et al.,* 2013,Mandala *et al.,* 2017). Median values for hemoglobin (HB) were low for age 1-15 years, and then increased between 15-18 years among males. This observation agrees with those reported by others (Bleyere *et al.,* 2013; Kiess *et al.,* 2016; Mandala *et al.,* 2017) who demonstrated changes of this parameter with advancement in age from childhood to adolescence. Median values for mean cell hemoglobin concentration (MCHC) were high between 1 and 5 years among children and adolescents, then decreased between 5 and 10 years among females, and returned to values of age 1-5 years and plateaued there between 10 and 18 years among females.

This observation contrasts the gender independence values of mean cell hemoglobin concentration (MCHC) of children and adolescents reported by Bleyere *et al.,* (2013) but agree with the gender dependence observation of this parameter reported by Mandala *et al.,* (2017) in this population. Median value of mean cell volume (MCV) were high between 1 and 5 years, then decreased in age 5-10 years among males, and then returned to values of age 1-5 years and plateaued there between 10 and 18 years among males; an observation agreeing with previous reports of other researchers (Bleyere *et al.,* 2013 ;Mandala *et al.,* 2017). Median values of red blood cell distribution width-

standard deviation (RDW-SD) and red blood cell distribution width-coefficient of **variation (RDW-CV) were high in age 1-5 years among children and adolescents, and then decreased and plateaued** between 5 and 18 years among **males**. These age-dependent values for red blood cells (RBC), hemoglobin (HB) and mean cell volume (MCV) agree with those reported by other researchers (Bleyere *et al.,* 2013; Kiess *et al.,* 2016).

The lower levels reference intervals of the iron-based hematological parameters based on either lower or upper limit or both limits (Lugada *et al.,* 2004; Buseri *et al*., 2010; Dosoo *et al.,* 2014; Bimerew *et al.,* 2018) compared with the literature-reported values could be due to taking diets which are nutritionally poor in iron that impairs hematopoiesis. This result could also be due to intermittent red blood cell loss due to malaria, helminthes infestation, or thalassemia, and sickle cell disease that are common in Taita-Taveta County, Kenya.

Median values for white blood cells (WBC) were high in age 1-5 years among children and adolescents, and then decreased and plateaued between 5 and 18 years among the males. These age-dependent median values for white blood cell agree with age-dependent values reported by others (Lugada *et al.,* 2004; Buseri *et al.,* 2010; Dosoo *et al.,* 2014) but contrast the age-independent values reported by Bimerew *et al.,* (2018) and Onwurah *et al.,* (2018). Median values for absolutes neutrophils (NEU) were high in age 1-5 years among children and adolescents, then decreased and plateaued in age 5-15 years among males, and

then returned to values of age 1-5 year and remained there in age 15-18 years among males.

This age dependent median values for neutrophils agree with those reported by others (Lugada *et al.,* 2004; Buseri *et al.,* 2010; Dacie and Lewis, 2017; North Bristol NHS) but contrast the age-independent values reported by Bimerew *et al.,* (2018). Interestingly, median values for percent neutrophils were low in age 1-15 years among children and adolescents, and then increased in age 15-18 years among females. Median values for absolute and percent lymphocytes were high in age 1-15 years among children and adolescents, and then decreased in age 15-18 years among females. These age dependent median value for absolute lymphocytes reported in this study agree with age dependent values reported by other researchers (Lugada *et al.,* 2004; Buseri *et al.,* 2010; Dosoo *et al.,* 2014; Dacie and Lewis, 2017; North Bristol NHS) but contrast the age independent values reported by Bimerew *et al.,* (2018).

Median values for absolute and percent monocytes were high in age 1-10 years among children and adolescents, and then decreased and plateaued in age 10-18 years among females; an observation agreeing with the age-dependent absolute and percent monocytes values reported by others (Lugada *et al.,* 2004; Buseri *et al.,* 2010; Dosoo *et al.,* 2014; Dacie and Lewis, 2017; North Bristol NHS) but contrasts the age independent results reported by Bimerew *et al.,* (2018). Median values for absolute eosinophils were low in age 1-15 years, then increased in age 15-18 among children and adolescents. The age dependent absolute eosinophil values reported in this study agree with those

reported by others (Lugada *et al.,* 2004; Buseri *et al.,* 2010; Dacie and Lewis, 2017) but contrasts the age-independent absolutes eosinophil values reported by Bimerew *et al.,* (2018). Median values for percent eosinophils were not affected by advancement in age though statistically differing. This difference was due to overweighting the ranks either near the 2.5 percentile or the 97.5 percentile. Median values for percent basophils (BAS) were high in age 1-5 years among children and adolescents, decreased in age 5-10 years among males, and then returned to value of age 1-5 years and remained there in age 10-18 years among children and adolescents. This age dependence of percent basophils in children and adolescents contrasts the age independent percent basophils previously reported by Bleyere *et al.,* (2013). The age dependent median values of white blood cells, absolute and percent neutrophils, absolute and percent lymphocytes, absolute and percent monocytes, absolute and percent eosinophils, and percent basophils in children and adolescents reported here contrasts the age independence values of these parameters reported by Bleyere *et al.,* (2013).

The observed age-independent median platelet count in age 1-18 years among children and adolescents in this study contrast that reported by others (Lugada *et al.,* 2004; Buseri *et al.,* 2010; Bleyere *et al.,* 2013;; Dosoo *et al.,* 2014 ; Mandala *et al.,* 2017) who reported age dependent platelet count. However, these results agree with the age independent values for platelets reported by Bimerew *et al.,* (2018) and Onwurah et al., (2018). The age independent median values of platelet distribution width reported in this study contrasts its

age dependence reported by Dosoo *et al.,* (2014). Interestingly, the median value of mean platelet volume (MPV) was high in age 1-5 years among males, then decreased between 5 and 10 years, increased in age 10-15 years and continued to decrease in age 15-18 years among the males.

Further, the median value of mean platelet volume (MPV) was low in age 1-15 years among females, and then increased in age 15-18 years among them. This age-related differences in platelets related indices could be caused the presence of estrogen in females which is associated with platelet function (Mandala *et al.,* 2017). The lower platelet count observed in this study in children and adolescent population of Taita-Taveta County, Kenya compared to the published literature (Dosoo *et al.,* 2014) could be associated with genetic or environmental factors or both (Mandala *et al.,* 2017). The other hematological parameters reference intervals including mean cell hemoglobin (MCH), platelets (PLT), and platelets distribution width (PDW) for male and female children and adolescents of Taita-Taveta County, Kenya are not affected by age.

This study had several limitations. One of the limitations of this study is that parasitic infections such as *Ascaris lumbricoides*, *Trichiuris trichiura*, filariasis, malaria, schistosomiasis and hookworm infections, and genetic diseases such as sickle cell anaemia, thalassemia, and micronutrient deficiencies were not investigated; these disease conditions could have affected hematological parameters. For example, the presence of parasitic intestinal helminths increases the level of eosinophils. The sample sizes for the various

age groups were not equal. They were below the required minimum of 120 referents for each age group due to lack of fulfillment of the inclusion and exclusion criteria. The behavioural, dietary, environmental factors that could affect the value of hematological parameters were not collected. The blood sampling time was not fixed at a specific period of the day hence effects due the circadian rhythm may have affected the results of this study.

5.3 Reference interval limits for serum biochemistry of children and adolescents of Taita-Taveta Ccounty, Kenya

This study established age and sex specific median and 95% reference interval limits for routine biochemistry for children and adolescents which can be used for interpreting clinical chemistry laboratory reports during routine healthcare practice and for screening and/or follow-up during clinical trials in Taita-Taveta County, Kenya in addition to other counties with similar population profiles. Development of reference interval limits for children and adolescents can be established based on either the continous reference interval approach which is suitable for parameters that continuously change with age for either males or females or both, or age subgrouped reference intervals (such as 1-5, 6-10, 11-15, and 16-18 years) for either males or females or both especially in the pubertal stage of growth and development. However, few clinical chemistry laboratory AutoAnalyzers are IT programmed to interpret laboratory results using continous reference interval approach (Bogner *et al.*, 2019). The continous approach helps avoid arbitrary subgrouping of age-based reference intervals and gives intervals that better represent physiological processes.

Presently, the most preferred reference interval partitioning approach is the age subgrouped approach. Establishment of combined male and female reference intervals for the measured analytes in children and adolescents is based on lack of sex differences and separate male and female reference intervals is based on significant sex differences. Review of literature on pediatric reference intervals revealed the absence of complete reference intervals for biochemistry analytes for children, pre-adolescents and adolescents in many African studies including Kenya. In addition, the developed reference intervals for pediatric population only involve few analytes.

Results indicating statistically similar serum biochemistry reference intervals for TP (g/L), ALB (g/L), ALT (U/L), AST (U/L), ALP (U/L), GGT (U/L), T-BIL (μmol/L), D-BIL (μmol/L), CREAT (μmol/L), BUN (mmol/L), UA (μmol/L), CL (mmol/L), K (mmol/L) and NA (mmol/L) for the male and female children and adolescent population of Taita-Taveta County imply that these parameters are gender independent. The developed reference intervals for TP is 45-85.5 g/L, ALB is 28.3-51.8 g/L, ALT is 4.2-61.8 U/L, AST is 10.9-129.3 U/L, GGT is 4.3-175 U/L, ALP is 0-492.4 U/L, T-BIL is 0.9-166 μmol/L, D-BIL is 0.3-16.3 μmol/L, CREAT is 18-131 μmol/L, BUN is 1.6-12.5 mmol/L, UA is 0.2-2.0 μmol/L, CL is 85-116.3 mmol/L, K is 3.7-6.0 mmol/L, and NA is 121.7-148.9 mmol/L.

The sex independent reference intervals for total protein (**TP**), reported here for children and adolescents contrast the sex dependent reference intervals for total protein reported by Buchanan *et al.,* (2015) for Tanzanian adolescents and

Sung *et al.,* (2021) for Korean children and adolescents but agrees with the sex independent reference intervals for total protein reported by Dosoo *et al*. ,(2014) for Ghanaian adolescents and Adeli *et al.,* (2015) for Canadian children and adolescents. The sex independent reference intervals for aspartate aminotransferase (**AST**) reported here for children and adolescents contrast the sex dependent reference intervals developed for this parameter reported by Zeh *et al.,* (2011); Soldin *et al.,* (2011) for American adolescents, for Tanzanian, Dosoo *et al.* (2014) for Kenyan, Buchanan *et al.,* (2015), Adeli *et al.,* (2015) for Canadian, Bogner *et al.,* (2019) for Austrian, and Sung *et al.,* (2021) for Ghanian children and adolescents. The sex independent reference intervals for γ-glutamyltransferase (**γ-GT**) reported here for children and adolescents agree with the sex independent reference intervals of this parameter reported by Dosoo *et al.,* (2014) for Ghanaian, Bogner *et al.,* (2019) for Austrian adolescents, but contrasts the sex dependent reference intervals for this parameter reported by Soldin *et al.,* (2011) for American adolescents, Adeli *et al.,* (2015) for Canadian children and adolescents. The sex independent reference intervals for total bilirubin (**T-BIL**) reported here for children and adolescents agree with the sex independent reference intervals reported for this parameter by Sung *et al.,* (2021) for Korean children and adolescents but contrast the sex dependent reference intervals reported by Zeh *et al.,* (2011) for Kenyan, Dosoo *et al.,* (2014) for Ghanaian adolescents, Buchanan *et al.,* (2015) for Tanzanian, and, and Adeli *et al.,* (2015) for Canadian children and adolescents. The sex independent reference intervals for direct bilirubin (**D-**

BIL) reported here for children and adolescents contrast the sex dependent reference intervals for this parameter reported by Dosoo *et al.*. (2014) for Ghanaian adolescents. The sex independent reference intervals for sodium (**NA**), potassium (**K**), and chloride (**CL**) reported here for children and adolescents agree with the sex independent reference intervals for these parameters reported by Dosoo *et al.* ,(2014) for Ghanaian adolescents, Buchanan *et al.,* (2015) for Tanzanian, and Sung *et al.,* (2021) for Korean children and adolescents for sodium (NA) but contrasts the sex dependent reference interval for sodium (NA) reported by Adeli *et al.,* (2015) for Canadian adolescents.

Median and 95% reference intervals for total protein (TP) were lower between 1 and 5 years among children, increased and plateaued between 5 and 18 years among children and adolescents. This observation contrasts with the sex and age independent reference intervals for this parameter reported by Buchanan *et al.,* (2015) but agrees with the age dependent reference intervals reported by Adeli *et al.,* (2015) for Canadian and Sung *et al.,* (2021) for Korean children and adolescents, respectively.

Median and 95% reference intervals for albumin (ALB) for children between 1 and 5 years was lower than that between 5 and 10 years which was followed by a decrease and plateauing to that of between 1 and 5 years and remained there between 10 and 18 years. The age dependent finding for albumin levels in this study agree with that reported by Dosoo *et al.,* (2014) for Ghanaian, Buchanan *et al.,* (2015) for Tanzanian, Adeli *et al.,* (2015) for Canadian children and

adolescents. Interestingly, these findings contrast the age independent albumin value reported by Sung *et al.,* (2021) for Korean children and adolescents. However, unlike this study's finding, these previous researchers observed progressive increase of albumin values with age.

Median and 95% reference intervals for alanine aminotransferase activity (ALT) for children between 1 and 15 years was higher than those of adolescents between 15 and 18 years. The findings agree with the age dependent values for this parameter reported by Buchanan *et al., (*2015) for Tanzanian, and Adeli *et al.,* (2015) for Canadian children and adolescents. However, these previous researchers (Buchanan *et al.,* 2015; Adeli *et al.,* 2015) reported progressive increase in alanine aminotransferase (ALT) with age. In addition, this study's findings on this parameter contrasts the age independent alanine aminotransferase activity values reported by Dosoo *et al.*, (2014) for Ghanaian, and Sung *et al.,* (2021) for Korean children and adolescents.

Median and 95% reference intervals for aspartate aminotransferase activity (AST) for children of between 1 and 5 years were higher but this was followed by a progressive decrease of this parameter levels between 5 and 18 years with age. The findings of this study agree with those reported by Dosoo *et al.,* (2014) for Ghanaian, Buchanan *et al*., (2015) for Tanzanian, Adeli *et al.,* (2015) for Canadian, and Sung *et al.,* (2021) for Korean children and adolescents. These researchers also reported a progressive decrease of aspartate aminotransferase activity (AST) with advancement with age.

Median and 95% reference intervals for alkaline phosphatase activity (ALP) was higher for children between 1 and 5 years compared to its lower plateaued value for children and adolescents of between 5-18 years. The finding for this parameter agrees with those reported by Buchanan *et al.,* (2015) for Tanzanian, Adeli *et al.,* (2015) for Canadian, and Sung *et al.,* (2021) for Korean children and adolescents. However, Buchanan *et al.,* (2015) findings for Tanzanian children and adolescents progressively increased with advancement in age. Further, Adeli *et al.,* (2015) findings for Canadian children progressively increased with age between 3 and 10 years. This was then followed by a progressive decrease with age between 11 and 18 for adolescents. In addition, Sung *et al.,* (2021) findings for Korean children and adolescents progressively increased between zero and 12 years. This was then followed by a progressive decrease between 13 and 18 years for adolescents.

Median and 95% reference intervals for γ-glutamyltransferase activity (γ-GT) was higher for children between 1 and 5 years compared to the decreased plateaued values for this parameter between 5 and 18 years for children and adolescents. The finding agrees with that reported by Adeli *et al.* (2015) for Canadian children and adolescents. However, Adeli *et al.,* (2015) reported a progressive increase in γ-glutamyltransferase activity (γ-GT) with advancement in age. This finding contrasts the age independent γ-glutamyltransferase activity (γ-GT) reported by Dosoo *et al.,* (2014) for Ghanaian children and adolescents.

Median and 95% reference intervals for total bilirubin (T-BIL) was higher for children between 1 and 5 years compared to children between 5 and 10 years. This then rose to the levels of children between 1 and 5 years and plateaued there for children and adolescents between 10 and 18 years. The finding for this age dependent total bilirubin (T-BIL) value agrees with those reported by Buchanan *et al.,* (2015) for Tanzanian, and Adeli *et al.,* (2015) for Canadian children and adolescents. However, these researchers (Buchanan *et al.,* 2015; Adeli *et al.,* 2015) reported progressive increase of this parameter with advancement in age. This age dependent finding for total bilirubin (T-BIL) here contrasts the age independent findings reported by Dosoo *et al.,* (2014) for Ghanaian, and Sung *et al.,(*2021) for Korean children and adolescents.

Median and 95% reference intervals for direct bilirubin (D-BIL) was higher for children between 1 and 5 years compared to children between 5 and 10 years. This was then followed by a rise to the level of children between 1 and 5 years and plateaued there for children and adolescents between 10 and 18 years. The age dependent finding of this parameter reported here agrees with that reported by Dosoo *et al.,* (2014) for Ghanaian children and adolescents which progressively decrease with advancement in age. However, the age dependent finding for direct bilirubin (D-BIL) reported here contrasts the age independent finding reported by Sung *et al.,* (2021) for Korean children and adolescents.

Median and 95% reference intervals for sodium (NA) was lower for children of between 1 and 5 years compared to children and adolescents between 5 and 15 years. This is then followed by return of sodium levels to those of children of

between 1 and 5 years and fairly plateauing at this level for adolescents between 15 and 18 years. The age dependent finding for this parameter in children and adolescents agrees with the age dependent findings reported by Dosoo *et al*., (2014) for Ghanaian, Buchanan *et al.*, (2015) for Tanzanian, Adeli *et al.,(*2015) for Canadian and Sung *et al.*, (2021) for Korean children and adolescents.

Median and 95% reference intervals for potassium (K) remained fairly unchanged for children and adolescents between 1 and 15 years, then decreased for adolescents between 15 and 18 years. The age-dependent finding of this parameter reported here agrees with the age dependent findings reported by Dosoo *et al.,* (2014) for Ghanaian, Buchanan *et al.*, (2015) for Tanzanian, and Adeli *et al.*, (2015) for Canadian children and adolescents; all the three researchers observed a progressive decrease of this parameter with aging. However, the age dependent finding of this parameter reported here contrasts the age independent finding reported by Sung *et al.,* (2021) for Korean children and adolescents.

Median and 95% reference intervals for chloride (CL) was higher for children of between 1 and 5 years compared to children and adolescents of between 5 and 15 years. This is then followed by a return of this electrolyte level to that of children of between 1 and 5 years and then remained fairly constant for adolescents of between 15 and 18 years. The age dependent finding for this electrolyte reported here contrasts the age independent finding reported by Dosoo *et al.,* (2014) for Ghanaian, Buchanan *et al.*, (2015) for Tanzanian, and

Adeli *et al.,* (2015) for Canadian, and Sung *et al.,* (2021) for Korean children and adolescents.

The small significant changes in electrolyte (NA, K, CL) levels with age from childhood to adolescents in this study supports the concept of the existence of a feedback mechanism that makes the levels of electrolytes relatively stable throughout pediatric life as reported by Adeli *et al.,* (2015) for Canadian children and adolescents. This small significant change in electrolytes levels with age has also been reported by Dosoo *et al.,* (2014) for Ghanaian children and adolescents and Buchanan *et al.,* (2015) for Tanzanian,. These small changes in electrolyteelectrolyte levels could reflectent stages of kidney function and regulation during aging. For example, the decrease in the upper limit of 7 mmol/L between 1 to 5 years to 5.6 mmol/L in age 15 to 18 years could be attributed to the change in kidney excretion caused by the hormones that regulate its clearance.

The decrease in alkaline phosphatase activity an enzyme involved in bone metabolism with advancement in age could be associated with both increased bone growth during childhood development. This was previously observed by Buchanan *et al.,* (2015) for Tanzanian and Adeli *et al.,* (2015) for Canadian children and adolescents.

Total protein and albumin rose in age 5-15 years, decreased to the level of age 1-5 years, and remained fairly stable at this level in age 15-18. Sex differences were observed for albumin between ages 15-18, with female values being lower than male values. Decreased albumin in adolescent females have been

previously reported by Dosoo *et al.,* (2014) for Ghanaian, Adeli *et al., (*2015) for Canadian, and Bogner *et al.,* (2019) for Austrian for adolescents. This decrease in albumin in females could be associated with blood loss through menstruation. The small and significant increase in albumin in age 5-15 years in male and female adolescents could be attributed to the growth and maturation of the liver.

Aspartate aminotransferase activity (AST) is expressed in the liver, heart, red blood cells, and kidney. This differential expression of aspartate aminotransferase (AST) in different tissues could explain its higher levels during childhood and decrease in adolescents, reflecting the period of growth during childhood development of organ systems. This progressive decrease of aspartate aminotransferase (AST) activity with age was also reported by Dosoo *et al.,* (2014) for Ghanaian, Buchanan *et al.,* (2015) for Tanzanian, Adeli *et al.,* (2015) for Canadian, and Sung *et al.,* (2021) for Korean children and adolescents.

The decrease in alanine aminotransferase (ALT) with age from childhood to adolescence reflects growth during the liver's childhood development. This decrease in the activity of ALT with age contrasts the progressive age dependent increase reported by Buchanan *et al.,* (2015) for Tanzanian, and Adeli *et al.,* (2015) for Canadian and its age independent reported by Dosoo *et al.,* (2014) for Ghanaian, and Sung *et al.,* (2021) for Korean children and adolescents. The sex differences alanine aminotransferase (ALT) observed in age 1-5 years with male value being higher than female values could be

reflecting its early release of liver cells at the period of high rapid cell turnover. The decrease in gamma-glutamyltransferase (GGT) with age from childhood to adolescentsadolescence reflects growth during the liver's childhood developmentease in the activity gamma-glutamyltransferase (GGT) with age contrasts the progressive age dependent increase of this enzyme reported by Adeli *et al.,* (2015) for Canadian and its age independent observation reported by Dosoo *et al.,* (2014) for Ghanaian children and adolescents.

The decrease in total (T-BIL) and direct (D-BIL) bilirubin with age in age 5-10 years compared to age 1-5 years and returning to age 1-5 years and stagnating at this level in age 10-18 years contrasts the progressive age dependent increase during childhood and adolescents reported by Buchanan *et al.,* (2015) for Tanzanian, and Adeli *et al.,* (2015) for Canadian, and its age independent observation reported by Dosoo *et al.,* (2014) for Ghanaian, and Sung *et al.,* (2021) for Korean children and adolescents.

The renal biomarker creatinine (CREAT), a product of muscle degradation, increased in pediatrics from childhood to adolescents with increasing age reflecting increasing skeletal and muscle mass caused by increased growth during childhood development. The cause of multiple sex differences in creatinine (CREAT) values with females having higher values than males at specific age (5-10 years and 15-18 years) subgroups is unclear and requires investigation. In agreement with this study Dosoo *et al.,* (2014) for Ghanaian, Buchanan *et al.,* (2015) for Tanzanian, Adeli *et al.,* (2015) for Canadian reported progressive increase in creatinine (CREAT) levels with age from

childhood to adolescents. The decrease in blood urea nitrogen (BUN), a degradative product of protein with age, especially in adolescents, seems to reflect growth during childhood development to adolescents. The sex differences in children of age 1-5 years with females having higher levels than males, could reflect its release from liver cells during rapid cell turnover during growth in early childhood development in females but not males. The reason for this sex related observation is unclear and requires investigation. In contrast, Dosoo *et al.,* (2014) for Ghanaian, Buchanan *et al.,* (2015) for Tanzanian and Adeli *et al.,* (2015) for Canadian childhood to adolescents reported a progressive increase in blood urea nitrogen levels with age.

The lower total protein and albumin levels observed in children and adolescents in this study compared to that reported by Soldin *et al.,* (2011) for American, Dosoo *et al.,* (2014) for Ghanaian, Buchanan *et al.,* (2015) for Tanzanian, Adeli *et al.,* (2015) for Canadian, Bogner *et al.,* (2019) for Austrian, and Sung *et al.,* (2021) for Korean children and adolescents could be associated with consumption diets that are poor in dietary proteins. Dietary proteins are digested to amino acids which are absorbed into the bloodstream and then used to synthesize proteins and other amino acid derivatives needed by humans. This consumption of diets poor in dietary proteins could also explain the lower levels of blood urea nitrogen observed in this study compared to that reported by Adeli *et al.,* (2015) for Canadian, Bogner *et al.,* (2019) for Austrian, and Bandesh *et al.,* (2019) for Indian children and adolescents. Blood

urea nitrogen is a synthesized from ammonia, a degradative product of amino acids obtained from protein catabolism.

The lower creatinine (CREAT) levels observed in this study in children and adolescents compared to that reported by Zeh *et al.,* (2011) for Kenya, Soldin *et al.,* (2011) for American children and adolescents , Adeli *et al.,* (2015) for Canadian, Bogner *et al.,* (2019) for Austrian and Sung *et al.,* (2021) for Korean could by associated with the reduced muscle mass in this Kenyan population. Creatinine (CREAT) is a degradative product of muscle mass (Bandesh *et al.,* 2019).

This study's total bilirubin (T-BIL) and direct bilirubin (D-BIL) reference interval values for children and adolescents is lower than that reported by Dosoo *et al.*. (2014) for Ghanaian, Buchanan *et al.,* (2015) for Tanzanian, Adeli *et al.,* (2015) for Canadian, Sung *et al.,* (2021) for Korean children and adolescents. This could reflect the higher amount of hemoglobin associated with these children and adolescents' populations. Bilirubin (BIL) is a degradative product of hemoglobin (HB). Low hemoglobin levels could be caused by diets low in iron, chronic blood loss resulting from parasitic infections (such as schistosomes (*Schistosoma haematobium* (18.9%) and *Schistosoma mansoni* (15.9%)), *Ascaris lumbricoides* (6.8%), hookworms (4.5%)), and hemoglobinopathies including genetic diseases of hemoglobin like α- and β-thalassemia, and sickle cell anemia (HbS) (Dosoo *et al.,* 2014). These parasitic infections, hemoglobinopathies and iron deficiency anemia (31.8 %) are present in Taita-Taveta County, Kenya (Ngaluma *et al.,* 2020).

The higher reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ-glutamyltransferase (γ-GT) activities in children and adolescents in this study compared to those reported by Zeh *et al.,* (2011) for western Kenya, Soldin *et al.,* (2011) for American, Dosoo *et al.,* (2014) for Ghanaian, Buchanan *et al.,* (2015) for Tanzanian, Adeli *et al.,* (2015) for Canadian, Bogner *et al.,* (2019) for Austrian, and Sung *et al.,* (2021) for Korean could be associated with decreased synthesis due to either genetics or consumption of diets poor in dietary proteins. In addition, this may also be explained by the observation that black children exhibit higher values of these enzymes than white children (Palmer, 2019). Further, it may also be explained by the fact that a hilly landscape, compared to a flat landscape of a geographical location, may promote exercise which also increases the level of these enzymes (Palmer, 2019).

This study's reference interval values for sodium (NA) and chloride (CL) for children and adolescents are lower than those reported by Dosoo *et al.,* (2014) for Ghanaian, Buchanan *et al.,* (2015) for Tanzanian, Adeli *et al.,* (2015) for Canadian, and Sung *et al.,* (2021) for Korean children and adolescents. This difference could be accounted for by consumption of diets with low quantities of sodium (NA) and chloride (CL) by children and adolescents of Taita-Taveta County, Kenya. This difference could also be caused by using instruments with different assay methodologies with different matrix effects. The levels of total protein (TP), total cholesterol (T-CHOL), and triacylglycerols (TG) could

cause differences in the levels of sodium (NA), potassium (K) and chloride (CL) when measured using direct and indirect ISE methods. However, while the total protein of the serum samples was estimated and noted to be lower than the reported literature values from other populations in this study, the levels of total cholesterol and triacylglycerols were not investigated.

This study had several limitations. One of these limitations was the use of less than 120 referents recommended by Clinical and Laboratory Standards Institute (CLSI, 2010) in age and sex subgroupings as a result of exclusion of some potential referents based on the screening tests. Sample size affects both the statistical power of the statistical analysis tool being used and subgroup partitioning. Further, blood was collected during any time of the day instead of a fixed period in the morning or a fixed period in the afternoon. This disregarded the influence of the circardian rhythm on some serum biochemistry analytes in a single blood collection day. Neither was a record of the diets of this study's pediatric population taken nor was its nutrients composition investigated. The health status for the studied pediatric population was not confirmed by clinical laboratory reports other than by the use of a self-administered questionnaire in addition to the physical and clinical observation by the projects nurse and physician. Data on the environmental and behavioural factors that can affect serum biochemistry analytes levels was also not collected.

5.4 Established reference ranges for pregnant mothers

Results of this study indicating statistically nonsignificant difference in the reference intervals for red blood cells (RBC) (3.2-5.2 $x10^{12}$/L), hemoglobin (HB) (7.8-14.3 g/dL), mean cell hemoglobin (MCH) (20.8-33.2 pg), mean cell hemoglobin concentration (MCHC) (28.0-36.5 g/dL), mean cell volume (MCV) (61.9-94.8 fL), red blood cell distribution width (RDW-CV) (11.27-20.85 %), red blood cell distribution width (RDW-SD) (38.0-69.5 %), white blood cells (WBC) (4.4-15.1 $x10^9$/L), lymphocytes (LYM) (1.0-4.4 $x10^9$/L; 10.8-44.0 %), neutrophils (NEU) (2.3-12.5 $x10^9$/L; 39.1-85.9 %), eosinophils (EOS) (0.07-0.63 $x10^9$/L; 0.30-6.21 %), monocytes (MON) (0.12-1.03 $x10^9$/L; 1.8-10.1 %), platelets (PLT) (128-388.4 $x10^9$/L), platelet crit (PCT) (0.12-0.34 %), platelet distribution width (PDW) (38.0-69.5 %), and mean platelet volume (MPV) (7.5-11.0 fL) in pregnant women in the second trimester compared to the third trimester imply that there are no trimester related alterations in these hematological parameters for this population in this environment. These results contrast the expected decrease in RBC, HB, and PLT due to increase in plasma volume which occurs faster than the increase in erythrocyte mass secondary to sodium and water reabsorption leading to hemodilution and the increased need for minerals and vitamins (iron, vitamin B12, folic acid) for fetal hematopoiesis. This is transmitted via renin-angiotensin-aldosterone pathway activation by increased secretion of progesterone and estrogen by the placenta during pregnancy. This is the modification of a pregnant woman's physiology

that occurs to compensate for the needs brought about by the fetus and its environment (Abdulqadir *et al.,* 2017).

The non-significant changes in the hematological parameters observed in this study could be explained by a fast hemodilution in the first trimester which seems to level off in the second and third trimesters as demonstrated in the reduced changes in the hematological parameters as reported by Balloch and Cauchi (1993), Purohit *et al.,* (2015), Odhiambo *et al.,* (2017) and Bakrim *et al.,* (2018). Alternatively, it could be that pregnant women on minerals and vitamins (iron, vitamin B12, folic acid) and antimalarial supplementation as occurs in this population for this County and the rest of Kenyan Counties have less pronounced hematological changes because their increase in red blood cell mass is proportional when compared to pregnant women not on minerals and vitamins (iron, vitamin B12, folic acid) and antimalarial supplementation (Purohit *et al.,* 2015; Genetu *et al.,* 2017).

In agreement with the current study, Balloch and Cauchi (1993) reported trimester independent reference intervals for HB, MCH, MCHC, MCV, MON, and EOS, Lurie *et al.,* (2008) reported trimester independent reference intervals for EOS and BAS, Mwinga *et al.,* (2009) reported trimester **independent** reference intervals for WBC and LYM (%), Purohit *et al.,* (2015) reported trimester **independent** reference intervals for RBC, HB, MCH, MCHC, MCV, NEU, LYM, MON and EOS, Odhiambo *et al.,* (2017) reported trimester **independent** reference intervals for RBC, MCV, MON and EOS, Genetu *et al.,* (2017) reported trimester **independent** reference intervals for RBC, HB,

MCH, MCHC, MCV, WBC, LYM and PLT, and Bakrim et al. (2018) reported trimester **independent** reference intervals for RBC, HB, MCH, MCHC, MCV, MPV, MON, BAS, and EOS for pregnant women in their second and third trimester. In contrast to the current study, Balloch and Cauchi (1993) reported trimester **dependent** decrease in the reference interval for RBC (3.43-4.49 g/dL versus 3.38-4.43 g/dL), and LYM (0.9-3.9 $x10^9$/L versus 1.0-3.6 $x10^9$/L), and an increase in the reference interval for WBC (6.6-14.8 $x10^9$/L versus 5.9-16.9 $x10^9$/L), NEU (3.8-12.3 $x10^9$/L versus 3.9-13.1 $x10^9$/L), MON (0.1-1.1 $x10^9$/L versus 0.1-1.4 $x10^9$/L), and PLT (171-409 $x10^9$/L versus 155-429 $x10^9$/L); Lurie *et al.,* (2008) reported trimester **dependent** increase in the reference intervals for WBC (5.22-12.20 $x10^9$/L versus 5.12-13.20 $x10^9$/L), NEU (3.37-9.05 $x10^9$/L versus 3.26-9.76 $x10^9$/L), LYM (0.85-2.69 $x10^9$/L versus 1.01-2.75 $x10^9$/L), and MON (0.163-0.705 $x10^9$/L versus 0.120-0.874 $x10^9$/L).

Mwinga *et al.,* (2009) reported a trimester **dependent** increase in the reference intervals for RBC (2.65-4.92 $x10^{12}$/L versus 2.87-5.34 $x10^{12}$/L), HB (8.2-13.2 g/dL versus 9.0-13.95 g/dL) and MON (1.7-17 % versus 1.1-23.1 %), and a decrease in PLT (97-350 $x10^9$/L versus 86.5-344.5 $x10^9$/L); Akinbami *et al*., (2013) reported trimester **dependent decrease** in reference intervals for HB (7.44-14.18 versus 7.89-12.87 g/dL), MCHC (34.37-38.61 g/dL versus 29.87-32.81 g/dL), MCV (67.17-89.57 fL versus 59.44-80.60 fL), PLT (104.09-351.05 $x10^9$/L versus 15.80-385.84 $x10^9$/L), and an increase in WBC (3.33-12.43 $x10^{12}$/L versus 4.10-12.52 $x10^{12}$/L); Purohit et al. (2015) reported

trimester **dependent** increase in RDW-CV (10.3-19.8 % versus 11.4-26.5 %) and WBC (4.9-14.5 $x10^9$/L versus 6.0-14.3 $x10^9$/L) and a **decrease** in PLT (234-390 $x10^9$/L versus 170-338 $x10^9$/L); Odhiambo *et al.*, (2017) reported trimester **dependent** reference intervals decrease in HB (6.1-13.0 g/dL versus 5.5-12.7 g/dL), WBC (3.6-11.1 x 10^9/L versus 3.3-10.7 x 10^9/L), and BAS (0.01-0.12 x 10^9/L versus 0.01-0.09 x 10^9/L), and increase in PLT (98-395 x 10^9/L versus 105-425 x 10^9/L), NEU (1.9-7.1 x 10^9/L versus 2.0-5.7 $x10^9$/L), and LYM (0.9-3.3 x 10^9/L 4versus 1.2-3.8 x 10^9/L); and Bakrim *et al.*, (2018) also reported trimester **dependent** increase in WBC (4.6-12.6 x 10^9/L versus 5.3-14.3 x 10^9/L), NEU (2.2-9.2 x 10^9/L versus 3.0-11.0 $x10^9$/L), LYM (1.2-3.6 x 10^9/L versus 1.1-3.8 x 10^9/L), and PLT (140-364 x 10^9/L versus 139-398 x 10^9/L) from the second to the third trimester. These differences highlight the need for the use of locally developed reference intervals for clinical management of pregnancy related disease conditions in pregnant women.

Results of this study indicating that the reference interval of the packed cell volume (PCV) (0.3-71.2 %), and basophils (BAS) (0.01-0.14 $x10^9$/L) of pregnant women of Taita-Taveta County population in their second trimester is significantly higher, and lower than that of their counter parts in the third trimester (0.3-45.0 %) and (0.0-0.60 $x10^9$/L), respectively, implies that these hematological parameters are trimester dependent for this referent population.

The significant decrease in the reference intervals for PCV in the third trimester of pregnant women of Taita-Taveta County population compared to those in the second trimester of the same county could be due to decreased red

blood cells (RBC) explained by hemodilution resulting from the increase in plasma volume. The increase in progesterone and estrogen secreted from the placenta during pregnancy induces release of renin from the kidneys and reduces the release of atrial natriuretic peptide from the heart. Renin stimulates the conversion of angiotensinogen released from the liver to angiotensin I which in turn is converted to angiotensin II by angiotensin converting enzyme (ACE) released from the endothelial cells of the lungs. Angiotensin II acts directly on blood vessels to induce vasoconstriction, and on the adrenal cortex to stimulate the production of aldosterone which in turn acts on the kidneys to promote sodium and water reabsorption which increase the plasma volume (blood volume).

The increase in blood volume and induction of vasoconstriction induces an increase in blood pressure. The increase in plasma volume (40 %) is more pronounced than the increase in red blood cell mass (20 %) and therefore leading to a decrease in maternal hemoglobin concentration causing physiological anemia (Kaur *et al.,* 2014; Purohit *et al.,* 2015). It may not be associated with malaria infection since all pregnant women in Taita-Taveta County were on antimalarial drugs (Fansidar) as a prophylactic measure after sixteen weeks of pregnancy when the fetal heart beat is noticeable. The significant increase in the reference intervals for BAS in the third trimester of pregnant women of Taita-Taveta County population relative to those in the second trimester of the same county could be explained by the increased production of basophil granule major basic protein (MBP) (Wasmoen *et al.,*

1987). The pregnancy associated major basic protein (MBP) localized in placental X cells and placental-site giant cells is indistinguishable from basophil granule major basic protein (MBP) immunochemically and biochemically: they have the same molecular weight, isoelectric point, and the same peptide map. Basophil granule major basic protein (MBP) is associated with tracheal smooth muscle contractility alteration. Together with alteration of the levels of histamine, estrogen, progesterone, prostaglandins, and oxytocin in complex interaction, basophil MBP could also be associated with induction of labor by altering myometrial smooth muscle contractility and cervical ripening (Wasmoen *et al.,* 1987). The elevated levels of basophils ensure its supply during the third trimester of pregnancy.

The observed **decrease** in PCV (0.3-71.2 % versus 0.3-45 %) in this study from the second to the third trimester is in **agreement** with the decrease reported by Odhiambo *et al.,* (2017) (21.4-38.1 % versus 17.7-38.6 %) for western Kenyans. However, it **contrasts** the increase (23.8-39.3 % versus 25.5-41.85 %) reported by Mwinga *et al.,* (2009), Akinbami *et al.,* (2013) (19.55-39.97 % versus 25.44-40.64 %), Purohit *et al.,* (2015) (27.1-38.7 % versus 27.3-40.1 %), and Genetu *et al.,* (2017) (39.63-41.44 % versus 41.17-42.75 %) even though the study subjects were on iron supplementation. The observed increase in BAS (0.01-0.14 $x10^9$/L versus 0.0-0.60 $x10^9$/L) in this study from the second to the third trimester **contrasts** the decrease (0.01-0.12 $x10^9$/L versus 0.01-0.09 $x10^9$/L) reported by Odhiambo *et al.,* (2017) and the non-significant alteration reported by Balloch and Cauchi (1993).

And Lurie *et al.,* (2008).

One limitation of this study was that it did not include a sample from non-pregnant women from the same referent population to generate baseline data. Another limitation was that it was not possible to recruit pregnant women early enough to capture enough of them in their first trimester. Other limitations included the variation in the number of pregnant women in each trimester due to exclusion of participants during screening, and the time for drawing blood varied daily which may have increased the reference intervals due to the circadian rhythms. Further, this study neither screened and removed iron deficient referent subjects nor identify and remove referent subjects with hemoglobin related diseases. However, the pregnant referent population were supplemented with mineral, vitamins and antimalarial drugs (Fansidar) immediately after the confirmation of the fetal heat beat and were therefore not expected to suffer from mineral and vitamin deficiency related diseases and malarial infection. This, however, required confirmation by measuring the levels of iron, transferrin, and iron binding capacity, which was not done.

5.5 Developed reference intervals for liver and kidney tests, and electrolytes for pregnant women of Taita-Taveta County, Kenya

Results of this study indicating that the developed reference intervals for liver and kidney tests, and electrolytes for total protein (TP), serum albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), blood urea nitrogen (BUN), potassium (K), chloride (CL), and calcium (CA) for pregnant women in the second trimester

are not significantly different from those in the third trimester indicates that these serum biochemistry parameters are trimester independent for this Taita-Taveta County population.

These trimester independent reference intervals for pregnant women are 55.6-72.2 g/L for TP, 29.1-46.7 g/L for ALB, 3.8-42.4 U/L for ALT, 9.7-41.9 U/L for AST, 1.7-79.9 U/L for GGT, 0.8-4.1 mmol/L for BUN, 89.9-105.7 mmol/L for CL, 3.6-5.2 mmol/L for K, and 0.1-2.1 mmol/L for CA. Other researchers have also reported trimester independent reference interval limits for CL (Abbassi-Ghanavati *et al.,* 2009), TP, K (Abbassi-Ghanavati *et al.,* 2009; Klajnbard *et al.,* 2010), and BUN (Klajnbard *et al.,* 2010) which were different from those reported in this study. Cheung and Lafayette (2013) also reported trimester-independent K levels in pregnancy. In contrast, other researchers reported trimester-dependent reference intervals for ALB and GGT (Abbassi-Ghanavati *et al.,* 2009), ALT (Abbassi-Ghanavati *et al.,* 2009; Klajnbard *et al.,* 2010 and Odhiambo *et al.,* 2017) , AST (Klajnbard *et al.,* 2010; Odhiambo *et al.,* 2017), ALP (Abbassi-Ghanavati *et al.,* ; 2009; Klajnbard *et al.,* 2010), BUN and CA (Abbassi-Ghanavati *et al.,* 2009). These are again different than those observed in the current study confirming the need for each clinical laboratory to develop their own reference intervals using the local population. These differences could be due to factors such as genetics, ethnicity, dietary habits, environment, the methods and reagents used in assessing the levels of the analytes, the comprehensiveness of the inclusion and exclusion criteria, among others. These findings are interesting especially when it is known that

the plasma volume and glomerular filtration rate increases during pregnancy resulting in a decrease in most serum constituents. The only serum biochemistry parameters that increase during pregnancy are those associated with the rising levels of oestrogen and progesterone, and placental and bone alkaline phosphatase and uric acid (risk marker for pre-eclampsia) in the third trimester (Sikaris, 2014).

Results of this study indicating that the developed reference intervals for liver and kidney tests, and electrolytes including alkaline phosphatase (ALP), total bilirubin (T-BIL), direct bilirubin (D-BIL), and creatinine (CREAT) are significantly higher, and sodium (NA), and uric acid (UA) are significantly lower in the second trimester relative to the third trimester, respectively, imply that these biochemical parameters are trimester dependent for this Taita-Taveta pregnant women population. These trimester dependent reference intervals for pregnant women are 0-133.9 U/L ALP in the second trimester and 0-165.8 U/L ALP in the third trimester, 0-14.3 μmol/L T-BIL in the second trimester and 0.4-17.0 μmol/L T-BIL in the third trimester, 0-3.1 μmol/L D-BIL in the second trimester and 0.1-6.5 μmol/L D-BIL in the third trimester, 29.6-113.4 μmol/L CREAT in the second trimester and 36.3-89 μmol/L CREAT in the third trimester, 0.18-0.35 μmol/L UA in the second trimester and 0.15-0.32 μmol/L UA in the third trimester, and 126.3-140.6 mmol/L NA in the second trimester and 121.9-139.9 mmol/L NA in the third trimester. Other researchers also reported trimester dependent intervals for ALP, UA (Abbassi-Ghanavati *et*

al., 2009 ; Klajnbard *et al.,* 2010), T-BIL, and CREAT (Abbassi-Ghanavati *et al.,* 2009 ;Odhiambo *et al.,* 2017).

Further, other investigators reported trimester independent reference intervals for NA (Abbassi-Ghanavati *et al.,* 2009 ; Klajnbard *et al.,* 2010), T-BIL (Klajnbard *et al.,* 2010), and D-BIL (Abbassi-Ghanavati *et al.,* 2009). The significant increase in ALP in the third trimester compared to the second trimester could be due to the increased production of both the placental and bone isoenzyme of this enzyme during the third trimester. The increase in creatinine (CREAT) in the third trimester relative to the second trimester could be due to a decrease in renal plasma flow in the third trimester of pregnancy towards preconception values leading to an increase in serum creatinine (CREAT). The decrease in serum NA levels in the third trimester compared to the second trimester of pregnancy could be due to its increased reabsorption in the proximal nephron due to a large increase in aldosterone and potassium excretion being counterbalanced by increased estrogen and progesterone levels with progesterone acting as a mineral corticoid antagonist in the distal nephron (Teasdale and Morton, 2018). It could also be due to hemodilution caused by vasodilation, secondary increase in water and sodium retention, and increased plasma volume and subsequent antidiuretic hormone (ADH) release during pregnancy (Cheung and Lafayette, 2013). Water homeostasis is controlled by thirst and secretion of antidiuretic hormone (ADH) which are regulated by serum osmolality.

The decrease in serum uric acid (UA) levels in the third trimester compared to the second trimester could be due to a continuous increase in renal plasma flow and glomerular filtration rate (GFR) and decreases in proximal tubular reabsorption from the first trimester to the third trimester above the preconception value resulting in reduction in serum uric acid (Cheung and Lafayette, 2013; Narelle, 2017; Teasdale and Morton, 2018). Increased T-BIL and D-BIL could be attributed to liver damage caused by elevated estrogen levels during pregnancy towards preconception levels. Pregnancy related increase in estrogen, progesterone and mutations in canalicular bile salts export pump (ABCB11) and multidrug resistant protein-3 (MDR3, ABCB4) gene leads to increased sensitivity to estrogen which impairs sulphation and transport of bile acids, decreases hepatocyte membrane permeability and uptake of bile acid by the liver (Glantz *et al.*, 2004). The raised bile acid concentration interferes with myometrial contractility and induces vasoconstriction of placental chorionic veins, which causes preterm deliveries, meconium staining of liquor, fetal bradycardia, distress and fetal loss correlated with fasting serum bile acid levels higher than 40 μmol/L (Glantz *et al.*, 2004). The differences between the serum biochemistry analytes reference intervals for pregnant women population of Taita-Taveta County and those previously reported in literature from other parts of the world could be due to dietary habits (nutritional status), genetics, health status, environmental, lifestyle including tobacco smoking, alcohol consumption, and sedentary lifestyle. Further, the analytical instruments and reagents used and the selection criteria

of referent pregnant women in different parts of the world may also contribute to the observed differences.

This study had several limitations. One limitation of this study was that it did not include a sample from non-pregnant women from the same referent population to generate baseline data. Another limitation was that it was not possible to recruit pregnant women early enough to capture them in their first trimester. Other limitations included the variation in the number of pregnant women in each trimester due to exclusion of participants during screening, and the time for drawing blood varied daily which may increase the reference intervals due to the circadian rhythms. However, this study generated the first reference intervals for biochemical parameters for pregnant women of Taita-Taveta County population of Kenya to be used locally in the clinical management of women disorders during pregnancy.

5. 6 Developed reference interval limits for hematological parameters for adult and geriatric population

Results indicating statistically similar hematological reference intervals for the adult and geriatric male and female population for red blood cells (RBC $x10^{12}$/L), hemoglobin (HB g/dL), packed cell volume (PCV %), mean cell hemoglobin (MCH pg), mean cell hemoglobin concentration (MCHC g/dL), mean cell volume (MCV fL), red blood cell distribution width-coefficient of variation (RDW-CV %), red blood cell distribution width-standard deviation (RDW-SD %), white blood cells (WBC $x10^9$/L), absolute lymphocytes (LYM $x10^9$/L), absolute and percent monocytes (MON $x10^9$/L & MON %), absolute

eosinophils (EOS x10^9/L), absolute and percent basophils (BAS x10^9/L & BAS %), platelets (PLT x10^9/L), plateletcrit (PCT %), platelet distribution width (PWD fL), and mean platelet volume (MPV fL) of Taita-Taveta County, Kenya imply that these parameters are gender independent. The developed gender independent hematological reference interval limits for adult and geriatric male and female population of Taita-Taveta County, Kenya for RBC is 2.8-5.7 x 10^{12}/L, HB is 6.6-16.6 g/dL, PCV is 0.2-44.9 %, MCH is 20.6-35.1 pg, MCHC is 27.3-36.1 g/dL, MCV is 70.0-106.2 fL, RDW-CV is 11.4-18.6 %, RDW-SD is 39.4-66.8 %, WBC is 2.8-14.6 x 10^9/L, LYM is 0.8-5.0 x 10^9/L, MON is 0.01-0.98 x 10^9/L, MON is 0.4-13.3 %, EOS is 0.02-0.77 x 10^9/L, BAS is 0.01-0.10 x 10^9/L, BAS is 0.2-3.04 %, PLT is 116.3-513 x 10^9/L, PCT is 0.1-0.4 %, PDW is 7.6-16.9 % and MPV is 6.9-10.9 fL. These gender independent findings agree with the previously reported gender independent findings for mean cell hemoglobin (MCH) (**Yip** *et al.,* 1984, **Tsang** *et al.,* 1998 ,**Böhler** *et al*., 2008, **Mugis**ha *et al.,* 2016, **Al-Mawali** *et al.,* 2018), mean cell hemoglobin concentration (MCHC) (**Yip** *et al.,* 1984 , Swaanenburg *et al.,* 1987, **Böhler** *et al.,* 2008, **Mugis**ha *et al.,* 2016, **Al-Mawali** *et al.,* 2018), mean cell volume (MCV) (**Yip** *et al.,* 1984, Swaanenburg *et al.,* 1987, **Woo** *et al.,* 1989, **Tsang** *et al.,* 1998, **Böhler** *et al.,* 2008, **Mugis**ha *et al.,* 2016, **Al-Mawali** *et al.,* 2018), and red blood cell distribution width-coefficient of variation (RDW-CV) (**Al-Mawali** *et al.,* 2018) for Burkina Faso, south-west Uganda, American[2], Australian, Oman, Dutch and Chinese populations.

Further, these gender independent findings agree with the previously reported gender independent findings for **wh**ite blood cells (WBC) (H**ale** *et al.,* 1983, **Woo** *et al.,* 1989, L**ugada** *et al.,* 2004, **Böhler** *et al.,* 2008, , **Al-Mawali** *et al.,* 2018), absolute lymphocytes (LYM) (Swaanenburg *et al.,* 1987, **Böhler** *et al.,* 2008, **Mugis**ha *et al.,* 2016, **Al-Mawali** *et al.,* 2018), absolute monocytes (MON) (L**ugada** *et al.,* 2004, **Mugis**ha *et al.,* 2016, **Al-Mawali** *et al.,* 2018), absolute eosinophils (EOS) (L**ugada** *et al.,* 2004, **Al-Mawali** *et al.,* 2018), absolute basophils (BAS) (Swaanenburg *et al.,* 1987, L**ugada** *et al.,* 2004, **Mugis**ha *et al.,* 2016, **Al-Mawali** *et al.,* 2018), and percent basophils (BAS %) (Swaanenburg *et al.,* 1987, **Mugis**ha *et al.,* 2016) for the Burkina Faso, American[1], Chinese, Oman, Dutch, east Uganda and south-west Uganda populations. In addition, these gender independent findings agree with the previously reported gender independent findings for **p**latelets (PLT) (**Böhler** *et al.,* 2008), platelet distribution width (PDW) (**Al-Mawali** *et al.,* 2018) and mean platelet volume (MPV) (**Al-Mawali** *et al.,* 2018) for the Burkina Faso, and Oman populations.

However, the gender independent results for red blood cells, hemoglobin, and packed cell volume (H**ale** *et al.,* 1983, **Yip** *et al.,* 1984, Swaanenburg *et al.,* 1987, **Woo** *et al.,* 1989, **Tsang** *et al.,* 1998, L**ugada** *et al.,* 2004, **Böhler** *et al.,* 2008, **Mugis**ha *et al.,* 2016, **Al-Mawali** *et al.,* 2018), mean cell volume (H**ale** *et al.,* 1983 ; L**ugada** *et al.,* 2004), white blood cells (Swaanenburg *et al.,* 1987,**Tsang** *et al.,* 1998, **Mugis**ha *et al.,* 2016), absolute and percent monocytes, and absolute eosinophils (Swaanenburg *et al.,* 1987), platelets

(Swaanenburg *et al.,* 1987, **Woo** *et al.,* 1989, **Tsang** *et al.,* 1998, L**ugada** *et al.,* 2004, **Mugis**ha *et al.,* 2016, **Al-Mawali** *et al.,* 2018) and plateletcrit (**Al-Mawali** *et al.,* 2018) contrast their gender dependence previously reported by other researchers for the Dutch, east Uganda, south-west Uganda, American[1], American[2], Chinese, Australian, Oman, and Burkina Faso populations.

Results indicating statistically different hematological reference interval limits for the adult and geriatric male and female population for NEU ($x10^9$/L), NEU (%), LYM (%), and EOS (%) of Taita-Taveta County, Kenya imply that these parameters are gender dependent. The developed gender dependent hematological parameters reference intervals for adult and geriatric population of Taita-Taveta County, Kenya for NEU is 2.3-6.9 $x10^9$/L and 22.6-86 % for males and 0.6-7.7 x 10^9/L and 22.3-86 % for females; both of which are higher in males than females, LYM is 10.2-63.6 % for males and 10.2-72.7% for females, and EOS is 0.2-11.92 % for males and 0.27-10.8 % for females; both of which are higher in females than males.

These gender dependent results of this study for absolute neutrophils (Swaanenburg *et al.,* 1987, L**ugada** *et al.,* 2004, **Mugis**ha *et al.,* 2016), percent neutrophils (**Mugis**ha *et al.,* 2016), and percent eosinophils (Swaanenburg *et al.,* 1987) agree with those reported by other researchers in the Dutch**,** eastern Uganda[L], and south-western Uganda[M] populations. In contrast, these gender dependent results for absolute neutrophils (**Al-Maw**ali *et al.,* 2018), percent neutrophils (Swaanenburg *et al.,* 1987, **Böhler** *et al.,* 2008), and percent lymphocytes (**Böhler** *et al.,* 2008) reported in this study contrast their gender

independent values reported for the Oman, Dutch, and Burkina Faso populations.

The observed decrease in the mean cell volume (MCV fL) and hemoglobin (HB g/L) in elderly adults especially in males in the seventh decade onwards compared to their counterparts in the sixth decade indicates that this elderly adult population has microcytic anemia. Microcytic anemia that is common in elderly adults could be due to iron deficiency, inflammatory disease or thalassemias. Iron deficiency anemia in the elderly adults is mostly caused by diets poor in iron or inflammatory disease that impairs iron uptake from the gastrointestinal mucosa or blood loss through the gastrointestinal tract. Poor iron uptake may be due to age related decrease in stomach hydrochloric acid that is responsible for iron uptake in the intestines.

Decreased serum concentration of vitamin B12 is a common problem in the elderly adults that is also caused by decreased secretion of hydrochloric acid in the stomach or chronic atrophic gastritis. Atrophic gastritis is a disease characterized by chronic inflammation of gastric mucosa of the stomach which occurs due to loss of gastric glandular cells and their replacement with intestinal fibrous tissue. This account for the low levels of intrinsic factor responsible for vitamin B12 uptake; this result in reduced levels of vitamin B12 absorption leading to its decreased concentration of serum vitamin B12 (Palmer, 2019).

This gender-based observation could be associated with sexual differentiation that is caused by genetic and epigenetic factors including acute sex hormone

actions as reported by Murphy (2014). Oestrogens vasodilate while androgens vasoconstrict renal microvasculature: vasodilation and vasoconstriction of vessels below 300 μm in diameter, respectively, increases and decreases the packed cell volume of arterioles, capillaries and venules, changing oxygen delivery per unit red blood cell mass, and providing a mechanism for varying the red blood cell mass without compensatory changes in erythropoiesis at the juxtaglomerular apparatus (JGA). There is no compensatory response in the JGA to the constitutively lower levels in healthy adult females.

The lower developed reference intervals for RBC, HB, and PCV for the male and female population for the Taita-Taveta County compared to the other previously reported reference intervals may be associated with low levels of dietary iron intake, which causes iron deficiency anemia, abnormal hematopoiesis, chronic loss of blood due to hookworm infection, skin infections, diarrhea, urinary tract infections, or chronic malaria infection. The differences between the developed hematological reference interval limits from the previously reported reference intervals in literature by other researchers from other parts of the world may be accounted for by ethnicity, dietary habits, genetics, altitude above sea level, and environmental factors such as pathogens. An increase in altitude lowers the plasma volume resulting in an increase in hemoglobin concentration and the packed cell volume, and increases the number of circulating red blood cells with a small mean cell volume.

This increase in plasma volume is due to increased erythropoiesis caused by reduced oxygen concentration in the atmosphere and hence in cells (hypoxia)

with increase in altitude and the decrease in plasma volume at high attitudes (Al-Sweedan and Alhaj, 2012 , Bimerew *et al.,* 2018). Decrease in altitude results in reversing the effects on the above-mentioned hematological parameters (Al-Sweedan and Alhaj, 2012).

The first limitation of this study is that the seasonal variations which can affect these reference interval limits for hematological parameters were not investigated; circadian rhythms can affect hematological parameters. The second limitation is that the study relied on the referent population's clinical history and physical examination and a self-assessment questionnaire without specifically screening for overt clinical conditions. However, the failure to exclude referents with overt clinical conditions may not significantly alter the developed reference intervals for hematological parameters of this adult and geriatric population. Thirdly, the sample size for the age stratified reference interval limits was below the minimum of 120 referent individuals recommended by CLSI (2010).

5.7 Developed reference interval limits adults and geriatric population of Taita Taveta County, Kenya

Results indicating statistically similar serum biochemistry analytes reference interval limits for the adult and geriatric male and female population of Taita-Taveta County for random blood glucose (RBS mmol/L), total protein (TP g/L), albumin (ALB g/L), aspartate aminotransferase (AST U/L), alkaline phosphatase (ALP U/L), total bilirubin (T-BIL μmol/L), direct bilirubin (D-BIL μmol/L), blood urea nitrogen (BUN mmol/L), sodium (NA mmol/L),

chloride (CL mmol/L), and potassium (K mmol/L) imply that these parameters are gender independent. The developed gender independent serum biochemistry reference interval limits for the adult and geriatric male and female population of Taita-Taveta County, Kenya is 4.2-10.8 mmol/L for random blood glucose (RBS), 57.4-88.5 g/L for total protein (TP), 33.0-49.5 g/L for serum albumin (ALB), 8.3-66.5 U/L for aspartate aminotransferase (AST), 0-147.6 U/L for alkaline phosphatase (ALP), 1-19.7 μmol/L for total bilirubin (T-BIL), 1.8-8.6 mmol/L for blood urea nitrogen (BUN), 122.2-148.9 mmol/L for sodium (NA), 3.2-5.7 mmol/L for potassium (K), and 81.8-112.3 mmol/L for chloride (CL).

These results are in agreement with those reported by Mu *et al.* (2013) and Adeli *et al.,* (2015) who reported gender-independent reference interval limits for albumin (ALB) (for 20-79 and 55-79 years old, respectively) and total protein (TP) (for 20-79 years old). Mu *et al.,* (2013) and Achila *et al.,* (2017) reported gender-independent reference interval limits for alkaline phosphatase (ALP) (for 20-79 and 60-80 years old, respectively). Adeli *et al.,* (2015) and Achila *et al.,* (2017) reported gender-independent reference interval limits for aspartate aminotransferase (AST) (for 55-79 and 60-80 years, respectively) and blood urea nitrogen (BUN) (for 60-79 and 60-80 years old, respectively). Adeli *et al.,* (2015), Jia *et al.,* (2015) and Achila *et al.,* (2017) reported gender-independent reference interval limits for potassium (K) (for 6-79, 20-80 and 60-80 years old, respectively) and chloride (CL) (for 6-79, 20-80 and 60-80 years old, respectively). Adeli *et al.,* (2015) and Jia *et al.,* (2015) reported

gender-independent reference interval limits for sodium (NA) (for 50-79 and 20-80 years old, respectively).

In contrast, these results disagree with those reported by Adeli *et al.,* (2015), Jia *et al.,* (2015) and Achila *et al.,* (2017) who reported gender-dependent reference interval limits for sodium (NA) (for 50-79, 20-80 and 60-80 years old, respectively), alkaline phosphatase (ALP) (for 22-79 and 60-80 years old, respectively), and albumin (ALB) (55-79 and 60-80 years old, respectively). Mu *et al.,* (2013), Adeli *et al.,* (2015) and Achila *et al.,* (2017) reported gender dependent reference interval limits for total bilirubin (T-BIL) (for 49-79 and 60-80 years old, respectively). Achila *el al.,* (2017) reported gender dependent reference interval limits for direct bilirubin (D-BIL) (for 60-80 years old).

Results indicating statistically different serum biochemistry analytes reference interval limits for the adult and geriatric male and female population for ALT (U/L), GGT (U/L), D-BIL (μmol/L), and CREAT (μmol/L) of Taita-Taveta County, Kenya imply that these parameters are gender dependent. The developed gender dependent serum biochemistry reference intervals for adults and geriatric male and female population of Taita-Taveta County, Kenya for ALT is 15 (4.8-52) U/L for males and 13.5 (5.9-37) U/L for females, GGT is 27 (9-186.3) U/L for males and 21.5 (8.8-160.5) U/L for females, D-BIL is 2 (0-6) μmol/L for males and 4 (0-6) μmol/L for females, and CREAT is 77.5 (44.3-163.5) μmol/L for males and 72 (34.1-134.8) μmol/L for females; three of them except D-BIL are higher in males than females.

These results agree with those reported by Mu *et al.* (2013) and Adeli *et al.,* (2015) who reported gender-dependent reference interval limits for alanine transaminase (ALT) (20-79 and 50-79 years, respectively) and gamma-glutamyltransferase (GGT) (36-79 years). Adeli *et al.,* (2015) reported gender dependent reference interval limits for creatinine (CREAT) (16-79 years). In contrast, Achila *et al.,* (2017) reported gender-independent reference interval limits for alanine transaminase (ALT).

The observed increase of random blood glucose (RBS) and decreased creatinine (CREAT) level with advancement age from the fifth decade to the sixth decade and plateauing thereafter and in the seventh decade onwards, respectively, especially in females indicates that these parameters are age dependent. This observation could be due to an age-related decrease in liver and kidney function resulting in elevated random blood glucose leading to increased insulin levels leading to insulin resistance. Insulin resistance decreases glucose tolerance due to higher total percent adipose mass and visceral fat accumulation, which contributes to insulin-signaling imbalances between liver and adipose cells (Adeli *et al.*, 2015). An elevated hemoglobin level is a biomarker for high levels of iron in humans which is associated with increased activity of alanine transaminase (ALT) (**Chen** *et al.*, 2010). The capacity of insulin receptors is reduced in geriatrics (Palmer, 2017). The observed decrease in creatinine (CREAT) levels with advancement in age in the seventh decade onwards is due to reduced muscle mass due to increased muscle mass necrosis and decreased creatine synthesis due to either disease or

non-disease processes (Palmer, 2017). The decrease in sodium (NA) concentration with advancement in age in the sixth decade onwards in females and combined males and females could be due to consumption of low quantities of diets poor in sodium (NA) levels (Charlton and Donald, 2001). Creatinine clearance decreases with age, especially in geriatrics (Palmer, 2017). The decrease in the activity of alanine transaminase (ALT) in the sixth decade and plateauing upto the seventh decade onwards relative to the fifth decade could be due to the decreased liver function due to muscle necrosis and decreased protein synthesis including alanine transaminase (ALT) with age especially in geriatrics (Palmer, 2017). Palmer (2017) reported increased alanine transaminase (ALT) activity in the fifth decade which decreases in the sixth decade to the levels of young adults.

The decreased aspartate transaminase (AST) activity in the sixth decade relative to the fifth decade and its reversion to the level of the fifth decade in the seventh decade onwards is supported by Palmer (2017) report indicating increases in aspartate transaminase (AST) activity between 60 to 90 years. This may be due to uptake of diets low in or lacking vitamin B6 in their sixth decade as reported by Yanagita *et al.,* (2020) reverting to the uptake of normal diets in the seventh decade onwards. These results contrast age independent reference interval limits for alanine transaminase (ALT) and aspartate transaminase (AST) reported by Mu *et al.,* (2013) and Achila *et al.,* (2017) for Chinese and Eritrea population. Further, Adeli *et al.,* (2015) reported age independent reference interval limits for glucose (GLU) (males [55-79 years]; females [40-

79 years]), alanine transaminase (ALT) (males [50-79 years]; females [50-79 years]), aspartate transaminase (AST) (males and females [55-79 years]), creatinine (CREAT) (males [16-79 years]; females [17-79 years]), and sodium (NA) (male and females [50-79 years]) levels for Canadian population.

Significant sex differences were seen in the median and 95% range for creatinine (CREAT), direct bilirubin (D-BIL) and alanine transaminase (ALT) in male and female adults of Taita-Taveta population in the fifth decade with males having higher values than females. This observation could be due to the greater muscle mass and hemoglobin in males compared to females in the fifth decade; creatinine is a catabolic product of muscles and direct bilirubin is a catabolic product of hemoglobin. The higher alanine transaminase (ALT) activity in males compared to females is related to the higher hemoglobin (HB) and body mass index (BMI) of adult males relative to adult females in this decade. The high hemoglobin in males compared to females is due to a direct stimulatory effect of androgen (testosterone) on erythropoietin production in the kidneys in adult men and an inhibitory effect of estrogen on the bone marrow in adult females, and increase in skeletal muscle cells and hence muscle tissue in males (Bimerew *et al.,* 2018). In addition to hormonal influences, iron deficiency due to blood loss during menstruation could also be a factor (Bimerew *et al.,* 2018).

The differences in the lower and upper limits between the developed serum biochemistry reference interval limits for adults and geriatrics of Taita-Taveta population relative to those previously reported in literature could be due to

several factors. These factors include inadequate dietary intake, genetics, race, ethnicity, lifestyles such as smoking, taking of herbal medicines contaminated with toxic compounds and heavy metals, living in environments infested with parasitic infections such as malaria, helminthes and environments polluted with heavy metals, altitude above sea level, geographical location, methods and instrumentation used in measuring analytes, and the robustness of the inclusion and exclusion criteria.

This study had several limitations. One of the limitations of this study is that the age stratification of reference interval limits was developed using a sample size of less than 120 referent individuals for each age category and sex as recommended by EP28 A3c guidelines. Secondly, the healthy status of the referent individuals was based on self-reported information through a questionnaire which was not validated clinically by laboratory reports; therefore, some referent individuals may have been recruited with subclinical conditions or infections which may have affected the measured biochemistry analytes. Further, the medical conditions associated with the elderly population which may affect the measured analytes may not have been thoroughly screened through laboratory medicine requests and reports. In addition, some factors such as dietary habits, history of smoking or alcoholism, and altitude, among others, which affect some of the analytes, were not collected and analysed. Finally, ultrasonography was not used to confirm the presence or absence of fatty liver disease (FLD) or non-alcoholic fatty liver disease (NAFLD).

5.8 Developed reference intervals for fasting lipid profile for adult and geriatric populations,

Results indicating statistically similar fasting reference intervals for total cholesterol (T-Chol), triacylglycerols (TG), and low-density lipoprotein cholesterol (LDL-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio for adult and geriatric male and female population of Taita-Taveta County, Kenya implies that these parameters are sex independent. This observation is contrary to the well-known fact that females have a higher fat body content than males (Bermúdez *et al.,* 2012). The developed sex independent reference intervals for adults and geriatrics of Taita-Taveta County, Kenya is 2-8 mmol/L for total cholesterol, 1-6 mmol/L for low density lipoprotein cholesterol (LDL-Chol), 1-4.2 mmol/L for triacylglycerols (TG), and 1-6 for low density lipoprotein cholesterol (LDL-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio.

This is in agreement with sex independent reference intervals for total cholesterol reported by Bermudez *et al.,* (2012) of 2.98-6.99 mmol/L, Agrawal *et al.*, (2014) of 2.21-5.49 mmol/L, Achila *et al.* (2017) of 3-7.71 mmol/L, , and for Venezuelan Maracaibo, Haryana and Asmara population, respectively. These results, however, contrast the sex-dependent reference intervals for total cholesterol reported by Durgawale et al. (2009) of 0-5.54 mmol/L for males and 0.48-3.95 mmol/L for females, Das and Saika (2009) of 2.51-6.05 mmol/L for males and 2.40-6.80 mmol/L for females, and Kaur *et al.,* (2012) of 4.57-

4.73 mmol/L for males and 4.70-4.84 mmol/L for females for Western Maharashtra, Assamese, and Punjab populations, respectively.

The sex independent reference intervals for low density lipoprotein cholesterol (LDL-Chol) reported in this study agree with those previously reported by Kaur *et al.,* (2012) of 2.9-3.0 mmol/L, Bermudez *et al.,* (2012) of 1.39-4.80 mmol/L, and Agrawal *et al.*,(2014) of 1.30-3.81 mmol/L for Punjab, Venezuelan Maracaibo and Haryana populations, respectively. Further, the sex independent reference intervals for triacylglycerols reported in this study agree with those previously reported by Durgawale *et al.,* (2009) of 0.31-1.69 mmol/L, Kaur *et al.,* (2012) of 1.36-1.41 mmol/L, and Agrawal *et al.,* (2014) of 0.69-1.76 mmol/L for Western Maharashtra, Punjab and Haryana populations, respectively.

In contrast, these results differ from the sex-dependent reference intervals for triacylglycerols reported by Das and Saika (2009) of 0.45-2.89 mmol/L for males and 0.46-2.54 mmol/L for females, and Bermudez *et al.,* (2012) of 0.37-2.35 mmol/L for males and 0.35-1.93 mmol/L for females with males having higher values than females for Assamese, and Venezuelan Maracaibo populations, respectively. The higher fasting lipid profiles and the associated ratios of the Taita-Taveta County, Kenya population reported in this study compared to those reported by Durgawale *et al.,* (2009), Das and Saika (2009), Kaur *et al.,* (2012), Bermudez *et al.,* (2012) and Agrawal *et al.,* (2014) could be associated with the consumption of high carbohydrate and high-fat diets including wheat products (sold at the markets), sweet potatoes, maize, sorghum

and millet, and fish, broilers, eggs, red meat and milk from cattle, goats and sheep all of which are available in Taita-Taveta County, Kenya.

Results indicating statistically different reference intervals for high density lipoprotein cholesterol, and total cholesterol: high-density lipoprotein cholesterol, and triacylglycerols: high density lipoprotein cholesterol ratios for adult and geriatric males and females of Taita-Taveta County, Kenya imply that theses parameters are sex dependent. The separate male and female reference intervals for high density lipoprotein cholesterol is 0-2 mmol/L for males and 0-2.28 mmol/L for females with males having a lower value than females, total cholesterol: high-density lipoprotein cholesterol ratio is 2-10.9 for males, and 1.73-8 for females with males having a higher value than females, and triacylglycerols (TG): high density lipoprotein cholesterol (HDL-Chol) ratio is 0-6.90 for males and 0-6.28 for females with males having a higher value than females. The sex-dependent reference interval limits for higher density lipoprotein cholesterol results agree with those reported by Durgawale *et al.,* (2009) of 0.58-1.63 mmol/L for males and 0.65-1.74 mmol/L for females, Das and Saikia (2009) of 0.59-1.53 mmol/L for males and 0.62-1.89 mmol/L for females, and Kaur *et al.,* (2012) of 1.10-1.13 mmol/L for males and 1.15-1.18 mmol/L for females with males having lower values than females for Western Maharashtra, Assamese, and Punjab populations, respectively.

In contrast, these results differ from the sex-independent reference intervals for high density lipoprotein cholesterol reported by Bermudez *et al.,* (2012) of

0.78-1.98 mmol/L, Agrawal *et al.,* (2014) of 0.52-1.63 mmol/L, and Achila *et al.,* (2017) of 0.81-2.07 mmol/L for Venezuelan Maracaibo, Haryana, and Asmara, Eritrea populations, respectively. The sex dependent reference interval for total cholesterol: higher density lipoprotein cholesterol ratio reported in this study contrasts the sex independent reference interval of this parameter reported by Kaur *et al.,* (2012) of 4.15-4.26 for Punjab population. The sex dependent reference interval limits for triacylglycerol (TG): high density lipoprotein cholesterol (HDL-Chol) ratio of 0-6.9 for male and 0-6.28 for female adults and geriatrics of Taita-Taveta County, Kenya has no previously developed and reported comparative data elsewhere in literature.

The developed higher fasting lipid profile reference intervals for the male and female adults and geriatrics population of Taita-Taveta County compared to the previously reported medical literature reference intervals developed from other referent populations from different parts of the world implies that this population is at a higher risk of developing coronary heart disease whose major risk factor is dyslipidemia (Bermúdez *et al*., 2012). These differences could be accounted for by differences in dietary habits (eating foods rich in saturated fatty acids and high carbohydrate levels), lifestyle characteristics such as sedentary lifestyles (lack of exercise), consumption of alcohol and smoking. In addition, socio-economic status, geographical location, inclusion criteria used to recruit the referents, ethnicity, race and genetics of the studied population could also be contributors. Populations eating foods rich in saturated fatty acids and high carbohydrate levels such as broilers, eggs, red meat and milk from

cattle, goats and sheep, and high carbohydrate foods such as sweet potatoes, maize, sorghum, and millet which are all available and consumed in Taita-Taveta County, and having sedentary lifestyles especially people living in urban areas have higher levels of fasting lipid profiles (Turley *et al.,* 1998; O'Neal *et al.*, 2019; Lee and An., 2020).

The statistically significantly higher total cholesterol: high density lipoprotein cholesterol, and triacylglycerol: high density lipoprotein cholesterol ratios in males compared to females in this study may imply that males of Taita-Taveta County, Kenya are at a greater risk of coronary artery disease than females. However, the risk of coronary heart disease, especially in males, was not investigated in this Taita-Taveta population. These high values of the two ratios (T-CHOL: HDL-CHOL and TG: HDL-CHOL) may indicate that the males of Taita-Taveta County are consumers of high carbohydrate and high fat diets with minimal exercise (Agrawal *et al.,* 2014).

Most Taita-Taveta County males are businessmen. Such carbohydrate-rich diets may include wheat products (sold in the markets), sweet potatoes, maize, sorghum, and millet while the high fat diets may include broilers, eggs, fish, red meat and milk (butter, ghee) from cattle, goats and sheep, all of which are available and consumed in Taita-Taveta County. Consumption of fish which is rich in omega-3-fatty acids leads to increased levels of high-density lipoprotein cholesterol (HDL-Chol; good cholesterol) in the blood (Agrawal *et al.,* 2014). At the family level (at home) males may also be consuming more broilers without removing the skin which has high fat content and eggs, and more red

meat (animal fat) and milk (ghee, butter) from cattle, goats and sheep. The males could also be taking medications which modify the fasting lipid profile levels and their ratios, as well as being alcoholics and smokers despite the use of the self-reported questionnaire to eliminate such participants in the exclusion criteria (KPHC IV, 2019). This study did not investigate the foods consumed, the amounts, and their composition by Taita-Taveta population. High intake of high carbohydrate low fat diet leads to reduced levels of total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol (HDL) and increased levels of triacylglycerols (Turley *et al.,* 1998 ; Lee and An., 2020). Intake of low carbohydrate high fat diet leads to increased levels of total cholesterol, high density lipoprotein cholesterol, and low-density lipoprotein cholesterol (O'Neal *et al.,* 2019).

Results of this study indicating statistically similar reference interval limits for total cholesterol, high density lipoprotein cholesterol, non-high density lipoprotein cholesterol, triacylglycerols and total cholesterol: high density lipoprotein cholesterol ratio for adult and geriatric population of Taita-Taveta County, Kenya with advancement of age implies that these parameters are not affected by age. These results contrast the well-known fact that body fat content increases with advancement of age because of reduced overall energy expenditure caused by reduced activity with age (Buffa *et al.* 2011).

These observations agree with those reported by Kaur *et al.,* (2012) for combined reference intervals for total cholesterol, high-density lipoprotein cholesterol, triacylglycerols and total cholesterol: high-density lipoprotein

cholesterol ratio for adults and geriatrics of Punjab with the advancement of age. However, Kaur *et al.*, (2012) also reported increasing reference interval limits for triacylglycerols (0.73-2.03 mmol/L at age 51-60 years and 0.93-2.08 mmol/L at age above 70 years for females) and decreasing reference interval limits for total cholesterol (3.17-6.46 mmol/L at age 51-60 years and 2.77-6.76 mmol/L at above 70 year in females) with advancement in age.

Further, the reference interval limits for total cholesterol (3.17-6.46 mmol/L for females versus 2.78-6.63 mmol/L for males at age 51-60 years, 2.91-6.88 mmol/L for females versus 2.74-6.31 mmol/L for males at age 61-70 years, and 2.77-6.76 mmol/L for females versus 3.15-5.85 mmol/L for males at above 70 years), high density lipoprotein cholesterol (0.93-1.46 mmol/L for females versus 0.75-1.49 mmol/L for males at age 51-60 years, 0.84-1.56 mmol/L for females versus 0.67-1.49 mmol/L for males at age 61-70 years, and 0.72-1.49 mmol/L for females versus 0.82-1.34 mmol/L for males at above 70 years), low density lipoprotein cholesterol (1.20-4.79 mmol/L for females versus 1.22-4.47 mmol/L for males at age 51-60 years, 1.12-4.89 mmol/L for females versus 1.16-4.44 mmol/L for males at age 61-70 years, and 1.40-4.56 mmol/L for females versus 1.60-4.17 mmol/L for males at above 70 years), and triacylglycerols (0.73-2.03 mmol/L for females versus 0.57-2.17 mmol/L for males at age 51-60 years, 0.66-2.30 mmol/L for females versus 0.75-2.04 mmol/L for males at age 61-70 years, and 0.93-2.08 mmol/L for females versus 0.55-1.89 mmol/L for males at above 70 years) were high in females than males at the three age categories reported by Kaur *et al.,* (2012). In addition,

Kaur *et al.,* (2012) reported that the total cholesterol to high-density lipoprotein cholesterol (2.43-5.57 for females versus 2.34-5.86 for males at age 51-60 years, 2.14-6.06 for females versus 2.14-6.46 for males at age 61-70 years, and 2.73-5.87 for females versus 2.63-5.77 for males at above 70 years), and low density lipoprotein cholesterol to high density lipoprotein cholesterol (0.93-4.07 for females versus 1.03-4.17 for males at age 51-60 years, 0.74-4.26 for females versus 0.84-4.36 for males at age 61-70 years, and 1.52-3.88 for females versus 1.33-4.07 for males at above 70 years) ratios were not affected by sex and advancement with age.

This study findings contrast those reported by Das and Saikia (2009) who demonstrated increasing reference interval limits for total cholesterol (4.33 (2.69-6.0) mmol/L for males versus 4.84 (3.23-6.54) mmol/L for females at 51-60 years and 4.09 (2.51-5.97) mmol/L for males versus 5.12 (3.52-6.34) mmol/L for females at above 70 years) and high density lipoprotein cholesterol (0.98 (0.67-1.37) mmol/L for males versus 1.14 (0.73-1.61) mmol/L for females at 51-60 years and 1.06 (0.62-1.53) mmol/L for males versus 1.30 (0.73-1.89) mmol/L for females at above 70 years), and decreasing values for triacylglycerols (1.34 (0.49-2.55) mmol/L for males versus 1.28 (0.51-2.33) mmol/L for females at 51-60 years and 1.16 (0.59-1.99) mmol/L for males versus 1.11 (0.72-2.54) mmol/L for females at above 70 years) for adult and geriatric population for Indian Assamese with advancement in age.

Some limitations were associated with this study. The first of these limitations is that the eating habits and the physical activity which can affect the fasting

lipid profile levels of the Taita-Taveta County referent population used in this study were not investigated. The second limitation is that the inclusion criteria used was based on a self-reporting questionnaire by the referent subjects who may not have given their true health status knowingly or unknowingly. This may have allowed participants who had diabetes mellitus, endocrine disorders, dyslipidemia, renal disease, hypertension, liver obstruction, cardiovascular disease, and those on medications that could influence fasting lipid profiles. It may also have allowed smokers, alcohol abusers, caffeine abusers, and pregnant women to participate in the study. However, participants with excessive body weight and those on strenuous exercise would have easily been identified and stopped from participating in the reference interval development study. In addition, because of differences in diet and geographic locations in the coastal counties, these reference intervals may not be adopted for all the coastal counties and the rest of Kenyan counties. Further, the sample size for the two age categories was lower than the recommended minimum for reference interval development of 120 referent individuals by CLSI {EP28 3c guideline}, (2010).

5.9 Developed thyroid profile parameters

Results indicating that the stimulating thyroid hormone (TSH), total thyroxine (T4) and total triiodothyronine (T3) reference interval limits for the adult and geriatric male and female population of Taita-Taveta County, Kenya were statistically similar implies that these parameters are gender independent. The developed age and gender-independent reference intervals for thyroid

hormones for both males and females of Taita-Taveta County, Kenya are 0-6 mU/L for TSH, 2-15 nmol/L for T4, and 0-4 mU/L for T3, respectively. This study findings agrees with those of Hickman et al. (2017) who reported gender and age independent reference intervals for TSH (0.43-3.28 mU/L). Wang *et al.,* (2017) also reported gender independent reference intervals for T4 of 73.48-138.93 nmol/L. Further, these results also agree with those reported by Kratzsch *et al.,* (2005) who reported gender independent reference interval for TSH of 1.36 (0.40-3.77) mU/L. However, these results contrast the gender dependent reference interval for total T4 with females (113 [71.4-166] nmol/L) having a significantly higher reference interval than males (93.4 [68.4-125] nmol/L), and T3 with females (1.94 [1.23-2.97] mU/L) having a significantly higher reference interval than males (1.72 [1.28-2.33] mU/L) reported by Kratzsch *et al.,* (2005). These results are also in agreement with those reported by Mirjani-Azaric *et al.,* (2017) who reported gender-independent reference interval limits for TSH of 0.75-5.32 mU/L, and T4 of 73.49-126.30 nmol/L and contrast the gender-dependent reference interval limits for T3 with males (0-4.97 mU/L) having a significantly higher reference interval limits than females (0-4.61 mU/L). Wang *et al.,* (2017) reported gender dependent referenc interval limits for TSH of 0.66-4.95 mU/L for males and 0.72-5.84 mU/L for females with females having higher values than males, and T3 of 1.24-2.18 nmol/L for males and 1.20-2.10 nmol/L for females with males having higher values than females, respectively. Li *et al.,* (2020) reported gender-dependent and age-independent reference interval limits for TSH of 0.65-3.92 mU/L for

males and 0.43-4.67 mU/L for females, T4 of 78.52-144.94 nmol/L for males and 64.49-145.20 nmol/L for females, and T3 of 1.17-2.04 nmol/L for males and 1.04-2.00 nmol/L for females with males having higher values tha females, respectively.

These results also differ from those reported by Gesing *et al.,* (2012) and Barbesino (2019) who reported age dependence of thyroid hormone levels. Mirjani-Azaric *et al.,* (2017) also demonstrated that the reference interval of TSH decreased with the advancement of age from age 20-30 years upto age 50 years after which its level reverts to that of age range 20-30 years and above 50 years. Musa *et al.,* (2018) reported gender dependent reference interval for TSH with males (1.7 [0.5-3.4] mU/L) having significantly higher reference interval limits than females (1.1 [0.5-3.0] mU/L), T4 with females (106.0 [63-165] nmol/L) having a significantly higher reference interval limits than males (96.5 [62.0-148.2] nmol/L), and gender independent reference interval for total T3 of 1.4 (0.8-2.7) mU/L. Musa *et al.,* (2018) also demonstrated age-dependent reference interval for T4 with increasing reference interval with the advancement of age (for age range 20-30 years to above 60 years) and age independence of reference interval for TSH and T3. Ali *et al.,* (2018) reported gender independent reference interval for TSH of 1.2 (0.50-3.0) mU/L, T4 of 111.0 (72.0-161.1) nmol/L and T3 of 1.5 (0.8-2.8) mU/L, respectively. Ali *et al.,* (2018) also demonstrated age dependence reference interval for T4 and T3 with increasing reference interval with the advancement of age. Völzke *et al*., (2005) reported a gender-independent reference interval for TSH of 0.25-2.12

mU/L.Völzke *et al.*,(2005) also demonstrated age dependence reference intervals for TSH with decreasing reference interval with the advancement of age with age 20-49 years having a reference interval of 0.27-2.15 mU/L and age 50-79 years having a reference interval of 0.19-2.09 mU/L, respectively. Hollowell *et al.,* (2002) reported a gender-independent reference interval for TSH of 1.39 (0.45-4.12) mU/L for the American population and a gender dependent reference interval for T4 with females (111.8 [66.9-165.9] nmol/L) having significantly higher reference interval than males (107.3 [64.4-156.0] nmol/L). Hollowell *et al.,* (2002) also demonstrated an age-dependent reference interval for TSH with the advancement of age from 50-80 years (1.50 [0.52-4.03] mU/L for 50-59, 1.67 [0.49-4.33] mU/L for 60-69, 1.76 [0.45-5.90] mU/L for 70-79, and 1.90 [0.33-7.50] mU/L for above 80 years, respectively);T4 reference interval was demonstrated to decrease with advancement of age.

The decrease of T4 concentration with age, especially in the seventh decade onwards in females but not in males could be associated with the observed corresponding increase in TSH concentration in females relative to its unchanging concentration in males resulting in overt hypothyroidism (Völzke *et al.,* 2005 ; Bensenor *et al.,* 2012) in females. This could be accounted for by the decrease in thyroid gland function with advancement with age especially in the females which is associated with hypothyroidism; that is, increased TSH levels and decreased T4 levels. For males, TSH levels are reported not to vary with aging as observed in this study population but increases with aging in

females as observed in this study in the seventh decade onwards (Yeap *et al.*, 2017). However, this increase in TSH levels in females with age is overcome by excluding females with antithyroid antibodies (Bensenor *et al.*, 2012). Both increased TSH levels and antithyroid antibodies are more common in females and increase with age. Elderly females have a higher prevalence of iodine deficiency than males and increased TSH levels is a sensitive biomarker of iodine deficiency in geriatrics (Bensenor *et al.,* 2012).

The difference between the age-independent thyroid function tests reference intervals developed in this study for the Taita-Taveta County population from the age and sex-dependent thyroid function tests previously developed and reported from other parts of the world by other researchers could be due to: differences in ethnicity, iodine supply and hence iodine nutritional status of the referent individuals, the selection criteria used in defining the referent individuals, the principles and procedures of the detection methods used, and genetic factors.

The limitations of this study were: firstly, urinary iodine which would have been used to assess the association between the developed reference interval for thyroid hormones and iodine nutritional status was not measured. Secondly, the presence of thyroid antibodies, anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG) antibodies which can result in higher than appropriate reference intervals of thyroid hormones, were not assessed in this study. Thirdly, ultrasound was not used to further assess the thyroid volume and enlargement to confirm the healthy status of the selected referent population.

The study used a health questionnaire, drug history, and clinical assessment to define thyroid disease-free referent individuals which could result in including individuals with a developing thyroid disease resulting in higher than appropriate reference intervals; recall bias may affect results when using a questionnaire. Further, the health questionnaire and clinical assessment were used to remove other factors such as smoking and obesity which influence the reference intervals of thyroid hormones. Fourthly, this study only developed reference intervals for total thyroxine (T4) and triiodothyronine (T3) without developing the reference intervals for free T4 and free T3 which are more sensitive indicators of iodine nutritional status as in other previously reported studies. Because samples were not collected at fixed times such as 08.00am to 12.00 noon, the developed reference intervals for thyroid hormones may be influenced by effects caused by circadian rhythm.

The study involved a referent population of Taita-Taveta County and thus covered a narrow scope. A larger sample size of referent population including clinical laboratories of the eight counties of Kenya is needed to generate national reference intervals for thyroid hormones. Finally, the effect of age on the reference intervals for thyroid hormones used a sample size below the recommended minimum of 120 referents for each age category which could bias the results.

5.10 Developed reference interval limits of the five selected cancer biomarkers for adult and geriatric population,

Results indicating that the CA 15-3 (breast cancer biomarker) reference intervals for the adult and geriatric male and female population of Taita-Taveta County, Kenya are not significantly different imply that this parameter is gender independent. The developed reference interval for CA 15-3 for adult and geriatric male and female population of Taita-Taveta County, Kenya for this parameter is 22.4 (0-56.9) U/mL.

This developed upper reference interval limit of CA 15-3 in this study differs from the upper reference interval limit for this parameter reported in literature of 30 U/mL; this difference could partly be due to the matrix effects of the detection method used. Slev *et al.,* (2006) obtained different reference intervals for CA 15-3 using seven different detection methods from serum samples from the same 120 referent individuals (Access 2': 97.5% upper reference limit of 23.3 U/mL; ADVIA Centaur: 97.5% upper reference limit of 30.8 U/mL, ARCHITECT i2000: 97.5% upper reference limit of 29.2 U/mL, AxSYM: 97.5% upper reference limit of 30.6 U/mL, Elecsys 2010: 97.5% upper reference limit of 41.2 U/mL, IMMULITE 2000: 97.5% upper reference limit of 42.3 U/mL; VITROS ECi: 97.5% upper reference limit of 51.7 U/mL). Hayes et al. (1989) established age and gender-independent reference interval for CA 15-3 of 1.5-25.1 U/mL as reported by Duffy (1999). The difference between the developed reference intervals for CA 15-3 for adult and geriatric males and females of the Taita-Taveta County, Kenya in this study and the

previously developed reference interval limits by other researchers could also be explained by differences in race, ethnicity, lifestyle, and geographical location of the referent individual used.

Results indicating that the CA 19-9 (pancreatic cancer biomarker), and CEA (cervical cancer biomarker) reference intervals for adult and geriatric male and female population of Taita-Taveta County, Kenya were significantly different with females having a higher CA 19-9 levels than males and lower CEA levels than males implies that these parameters are gender dependent. The developed age-independent but gender-dependent reference interval for adult and geriatric male and female population of Taita-Taveta County, Kenya for CA 19-9 in their fifth decade are 10 (0-63.08) U/mL for males and 12 (0-82.5) U/mL for females, and in their sixth decade is 1 (0-23,75) U/mL for males and 11 (0-75.2) U/mL for females, respectively, CEA in their sixth decade is 2 (0-11.95) ng/mL for males and 1 (0-6.8) ng/mL for females, and CA 15-3 in their fifth decade is 20 (0-56.65) ng/mL for males and 25.5 (0-32.65) ng/mL for females, respectively.

The higher levels of CA 19-9 observed in females relative to males in adults and geriatrics of Taita-Taveta County, Kenya, could be associated with sexual differentiation which involves genetics and epigenetics in addition to acute sex hormone actions as reported by Rubin *et al.*, (2020). This same reason could also explain the higher levels of CA 15-3 and CEA in adult males and geriatrics, respectively, compared to females of Taita-Taveta County, Kenya (Rubin *et al.*, 2020). The decrease in the levels of CA 19-9 with age for males

and not females in this study is in contrast to its increase in the study reported by Zhang *et al.,* (2017) and could be related to the uterus and ovaries in females who are also releasing CA 15-3 and CA125 (Zhang *et al.,* 2017).

The **develope**d CA 19-9 reference interval in this study differ from the age independent reference intervals reported by Vestergaard *et al.,* (1999) of 5.2 (upper limit of 30.2) U/mL for females and 3.6 (upper limit of 23.0) U/mL for males. This also differs from the reported age and gender independent literature reference interval limits for CA 19-9 of 35-37 U/mL. Bjerner *et al.,* (2008) also reported age and gender independent upper limit reference interval for CA 19-9 of 28.3 U/mL. This difference between the developed reference interval limits for CA 19-9 for Taita-Taveta population in this study from those previously reported by others (Vestergaard *et al.,* 1999; Bjerner *et al.,* 2008) could be attributed to the race, the geographical location of the referent individuals, and the detection method and reagents used. La'ulu and Roberts (2007) obtained different reference interval limits for CA 19-9 using five different detection methods from serum samples from the same 127 referent individuals (ADVIA Centaur: 9.7 (4.7-37.1) U/mL, ARCHITECT i2000: 4.4 (2.0-26.4) U/mL, IMMULITE 2000: 3.3 (2.5-17.0) U/mL; Elecsys E170: 6.9 (0.6-31.9) U/mL; UniCel Dxl 800: 7.0 (1.0-33.2) U/mL). The age-independent but gender dependent reference interval for CEA in this study differs from age independent and gender-independent upper reference interval limit for CEA reported by Bjerner *et al.,* (2008) of 4.12 ng/mL for referent individuals of 50-70 years. Qin *et al.,* (2011) also reported reference interval upper limit of 6.94

ng/mL for CEA for Chinese males of 45 to 70 years. Further, Zhang *et al*., (2016) also reported gender dependent reference interval limits for CEA of 1.88 (0.60-5.43) ng/mL for males and 1.44 (0.43-4.26) ng/mL for females of 50-90 years with males having higher values than females. The difference between the developed reference interval for CEA in this study and those reported in medical literature previously by other researchers could be explained by the differences in race, lifestyle, and geographical location of the referent population and the differences in the detection methods and reagents used to assay this parameter.

The developed age independent PSA (prostate cancer biomarker) reference interval limit for the adult and geriatric male population of Taita-Taveta County is 0-6.8 ng/mL while that of age independent CA 125 reference interval limit for the female population of Taita-Taveta County is 0-25 U/mL. The age independence of the developed PSA reference interval limit for the male population for Taita-Taveta County could be due to a similar prostate gland volume and similar PSA density of age 50-95 years. The prostate gland volume and PSA density increase with age (Khezri *et al.,* 2009). The age independence of developed reference interval for PSA for the Taita-Taveta County male population differs from the findings of other researchers (Luboldt et al. (2007) [upper limit: 2.27 ng/mL for 50-59 years, 3.46 ng/mL for 60-69 years, 4.26 ng/mL for 70-79 years]; Liu *et al.,* (2013) [upper limit: 2.92 ng/mL for 50-59 years, 4.11 ng/mL for 60-69 years, 5.56 ng/mL for 70-79 years, 7.29 ng/mL for above 80 years]; Rahman *et al.*, (2014) [2.1-3.7 ng/mL for 50-59 years, 2.6-3.4

ng/mL for 60-69 years, 3.6-6.0 ng/mL for 70-79 years and 0.9-9.5 ng/mL for 80-89 years]) who reported increasing PSA levels with advancing age. The age independence and upper limit difference of the present findings from the international age-dependent reference interval for PSA (3.5 ng/mL for 50-59 years, 4.5 ng/mL for 60-69 years, and 6.5 ng/mL for 70-79 years for Caucasians) could be explained by the genetic differences between the different races, method and reagents used in its detection, and geographical location of the referent individuals. Hyeon (2005) reported increasing PSA reference interval limits with advancing age from different races (African Americans [4.0 ng/mL for 50-59 years, 4.5 ng/mL for 60-69 years, 5.5 ng/mL for 70-79 years], Japanese [3.65 ng/mL for 50-59 years, 4.06 ng/mL for 60-69 years, 5.09 ng/mL for 70-79 years, 5.66 ng/mL for 80-89 years], Chinese [2.35 ng/mL for 50-59 years, 3.20 ng/mL for 60-69 years, 3.39 ng/mL for 70-79 years, 3.39 ng/mL for 80-89 years], Taiwanese [3.31 ng/mL for 50-59 years, 5.05 ng/mL for 60-69 years, 5.73 ng/mL for 70-79 years], Singaporeans [2.3 ng/mL for 50-59 years, 4.0 ng/mL for 60-69 years, 6.3 ng/mL for 70-79 years, 6.6 ng/mL for 80-89 years], and Koreans [2.5 ng/mL for 50-59 ng/mL, 3.9 ng/mL for 60-69 years, 5.8 ng/mL for 70-79 years]). Khezri *et al.,* (2009) reported increasing PSA levels with advancing age for adult and geriatric Iranian men (2.61 ng/mL for 50-59 years, 3.59 ng/mL for 60-69 years, 4.83 ng/mL for 70-79 years).

The difference between the developed PSA reference intervals of this study and those reported in medical literature of people of different races could be

explained by differences in changes in prostate gland volume and PSA density. Khezri *et al.,* (2009) reported different prostate gland volume and prostate gland PSA density in USA whites, Japanese and Arab males of similar age, respectively. The developed age independence of CA 125 (ovarian cancer biomarker) reference interval for the female population of Taita-Taveta County agrees with the findings reported by Park *et al.,* (2012) who demonstrated age independent reference interval of CA 125 of 27.8 [22.1-39.1] U/mL for Asian female populations of 50-65 years.

The reported age independent literature upper reference interval limit for CA 125 is 35 U/mL. Bjerner *et al.,* (2008) also reported age independent upper reference interval limit of CA 125 of 35.8 U/mL. The difference in this study's CA 125 reference interval from the previously reported reference intervals for this parameter could be attributed to the genetic differences between the races, lifestyle, and the geographical location of referent individuals. Pauler *et al.,* (2001) demonstrated a race-dependent CA 125 reference interval in postmenopausal women: 9.0 (4.0-26.0) U/mL for African women, 13.0 (5.9-33.3) U/mL for Asian women, and 14.2 (6.0-41.0) U/mL for Caucasian women.

The limitations of this study are that these developed breast, ovarian, cervical, prostate and pancreatic cancer biomarkers reference intervals may not be suitable to the adult and geriatric healthy male and female population of other Counties other than those of Taita-Taveta County, Kenya. Secondly, the other limitation was that the developed reference intervals for the five selected tumor

markers based on age have smaller sample sizes than the 120 referent individuals recommended by CLSI EP28-A3c guideline. Thirdly, the healthy status of the referent individuals was based on self-reporting by the referent individuals who were not confirmed medically. Fourthly, the life-style status such as smokers or non-smokers, and alcoholics or non-alcoholics was also based on self-reporting by the referent individuals.

5.11 CONCLUSIONS

In conclusion, this study has developed age and gender-specific reference interval limits for body mass index (kg/m^2), and vital signs temperature (^0C), systolic and diastolic blood pressure (mmHg), peripheral oxygen saturation ($S\rho O_2$) (%), and pulse rate (beats per minute) for the male and female population of Taita-Taveta County, Kenya which are different from those reported in medical literature for other populations. This difference in the developed reference intervals for BMI and four vital signs for the Taita-Taveta County, Kenya population from those reported in medical literature supports the need for use of locally developed reference interval limits for these parameters to allow early accurate clinical detection, management and/or treatment, and monitoring the performance of therapeutic treatment regimens on recovery or deterioration of the acutely ill patients.

This study has developed age and gender-specific reference interval limits for body mass index (kg/m^2), and vital signs temperature (^0C), systolic and diastolic blood pressure (mmHg), peripheral oxygen saturation ($S\rho O_2$) (%), and pulse rate (beats per minute) for male and female population of Taita-Taveta

County, Kenya which are different from those reported in medical literature for other populations. This difference in the developed reference intervals for BMI and four vital signs for the Taita-Taveta County, Kenya population from those reported in medical literature supports the need for use of locally developed reference interval limits for these parameters to allow early accurate clinical detection, management and/or treatment, and monitoring the performance of therapeutic treatment regimens on recovery or deterioration of the acutely ill patients.

Current study has established the first gender and age based hematological parameters reference intervals for children and adolescent population of Taita-Taveta County which is different from that developed and reported in medical literature from other regions of the world. The established hematological parameters laboratory medicine professionals can use reference interval limits, research scientists, and practicing physicians in Kenya to improve research quality and provide quality medical care to children and adolescent patients in Taita-Taveta County, Kenya.

In this study has developed age and sex specific reference intervals for 13 serum biochemistry analytes for children and adolescents of Taita-Taveta County, Kenya which if adopted can be used for routine clinical evaluations. This study's reference intervals for serum biochemistry analytes differ from those of Caucasians, African children, and adolescents. In addition, these biochemistry reference values differ from one African pediatric population to another. Even within the same African country these biochemistry reference

values differ from one region to another. This supports the recommendation that every country should develop its own biochemistry reference intervals based on the local population of its different regions.

In conclusion, this study developed the first trimester specific reference intervals for hematological parameters for healthy pregnant women living in Taita-Taveta County, Kenya which differs from those reported in medical literature. This difference of the developed reference intervals for pregnant women of Taita-Taveta County from those reported in literature supports the need for use of locally developed reference intervals for managing pregnancy related challenges for the pregnant women of Taita-Taveta County, Kenya. These pregnancy induced physiological changes of hematological parameters reference intervals require consideration during the assessment of the health status of normal pregnancy and for diagnosing conditions or challenges occurring during pregnancy for optimal maternal and fetal medical care. In addition, these developed reference intervals for pregnant women can also be adopted and used by researchers in this specific area.

The research has developed trimester-specific reference intervals for selected serum biochemistry analytes for pregnant mothers in their second and third trimester of Taita-Taveta County which differ from those reported in medical literature. These can be adopted and used to provide cutoff points for diagnosing and managing pregnancy-related pathological conditions.

This research work has developed the first age and gender specific reference interval limits for hematological parameters for the adult and geriatric

population of Taita-Taveta County, Kenya which are different from those previously reported by other researchers in medical literature. It is therefore appropriate to use the developed reference interval limits for hematological parameters in order to define the correct cutoff point for diagnosis, treatment and management of anemia for this study population.

In conclusion, this study has established age and sex specific reference interval limits for serum biochemistry analytes for adult and geriatric population of Taita-Taveta County, Kenya, which are different from those previously reported in medical literature; it is therefore inappropriate to adopt and use reference interval limits for serum biochemistry analytes provided by the manufacturers without verification. These developed reference interval limits for adult and geriatric population of Taita-Taveta County, Kenya, should be adopted and used for accurate diagnosis, treatment and monitoring the performance of the treatment regimen of serum biochemistry analytes related diseases.

This study generated sex and age specific fasting lipid profile reference interval limits for adults and geriatrics of Taita-Taveta County, Kenya which was different from those previously reported in medical literature from other parts of the world. These developed fasting lipid profiles reference intervals for adult and geriatric population of Taita-Taveta County, Kenya can be adopted and used to make appropriate clinical decisions leading to improved diagnosis of cardiovascular diseases.

In **conclusion**, the developed reference interval limit for TSH, and T3 for the Taita-Taveta County, Kenya population are both gender and age independent, while those of T4 are age dependent for females and gender dependent for age 70-95 years. However, these developed reference interval limits for TSH, T4 and T3 are different from those indicated in the manufacturers' inserts reagent kits, and those developed and reported previously in medical literature in their lower and upper limits using different referent individuals and analytical methods of different parts of the world. These developed reference intervals for thyroid hormones can therefore be adopted for use in the Department of Clinical Chemistry, Moi Subcounty Hospital, Voi, Taita-Taveta County, Kenya and other hospitals within the County.

The current investigation has developed age and gender specific 95% (double-sided) reference interval limits for breast, ovarian, cervical, prostate and pancreatic cancer biomarkers which are different from those previously reported in medical literature using other communities from different parts of the world. These reference intervals can be adopted and used as cutoff points to allow more accurate diagnosis of various forms of cancers and rule out benign diseases for the Taita-Taveta County population. This confirms the need for each community to develop their own reference intervals limits for breast, ovarian, cervical, prostate and pancreatic cancer biomarkers appropriate for the accurate diagnosis and management of various types of cancers.

5.12 RECOMMENDATIONS

I. Laboratory medicine is crucial in diagnosing and managing various pathological ailments. The current research work has established normal reference ranges for vital signs, biochemistry and hematological parameters. The current research has demonstrated that the current ranges used to manage the Taita Taveta population differed with the current study. Therefore, its be prudent that other health and medical research facilities within Coastal region and Kenya as a whole should develop laboratory specific reference values.

II. For those healthy centres located in Taita Taveta County and not in position to develop reference ranges either directly or indirectly, the present reference values should be used for clinical management of patients and interpretation of laboratory data in research.

III. Similar studies should be carried out to establish reference values for other biochemical parameters not done in this study like fertility hormones, fasting blood sugars and arterial blood gas parameters.

IV. Future studies should target paediatric and develop their specific reference intervals

V. It is essential to adopt the current normal establish reference interval different for fdifferent age categories of Taita Taveta population. Once this is accomplished, diagnosing and management of Kenyan

population will no longer be dependent on western reference intervals as a tool in decision making

VI. Further studies should be done to evaluate the effect of nutritional status on reference ranges

VII. In future studies, the number of subject used in stratification should be adde to meet the minimum requirement of 120 as recommened by NCCl

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APPENDICES

Appendix I: Introduction letter and a consent form

Name of Investigator: RICHARD GITIMU

Postal Address: P.O. Box 635 80300 Voi.

Cell phone: 0721 388 454.

E-mail: richardmainag@gmail.com

Supervisors

Prof Eliud Nyaga Mwaniki Njagi,

Department of Biochemistry, Microbiology & Biotechnology,

School of Pure and Applied Sciences,

Kenyatta University

P.O. Box 43844-00100

Nairobi, Kenya.

Prof Joseph Kangangi Gikunju

Department of Medical Laboratory Science,

Institute of Tropical Medicine and Infectious Diseases (ITROMID),

Jomo Kenyatta University of Agriculture and Technology,

P.O. Box 62000-00200

Nairobi Kenya

Dr Stanley King'e Waithaka

Department of Medical Laboratory Sciences,

School of Health Sciences,

Mount Kenya University,

P.O. Box 342-01000,

Thika, Kenya

Purpose of study

This study is meant to sample and analyze blood for biochemical parameters from those in good health. The results will be used to determine reference values to enable the hospital to compare with those not in good health.

Participation

Participation is voluntary and one is free to reject. If you choose to participate, you have to read and understand the contents of this document then I will provide you with a donor questionnaire to complete before sample collection. Sample will be obtained during blood donation after which it will be screened for HIV, Syphilis and Hepatitis B and C.

Those unwilling to consent will be excluded from the study. Participants will include those who have lived in TaitaTaveta County for less than six months.

Sample collection

This study will collect blood from the vein using a sterile needle and a syringe. This will only be done once for the entire study.

Risks and discomforts

Participants may experience some pain during vene-puncture. There will be no risks involved since the Study requires 5 ml of blood from volunteer blood donors.

Benefits and costs

Participants will not spend any money on this study. In case the participant is found with any clinical condition he/she will be informed upon his/her own request. This study will be of benefit to the hospital and entire community since its success will aid in proper clinical decision making and treatment of patients.

Confidentiality

Results will be confidential.

Enquiries

For further explanation and queries contact the investigator on above address. For more about your rights contact the Ethical Institution of Kenyatta university box 43844 00100 Nairobi, MOH TaitaTaveta Box 1216 Wundanyi, NACOSTI.

I……hereby agree that I have read, explained to, allowed to ask questions concerning this study, understood and consented.

Signature of parent/ guardian ……Date……..

I have clearly explained the above study to the participant and he/she has understood and consented.

Signature of researcher……..Date……

Appendix II: Blood Sampling Questionnaire

Name…… Study case number……

Age……. Place of birth……

Sex……. Date…….

Whether suffered from any of the following ailments	Yes	No	Whether having any of the following conditions	Yes	No
High blood pressure			Pregnancy		
Diabetes mellitus			Lactation		
Renal disease			Family planning devices		
Tuberculosis			Menses		
Any allergy			On any medication		
Epilepsy			Drug abuse		
Stomach ulcers			Cigarette smoking		
Jaundice			Alcohol consumption		
Hepatitis B and C			Recent Surgery/hospitalization		
Heart disease			Frequent blood donor		
Surgery					
Malaria Syphilis HIV/AIDS					

Appendix III Approval letter to carry out research from Kenyatta University

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 19th July, 2016

TO: Mr. Richard Gitimu
C/o Department of Biochemistry & Biotechnology
KENYATTA UNIVERSITY

REF: 184/31987/15

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the Graduate School Board at its meeting 8th July, 2016 approved your Ph.D. Research Proposal **entitled "Establishment of Reference Values of Biochemical and Hematological Parameters and Tumour Makers for Coastal Population, Kenya".**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking Forms per semester. The form has been developed to replace the progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

KENYATTA UNIVERSITY OFFICE OF DEAN 26 AUG 2016 GRADUATE SCHOOL P.O Box 43844-00100 NAIROBI

Thank you.

REUBEN MURIUKI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Biochemistry & Biotechnology

Supervisors:

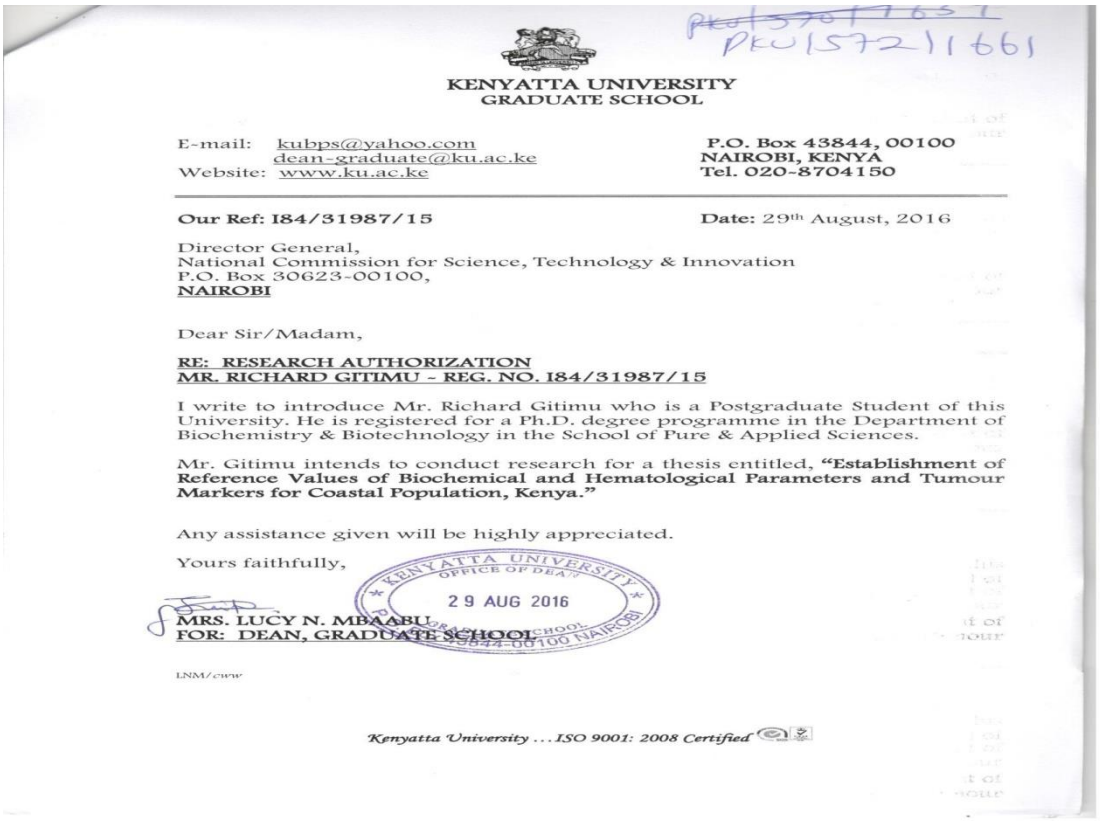
1. Prof. Eliud NM. Njagi
C/o Department of Biochemistry & Biotechnology
KENYATTA UNIVERSITY

2. Prof. Joseph Gikunju
Department of Medical Laboratory Sciences
C/o Department of Biochemistry & Biotechnology
KENYATTA UNIVERSITY

3. Dr. Stanley K. Waithaka
Mount Kenya University
C/o Department of Biochemistry & Biotechnology
KENYATTA UNIVERSITY

RM/cao

Appendix IV Authorization letter to carry out research from Kenyatta University



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Our Ref: I84/31987/15 **Date:** 29th August, 2016

Director General,
National Commission for Science, Technology & Innovation
P.O. Box 30623-00100,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION
MR. RICHARD GITIMU - REG. NO. I84/31987/15

I write to introduce Mr. Richard Gitimu who is a Postgraduate Student of this University. He is registered for a Ph.D. degree programme in the Department of Biochemistry & Biotechnology in the School of Pure & Applied Sciences.

Mr. Gitimu intends to conduct research for a thesis entitled, **"Establishment of Reference Values of Biochemical and Hematological Parameters and Tumour Markers for Coastal Population, Kenya."**

Any assistance given will be highly appreciated.

Yours faithfully,

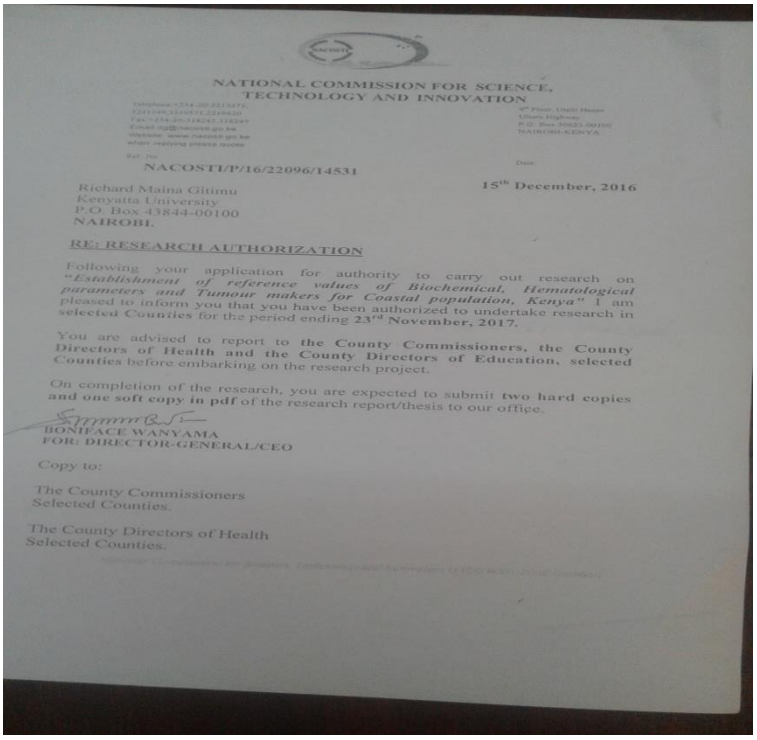
KENYATTA UNIVERSITY OFFICE OF DEAN 29 AUG 2016 GRADUATE SCHOOL P.O. Box 43844-00100 NAIROBI

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

LNM/cww

Kenyatta University ... ISO 9001: 2008 Certified

Appendix: IV permit to carry research, NACOSTI



NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

NACOSTI/P/16/22096/14531

15th December, 2016

Richard Maina Gitimu
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on *"Establishment of reference values of Biochemical, Hematological parameters and Tumour makers for Coastal population, Kenya"* I am pleased to inform you that you have been authorized to undertake research in **selected Counties** for the period ending **23rd November, 2017.**

You are advised to report to **the County Commissioners, the County Directors of Health and the County Directors of Education, selected Counties** before embarking on the research project.

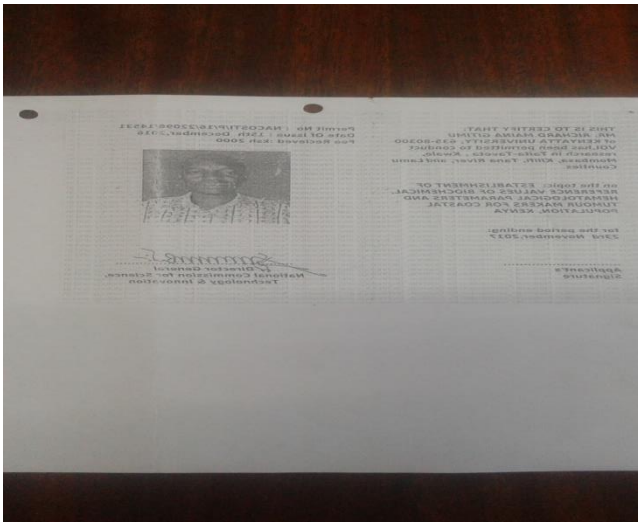
On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioners
Selected Counties.

The County Directors of Health
Selected Counties.



Appendix V Biochemicalreference values of manufacturer's reagents used at Moi district hospital (MDH)

Name of test	Manufacturers' reference values	SI units
Total proteins	64-83	mmol/L
Albumin	35-50	mmol/L
Alkaline phosphatise	Female: 35-104 Male: 40 - 129	U/L
Alanine aminotransferase	Female: 0- 32 Male: 0- 41	U/L
Aspartate aminotransferase	Female: 0- 31 Male: 0- 38	u/l
Total bilirubin	0- 17.1	µmol/L
Direct bilirubin	0- 4.0	µmol/L
Urea	0- 8.3	mmol/L
Creatinine	Female: 44- 80 Male: 62-106	µmol/L
Sodium	136-145	mmol/L
Potassium	3.5-5.1	mmol/L
Chloride	98-107	mmol/L
Calcium	2.20-2.55	mmol/L
Inorganic phosphorus	0.87-1.45	mmol/L
Uric acid	Female: 0- 340 Male: 0- 420	µmol/L
Amylase	28-100	U/L
Lactate dehydrogenase	Female: 135-214 Male: 135-225	U/L
Gamma glutamyl transferase	Female: 5-39 Male: 10-66	U/L
Creatine kinase	Female: 0- 167 Male: 0- 190	U/L

Data from Roche Diagnostics GmbH (Heil, W., Koberstein, Zawta, B. (2002)

Appendix VI Hematological reference values of manufacturer's reagents used at Moi district hospital (MDH)

Name of test	Manufacturers'reference values	SI units
HB	11.0-16.0	g/dl
RBC	3.50-5.50	$X10^{12}$/l
HCT	0.37-0.54	L/L
MCV	80.0-100.0	Ft
MCH	27.0-34.0	Pg
MCHC	32.0-36.0	g/dl
RDW-CV	11.0-16.0	%
RDW-SD	35.0-56.0	Fl
WBC	4.00-10.00	$x10^9$/L
NEUT %	50.0-70.0	%
LYM %	20.0-40.0	%
MONO %	3.0-12.0	%
ESO%	0.5-5.0	%
BASO%	0.0-1.00	%
NEUT	2.0-7.00	$x10^9$/L
LYM	0.8-4.00	$x10^9$/L
MONO	0.12-1.20	$x10^9$/L
ESO	0.02-0.50	$x10^9$/L
BASO	0.00-0.10	$x10^9$/L

PLT	100-300	$x10^9/L$
MPV	6.5-12.0	Ft
PDW	9.0-17.0	%
PCT	0.108-0.282	%

Appendix VII Special chemistry reference values of manufacturer's reagents used at Moi district hospital (MDH)

Name of test	Manufacturers' reference values	SI units
TSH	0.2-8.0	µU/ml
T3	0.5-2.8	nmol/L
T4	42-130	nmol/L
CA125	11-42	U/L
CA 19-9	11-35	U/L
CA15-3	9-37	U/L
CEA	0-3.4	U/L
PSA	1.1-4.3	(ng/mL)
T CHOL	2.9-6.4	(mmol/L)
HDL	1.1-2.1	(mmol/L)
LDL	1.1-4.3	(mmol/L)
T CHO: HDL RATIO		(mmol/L)

Data from Roche Diagnostics GmbH (Heil, W., Koberstein, Zawta, B. (2002)
5 level, Sig. = significance, CI= confidence interval.