REFERENCE RANGES FOR BIOCHEMICAL PARAMETERS, CREATININE CLEARANCE, ORAL GLUCOSE TOLERANCE TEST, LIPIDS, TUMOUR MARKERS AND HORMONES FOR ADULT KENYANS

By

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A thesis submitted in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy (Medical Biochemistry) in the School of Pure and Applied Sciences, Kenyatta University

AUGUST, 2012
DECLARATION

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

Signature........................................... Date ..........................

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DEDICATION

This thesis is dedicated to my dear wife Dr Rebecca Wanjiku and my lovely sons Fredrick Waithaka, Robert Waihenya and Lawrence Githengu for their total support and encouragement throughout the study period. I would also like to dedicate this work to my eldest brother Lawrence Githengu Waithaka and my late dear mother Jane Ngoiri for giving me the gift of education. Mother and Brother, you denied yourselves many things to make sure that I get the best education. I made a promise to you my brother that one day I will get the highest degree on earth and with this thesis I have kept my promise. My mother and brother your efforts were not in vain.
ACKNOWLEDGMENTS

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ABBREVIATIONS AND ACRONYMS

AACP  American Association of Clinical Endocrinologists
AAP   Aminoantipyrine
ADA   American Diabetic Association
ADH   Antidiuretic hormone
ADP   Adenosine diphosphate
AIDS  Acquired immune deficiency syndrome
AITD  Autoimmune thyroid diseases
AJCC  American Joint Committee on Cancer
ALB   Albumin
ALP   Alkaline phosphatase
ALT   Alanine aminotransferase
AMP   2-amino-2-methyl-1-propanol
AMY   Amylase
ARF   Acute renal failure
AST   Aspartate aminotransferase
ATP   Adenosine triphosphate
BCG   Bromocresol green
BMI   Body Mass Index
BUN   Blood urea nitrogen
Ca 125 Cancer 125
Ca 15-3 Cancer 15-3
Ca 19-9 Cancer 19-9
CAL   Calcium
CAPD  Continuous ambulatory peritoneal dialysis
CDC   Centre for disease control
CEA   Carcinoembryonic antigen
CF    Cystic fibrosis
CHD   Coronary heart disease
CHOL  Cholesterol
CK    Creatine kinase
CKF   Chronic kidney failure
CPK   Creatine phosphokinase
CLSI  Clinical Laboratory Standards Institute
CNP   2-chloro-4-nitrophenol
CNPG₃  2-chloro-4-nitrophenyl-α-D-maltotrioside
CNS   Central nervous system
CrCl  Creatinine Clearance
CREAT  Creatinine
CRF   Chronic renal failure
CV    Coefficient of variation
DBILI  Direct bilirubin
DCHBS  3, 5-dinchloro-2-hydroxybenzene sulfonate
DHBS  3,5-dichloro-2-hydroxydenzuesulfonic acid
DIDMOAD Diabetes insipidus, diabetes mellitus, optic atrophy, and deafness

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DM  Diabetes Mellitus
DPD  3, 5-dichlorophenyldiazonium
E2   Estradiol 2
Eercl Estimated creatinine clearance
ELFA Enzyme linked fluorescent assay
EQC  External quality control
ESRD End-stage renal disease
FBG  Fasting glucose
FSH  Follicle stimulating hormone
FT3  Free tri-iodothyroxine
FT4  Free thyroxine
G6PDH Glucose-6-phosphate dehydrogenase
GDM  Gestational diabetes mellitus
GFR  Glomerular filtration rate
GLP  Good laboratory practice
GLUDH Glutamate Dehydrogenase
GMP  Good manufacturing practice
GnRH Gonadotropin-releasing hormone
GoD  Glucose oxidase
H2O2 Hydrogen peroxide
H3PO4 Hydrogen phosphate
HBsAg Hepatitis b Surface Antigen
HCC  Hepatocellular carcinoma
HCG  Human chorionic gonadotropin
HCO3 Bicarbonate
HDL  High density lipoprotein
HIV  Human Immunodeficiency Virus
HK   Hexokinase
IDF  International Diabetes Federation
IFCC International Federation of Clinical Chemistry
IFG  Impaired fasting glucose
IGT  Impaired glucose tolerance
INN  International nonproprietary names
IQC  Internal quality control
IRM  International reference material
KACP Kenya association of clinical pathologists
LDH  Lactate dehydrogenase
LDL  Low density lipoprotein
LFT  Liver function tests
LH   Luteinizing hormone
LP   Lipid profile
MADS 3, 5-dimethylaniline, disodium salt
Merel Measured creatinine clearance
MDH  Malate dehydrogenase
MG   Magnesium
NAD  Nicotinamide Adenine Dinucleotide (oxidized form)
<table>
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<th>Abbreviation</th>
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<tr>
<td>NADH</td>
<td>Nicotinamide Adenine Dinucleotide (reduced form)</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease</td>
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<tr>
<td>NKF</td>
<td>National Kidney Federation</td>
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<tr>
<td>oCPC</td>
<td>o-Cresolphthalein complexone</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>PEP</td>
<td>Phosphoenolpyruvate</td>
</tr>
<tr>
<td>PEPC</td>
<td>Phosphoenolpyruvate Carboxylase</td>
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<tr>
<td>PHOS</td>
<td>Phosphorus</td>
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<tr>
<td>PKD</td>
<td>Polycystic kidney disease</td>
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<tr>
<td>POD</td>
<td>Peroxidase</td>
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<td>PPGT</td>
<td>Postprandial glucose test</td>
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<td>PRG</td>
<td>Progesterone</td>
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<td>Prolactin</td>
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<td>RBG</td>
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<td>SD</td>
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<td>Screening glucose challenge test</td>
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<td>SOP</td>
<td>Standard operating procedure</td>
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<td>SPR</td>
<td>Solid Phase Receptacle</td>
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<td>SPSS</td>
<td>Statistical package for social science</td>
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<tr>
<td>T1DM</td>
<td>Type 1 diabetes mellitus</td>
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<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
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<td>T3</td>
<td>Triiodothyroxine</td>
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<td>T4</td>
<td>Thyroxine</td>
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<td>TBILI</td>
<td>Total bilirubin</td>
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<td>TFT</td>
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<td>TG</td>
<td>Triglycerides</td>
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<td>TP</td>
<td>Total protein</td>
</tr>
<tr>
<td>TPSA</td>
<td>Total prostatic specific antigen</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid stimulating hormone</td>
</tr>
<tr>
<td>UA</td>
<td>Uric acid</td>
</tr>
<tr>
<td>UGT</td>
<td>Uridine-diphosphate glucuronosyltransferase</td>
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<tr>
<td>UV</td>
<td>Ultra violet</td>
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<tr>
<td>VDRL</td>
<td>Venereal Disease Research Laboratory</td>
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<tr>
<td>VLDL</td>
<td>Very-low-density lipoprotein</td>
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<td>WHO</td>
<td>World health organization</td>
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ABSTRACT

Clinical chemistry reference ranges vary due to factors such as age, sex, race, diet, climate, altitude, genetics, geographical location, instruments, analytical methods, and sample type. Due to this, International Federation of Clinical Chemists recommends that each laboratory establishes its own references ranges for the biochemical parameters and not rely on the Manufacturer’s reference range values. There is little information in the literature on biochemical reference values for adult Kenyans. The aim of this study was to determine age, sex, and region based reference ranges for 45 biochemical parameters for the adult Kenyans. This was a population based cross-sectional prospective study carried out at Kenyatta National Hospital. 6768 participants (3389 males and 3379 females) from the eleven counties were used in the establishment of reference ranges for 45 biochemical parameters. Reference ranges were constructed using parametric methods to estimate 2.5 and 97.5 percentiles of distribution as lower and upper reference limits, respectively. The performance of manual spectrophotometer was assessed by comparing levels of total protein, albumin, total bilirubin, and alkaline phosphatase using auto analyzer as the gold standard. Results show that some of the established reference ranges were sex and age specific, and were different from American, Germany and French based reference ranges used in Kenyan hospitals, except for calcium which was similar. Glucose and uric acid showed significant regional differences. Results of the selected analyte determined using the manual spectrophotometric method were significantly lower than those obtained using the automated analyzer. In conclusion, the findings of this study provide adult sex, age, and region specific reference range values to be used in Kenya; it also shows that automated methods are superior to the manual methods in determining analyte levels. In view of the differences in reference ranges of the study population and of the others quoted in literature, it is important that clinical laboratories should establish their own biochemical parameter reference ranges based on the population they serve. Due to age and sex differences expressed in this study, there is need to consider these two factors whenever test requisition is made and during the construction of reference ranges for other biochemical parameters. There is great need to revolutionize the analytical methods by replacing manual methods with automated methods in all clinical laboratories.
CHAPTER ONE
INTRODUCTION

1.1 Background information

Clinical laboratories worldwide are integral part of the health care systems that have the responsibility of the health of the human population. These responsibilities involve analysis of materials derived from the human body for the purpose of providing information on diagnosis, prevention, or treatment of ailments (Bruns, 2001). World health organization (WHO) monitors health care and the status of health of the population in each of its member states. The statistics provides valuable information for the comparison of the effectiveness of different health systems. As part of its global responsibility on health WHO issues international reference materials (IRMs), assigns international nonproprietary names for therapeutics (INNs), publishes guidelines for good manufacturing practice (GMP) and good laboratory practice (GLP) and, in collaboration with international professional organizations, makes recommendations towards achieving global harmonization of clinical laboratory investigations (Ezzelle et al., 2008; Guy et al., 2010).

There are six major disciplines in clinical laboratory medicine sector namely clinical chemistry, haematology/blood transfusion, parasitology, microbiology, histology and immunology. Though all disciplines of laboratory sciences are contributing in mitigating the misery and mortality of human beings, clinical chemistry has been in use for the longest period and is accessible even in the peripheral areas of most of the developing countries. This has been possible because most of tests conducted to assess the
biochemical status of the human body are simple, easy to perform, rapid, economical and
do not demand a sophisticated infrastructure. However, in the absence of uniformity in
the performance of these tests, the results generated would not meet the desired purpose
and might have an adverse impact on the diagnosis as well as treatment. To obviate this
problem, and to ensure quality of results, standard operating procedures (SOPs) play a
vital role. Every laboratory has to develop SOPs and use them in-house. The absence of
(SOP) makes it very difficult for many laboratories to generate reliable results. The
World Health Organization has been advocating the use of standard operating procedures
in all laboratory activities. To achieve this objective, a series of subject-specific
guidelines to develop SOPs suiting the requirements of particular laboratories have been
developed (Oosterhuis et al., 2004; Jonna Skov et al., 2008).

Laboratory investigations pertaining to clinical chemistry have been yielding useful
information to clinicians, both in the diagnosis of illness as well as the monitoring of
treatment. Laboratory medicine being an integral part of health sector has a great
influence on clinical decisions and 60-70% of the most important decisions on admission,
discharge, and medication are based on laboratory results. People in the developing
countries are also deriving benefit from these simple and economical tests. The utility of
these tests has been especially recognized in the management of lifestyle diseases such as
diabetes mellitus and other cardiovascular ailments - the incidence of which is increasing
steadily even in developing nations (Solomon et al., 1998). The extensive use of these
tests has resulted in the marketing of a variety of test reagents with variable technology.
The interpretation of any clinical laboratory analytical test report involves an important concept in comparing the patient's results to the test's reference range (Rustad et al., 2004). In any qualitative analytical reporting the patient's test result is either indicated as positive or negative. For the quantitative analytical reporting the patient's result's is followed by a reference range. The term reference range is preferred over normal range because the reference population can be clearly defined. Rather than implying that the test results are being compared with ill-defined concept of "normal", the reference range means the results are being considered in the most relevant context. When test results from different populations are examined it is quickly discovered that what is normal for one group is not necessarily normal for another group. For example, pregnancy changes many aspects of the body's chemistry so pregnant women have their own set of reference ranges (Sacher et al, 2000).

Age, sex, race, geographical location, instruments, analytical methods, sample type and diet are factors known to affect the reference ranges (Glick et al, 1986). Winsten (1976) divided the various age groups into the following categories: new born, the prepubertal group, the adult population and the older adult population. A comparison between a group of subjects in the pre-puberty and a comparable group of adults showed that the pre-pubertal subjects exhibited higher values of alkaline phosphatase, inorganic phosphate, aspartate aminotransferase and lactate dehydrogenase, but significantly lower serum uric acid, cholesterol, creatinine and total protein values. For both males and females there are common changes noted with advancing age, which includes a decrease in serum albumin and total proteins and an increase in urea, creatinine, glucose
cholesterol and alkaline phosphatase (O-Carroll, 1975). Serum uric acid, creatinine, and urea are higher in healthy males than in female counterparts. Before the sixth decade males have higher values for serum triglycerides, cholesterol, sodium and calcium (Leonard, 1973).

A reference range is constructed to include the range of values found in 95% of a reference population of healthy individuals. Presently, only a few clinical laboratories have established their own reference ranges, while great majority use reference ranges reported in literature (Ferre et al., 1999). The International Federation of Clinical Chemistry (IFCC) recommends that each clinical chemistry laboratory establish its own reference ranges for the biochemical parameters (Ferre et al., 1999). An alternative to this unsatisfactory situation is for laboratories in a given region to establish their own reference ranges using either direct or indirect method as recommended by the IFCC (Jagalinec et al., 1998). Direct method involves the use of data obtained from a healthy population accessible to hospital, such as blood donors. A lot of work has been done in the production of reference ranges using direct methods (Grasbeck, 1969; Martin et al., 1975). The indirect method involves the use of data obtained from patients’ records. This data is used with the assumption that the majority of values come from subjects whose values are compatible with those of healthy subjects (in the sense that their values have not been altered by disease) (Ferre et al, 1999).

The study was undertaken using direct method for the establishment of national reference ranges. The study was based on recruited healthy subjects from twelve counties
representing nine regions of Kenya namely, Kiambu and Nyeri (Central region), Mombasa (Coast region), Meru and Machakos (Eastern region), Nairobi (Nairobi region), Garissa (North Eastern region), Kisumu (Nyanza region), Uasin Gishu (North Rift Valley region), Nakuru (Central Rift valley), Kajiado (South Rift Valley region) and Kakamega (Western region).

The commonly requested biochemical analytical profiles in the Clinical Chemistry includes; renal function tests, liver function tests, lipid profiles, cardiac tests, glucose (random and fasting), thyroid function test, fertility hormones, tumor markers, bone metabolism tests, pancreatic function tests and body fluids biochemical tests. Other biochemical procedures carried out in clinical chemistry laboratories whose reference guidelines were established in the current study includes; creatinine clearance, fasting lipid profile and oral glucose tolerance test.

Reference ranges were established for the following biochemical analytes namely, alanine aminotransferase \((\text{ALT}) (\text{EC 2.6.1.2})\), (aspartate aminotransferase \((\text{AST}) (\text{EC 2.6.1.1})\)), alkaline phosphatase \((\text{ALP}) (\text{EC 3.1.3.1})\), gamma glutamyl transferase \((\gamma \text{GT or GGT}) (\text{EC 2.3.2.2})\) albumin (ALB), bicarbonates \((\text{HCO}_3^-)\), total bilirubin \((\text{TBILI})\), direct bilirubin \((\text{DBILI})\), creatinine \((\text{CREAT})\), creatinine clearance \((\text{CrCl})\), random blood glucose \((\text{RBG})\), fasting glucose \((\text{FBG})\), oral glucose tolerance test \((\text{OGTT})\), potassium \((\text{POT})\), sodium \((\text{SOD})\), blood urea nitrogen \((\text{BUN})\), total fasting cholesterol \((\text{CHOL})\), high density lipoprotein \((\text{HDL})\), low density lipoprotein \((\text{LDL})\), fasting triglycerides \((\text{TG})\), total protein \((\text{TP})\), uric acid \((\text{UA})\), phosphorus \((\text{PHOS})\), alpha amylase \((\text{AMY})\)
(EC 3.2.1.1)}, lactate dehydrogenase \{(LDH) (EC 1.1.1.27)\}, creatine kinase \{(CK) (EC 2.7.3.2)\} and magnesium (MG). Other tests were total prostatic specific antigen (TPSA) carcinoembryonic antigen (CEA), Ca 125, Ca 15-3, Ca 19-9, Testosterone (TESTO), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol 2(E2), progesterone (PRG), Prolactin (PRL), Thyroid function tests, Thyroid stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3) free tri-iodothyroxine (FT3) and free thyroxine (FT4). The analytical work was done using clinical chemistry automated analyzer, Olympus (AU 640 and AU 400), spectrophotometer (Hitachi), and glucose meter (accu-check) instruments.

1.2 Justification

Clinicians in our Kenyan health institutions either use biochemical reference ranges from books or those stated in the laboratory diagnostic kits. Today, as a result of liberalization our Kenyan markets are flooded with reagent diagnostic kits from different parts of the world especially from Europe, America and Asia. The reference ranges for various biochemical analytes quoted in these diagnostic kits are based on reference ranges establishment work done on healthy adult population from these different geographical regions. The reference ranges are published in medical textbooks or by manufacturers of diagnostic kits. There is need to establish reference ranges for biochemical analytes determined in Clinical Chemistry laboratories of Kenyan health institutions based on healthy adult Kenyan population.
In the diagnosis and management of renal and cancer cases, the knowledge of the patient's glomerular filtration rate is a very important factor. The kidney's role of clearing the metabolic waste products and cancer drugs toxic products was achieved by the determination of the reference ranges of creatinine clearance for the adult Kenyan population. The establishment of a reference oral glucose tolerance curve for the adult Kenyan population is expected to be used in the early diagnosis and management of diabetic mellitus. This was done in Kenyatta National hospital since it has a diabetic referral clinic.

The most common instruments used for analysis of biochemical tests in Kenyan provincial and district hospitals are simple colorimeters and spectrophotometers. Automated clinical chemistry analyzers are found only in the big health institutions such as Kenyatta National hospital. There was need therefore to establish the effect instrumentation have on reference ranges for the biochemical analytes.

Some biochemical analytes are determined using different biochemical analytical methods eg in the determination of serum/plasma electrolytes, flame photometry, ion selective electrode and calorimetric methods can be used. There was need therefore to establish whether these biochemical methods had an effect on the reference ranges.

The achievement of the above objectives is aimed at helping the clinicians to interpret the laboratory data more appropriately. The establishment of national reference ranges for the various performed biochemical tests in clinical chemistry form the basis of this work and
to achieve this goal the internal quality assurance programme in the clinical chemistry laboratories was totally adhered in order to achieve quality results.

1.3 Research hypotheses

(1) There are no sex and age specific national reference ranges for some routine biochemical parameters for healthy adult Kenyan population.

(2) There are no sex and age specific reference ranges for some special biochemical parameters from a selected healthy adult Kenyan population.

(3) There are no sex and age specific reference ranges for fasting profiles and oral glucose tolerance test for healthy adult Kenyan population.

(4) There are no differences in the performance of analytical methods used to determine the levels of selected analytes using different reagents.

(5) There are no differences in the performance of automated and manual spectrophotometers in determining the levels of selected analytes.

1.4 Research objective

The objective of this study was to establish national sex and age specific reference ranges for some routine and special biochemical parameters using automated and manual analysers for the adult Kenyan population.

1.4.1 Specific objectives

(1) To establish sex and age specific national reference ranges for routine biochemical parameters for healthy adult Kenyan population.
(2) To establish sex and age specific reference ranges for special biochemical parameters from a selected healthy adult Kenyan population.

(3) To establish sex and age specific reference ranges for fasting profiles and oral glucose tolerance test for healthy adult Kenyan population.

(4) To establish the performance of analytical methods used to determine the levels of selected analytes using different reagents.

(5) To establish the performance of automated and manual spectrophotometers in determining the levels of selected analytes.

1.5 Impact of the results

The study is expected to establish the national reference ranges for the routinely determined biochemical tests for the adult Kenyan population. The study will also establish the glomerular filtration rate and the normal oral glucose tolerance curve for the adult Kenyan population. Recommendations will be made to the routine users of the reports from the laboratory. The study findings may be extended to open ways for the establishment of national reference ranges for other analytical parameters in other disciplines of laboratory medicine.
2.1 Reference ranges

Reference ranges are medical decision-making tools that are provided by a clinical laboratory to aid the health provider in differentiating a diseased patient from a healthy individual. Establishing correct reference ranges is an important task for a clinical laboratory. However, a major problem that the laboratory faces is obtaining a sufficient number of specimens from healthy individual representative of the population that the laboratory serves. The complexity of establishing reference ranges and the cost and labour are additional difficulties (Alex and David 2010).

It has been concluded by most of the European learned societies on clinical chemistry that each clinical laboratory should produce its own reference values (Morrow et al., 2007). However, very few laboratories actually do so. To make things easier, the International Federation of Clinical Chemistry (IFCC) published recommendations for the transferability of the reference values from one institute to another, but even in this case, the laboratories involved should obtain comparable results and this can only be achieved by conducting long-term inter-laboratory studies of the analytical methods in use, in terms of precision and accuracy (Solberg, 2004, Gian et al., 2010). According to the International Federation of Clinical Chemistry (IFCC), these reference ranges are constructed from 95% of the healthy population (Ferre et al., 1999). There are two types of reference ranges namely: the group based and subjects based reference ranges. In the group based reference ranges, the clinician uses these by estimating the probability that
the subject (patient) from whom the given laboratory report was obtained belongs to a
group of healthy subjects from whom the reference ranges were obtained. If the reference
values are from the same subject when he/she was in a defined state of health, the
clinician compares the present value with the past values to estimate whether the subject
is still in the same state of health (Grasbeck, 1969; Martin et al, 1975). One study carried
out in Spain to establish reference values for 14 biochemical analytes using indirect
method concluded that reference values for bilirubins, potassium, alanine
aminotransferase, aspartate aminotransferase, were not suitable after validation by
statistical comparison with reference values estimated from a reference sample according
to recommendations of the IFCC (Ferre et al., 1999).

Two major problems are encountered in establishing reliable reference ranges from a
group of healthy subjects. One is involved in obtaining specimens from a sufficiently
large number of healthy subjects of whom, Martin (1975) recommended a minimum of
300 healthy subjects. The other obstacle is to ascertain that the factors involved in the
preparation of the subjects and the analytical procedures, noted during the establishment
of reference values are the same factors present during day to day routine analysis of
patient specimen. It may well be true that the best reference values for an individual are
so-called “subject-based reference values,” derived from their own prior data, but again,
such data are not often available. Additionally, the IFCC definition of healthy ambulatory
individuals may not be optimal references for hospitalized patients, because of
differences in physical activity, diet, level of stress, diurnal rhythms, or other factors
related to hospital stay. From this point of view, a hospitalized patient, not affected by the
disease in question, but subject to the same conditions would be a better reference for a patient having a certain disease (Ferre et al., 1999).

For all these reasons, some scientists working in the area have investigated the possibility of establishing reference values from large collections of laboratory data, using sophisticated laboratory information systems and statistical programs. The major advantage of using such an approach is that it saves a significant amount of money and work by using data that already exist (Solberg, 2006).

The use of single laboratory parameter to make a conclusive diagnosis of certain pathological disorder has not been adequate. This necessitated the introduction of clinical chemistry analytical multi-test profiles and organ- or disease-specific test profiles which has been in place since mid last century. The introduction of automation at the same time contributed a lot in the use of tests profiles in the management of various pathological disorders (CLSI 2008). Clinical chemistry analytical test profiles combines more than two parameters aimed at revealing the health status of a particular body organ (designed to measure and evaluate the body's major organ functions such as the heart, liver, kidney, gland, nerve, bone and muscle functions). The use of analytical tests profiles has revolutionized the diagnosis and management of various pathological disorders affecting man (Grossi et al., 2005). Some of the analytical tests profiles investigated in clinical chemistry laboratory includes:- glucose metabolism, lipids metabolism, renal function, liver function, bone metabolism, cardiac enzymes, thyroid function, gonad function (fertility hormones) and tumuor makers (Bock et al., 2003).
2.2 Glucose metabolism

Glucose is the end product of carbohydrate metabolism and it provides energy to all cells in human body. To maintain a constant blood-glucose level, the body relies on two hormones produced in the pancreas that have opposite actions: insulin and glucagon (Dunning, et al., 2006). Insulin is a protein hormone made and secreted by the beta cells of the pancreatic islets (Islets of Langerhans). It is required by almost all of the body's cells, since it enhances glucose entry into these cells. As such, insulin stores nutrients right after a meal by reducing the concentrations of glucose, fatty acids and amino acids in the bloodstream (McLarty, et al., 1993). Glucagon is another protein hormone that is made and secreted by the alpha cells of the pancreatic islets (Islets of Langerhans). Glucagon acts on the same cells as insulin, but has the opposite effects: Stimulates the liver and muscles to break down stored glycogen (glycogenolysis) and release the glucose. It stimulates gluconeogenesis in the liver. In contrast to insulin, glucagon mobilizes glucose from stores inside the body and increases the concentrations of glucose in the bloodstream. Insulin and glucagon have opposite effects on liver and other tissues for controlling blood-glucose level (Abdul, et al., 2006).

2.2.1 Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia (elevated blood glucose concentration) resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Eddy, et al., 2005). Several pathogenic processes are involved
in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action (De León, et al., 2006). Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular symptoms and sexual dysfunction. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (Torgerson, et al., 2004)

The incidence of diabetes mellitus is rapidly growing in the world. By the end of 2006, the incidences had increased to 230 million, representing 6% of the world population (King, et al., 1995; Roglic, et al., 2005). It is estimated that, during the next 35 years, diabetic world-wide prevalence will reach 25%, with India being the hardest hit. The figure of Africans suffering from diabetes is expected to rise to 18.6 million by 2030 (Wild, et al., 2004). Data from the Ministry of Health in Kenya indicated that, of all humans living in Africa, an estimated 1.2 million Kenyans live with diabetes, and if the trend continues, by 2025 that number is expected to rise to 1.5 million (McFerran, 2008).

The racial and ethnic puzzle of diabetes has led scientists to suggest that genetic predisposition might be a major factor, along with environmental factors, diet, lifestyle
(inactivity), and residence (Swai et al., 1990; Cruickshank et al., 2001). It is well known now that obesity is one of the most significant contributors to increased prevalence of diabetes, leading to the use of the word “diabesity, (International Diabetes Federation (IDF), (2007), Sobngwi et al., 2004), both in rural and urban areas. In comparison to the rural areas, the urban setting also presents an increased prevalence of obesity (Kamadjeu, et al., 2006; Hossain, et al., 2007).

2.2.2 Categorization of hyperglycaemic disorders

Hyperglycaemic disorders can be categorized into: diabetes mellitus (Type 1, Type 2 and gestational), impaired glucose tolerance, impaired fasting glucose, previous abnormality of glucose tolerance and potential abnormality of glucose tolerance. Type 1 (juvenile diabetes or insulin-dependent diabetes) is caused by an absolute lack of insulin. This type is found in 5% to 10 % of diabetics and usually occurs in children or adolescents. In Type 1 diabetics, the beta cells of the pancreatic islets are destroyed, possibly by the person's own immune system, genetic or environmental factors. Type 2 diabetes mellitus (T2DM) or (adult-onset diabetes or non-insulin-dependent diabetes) occurs when the body does not respond or can't use its own insulin (insulin resistance). Type 2 occurs in 90 % to 95 % of diabetics and usually occurs in adults over the age of 40 years, most often between the ages of 50 years and 60 years (Mozaffarian et al., 2009). Incidence varies substantially in different parts of the world, almost certainly because of environmental and lifestyle factors, though these are not known in detail (Zimmet et al., 2001). About 55 percent of type 2 diabetes patients are obese at diagnosis (Eberhart et al., 2004). Chronic obesity leads to increased insulin resistance that can develop into type 2 diabetes, most likely
because adipose tissue (especially that in the abdomen around internal organs) is a (recently identified) source of several chemical signals to other tissues (hormones and cytokines). Other research shows that type 2 diabetes causes obesity as an effect of the changes in metabolism and other deranged cell behavior attendant on insulin resistance (Camastra et al., 1999). However, environmental factors (almost certainly diet and weight) play a large part in the development of type 2 diabetes in addition to any genetic component. This can be seen from the adoption of the type 2 diabetes epidemiological pattern in those who have moved to a different environment as compared to the same genetic pool who have.

There is a stronger inheritance pattern for type 2 diabetes. Those with first-degree relatives with type 2 diabetes have a much higher risk of developing type 2 diabetes, increasing with the number of those relatives. Concordance among monozygotic twins is close to 100%, and about 25% of those with the disease have a family history of diabetes foot (Cotran and Collins, 1999). Genes significantly associated with developing type 2 diabetes, include TCF7L2, PPARG, FTO, KCNJ11, NOTCH2, WFS1, CDKAL1, IGF2BP2, SLC30A8, JAZF1, and HHEX. Gene expression promoted by a diet of fat and glucose as well as high levels of inflammation related cytokines found in the obese results in cells that "produce fewer and smaller mitochondria than is normal," and are thus prone to insulin resistance (Walley et al., 2006).

Gestational diabetes mellitus (GDM) can occur in some pregnant women and is similar to Type 2 diabetes mellitus. GDM has also been defined as any degree of glucose
intolerance with onset or first recognition during pregnancy, to differentiate it from pre-diagnosed type 1 or type 2 diabetes or maturity-onset diabetes of the young (MODY) in women that get pregnant (American Diabetes Association, 2010). This broad definition of GDM therefore includes women whose glucose intolerance develops during pregnancy and those that had pre-existing diabetes which had not been diagnosed before pregnancy. Insulin resistance usually begins in the second trimester and progresses throughout the remainder of the pregnancy. Insulin sensitivity is reduced by as much as 80% (Carr and Gabbe, 1998). Placental secretion of hormones, such as progesterone, cortisol, placental lactogen, prolactin, and growth hormone, is a major contributor to the insulin-resistant state seen in pregnancy. Depending on the population sample and diagnostic criteria, the prevalence may range from 1 to 14%. Of all pregnancies complicated by diabetes, GDM accounts for ~90% (Buchanan and Xiang, 2005).

Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) is a category of hyperglycaemic disorder which identifies individuals whose glucose levels, although not meeting criteria for diabetes, are nevertheless too high to be considered normal (The Expert Committee (Wild et al., 2004). Patients with IFG and/or IGT are now referred to as having “pre-diabetes” indicating the relatively high risk for development of diabetes in these patients. A study in Iranian population showed that more people have IFG (16.8%) than DM (7.7%) (Alireza et al., 2008). Previous abnormality of glucose tolerance is a category of hyperglycaemic disorder which identifies individuals known to have had an abnormality of glucose tolerance at one time but have been shown to have normal results on repeat testing. Someone who had Type 2 diabetes mellitus was obese, not treated with
insulin, and after weight reduction had a normal glucose tolerance test falls under this
category (Green et al., 2003). Potential abnormality of glucose tolerance is a category of
hyperglycaemic disorder which identifies individuals who are at greater than average
theoretical risk for later development of diabetes mellitus. Identical twin of a patient with
type 2 diabetes or a child of parents who each had type 2 diabetes falls under this
category (Inzucchi and Sherwin, (2005).

Regardless of the type of diabetes, diabetics exhibit several (but not necessarily all) of the
following symptoms: Excessive thirst (polydipsia), frequent urination (polyuria), extreme
hunger or constant eating (polyphagia), unexplained weight loss, presence of glucose in
the urine (glycosuria), tiredness or fatigue, changes in vision, numbness or tingling in the
extremities (hands, feet), slow-healing wounds or sores and abnormally high frequency of
infection (Gunderson et al., 2007).

2.2.3 Hypoglycaemia

Hypoglycaemia is the medical term for a state produced by a lower than normal level of
blood glucose. It can produce a variety of symptoms and effects but the principal
problems arise from an inadequate supply of glucose to the brain, resulting in impairment
of function (neuroglycopenia). Effects can range from vaguely "feeling bad" to seizures,
unconsciousness, and (rarely) permanent brain damage or death. The most common
forms of hypoglycemia occur as a complication of treatment of diabetes mellitus with
insulin or oral medications. Hypoglycemia is less common in non-diabetic persons, but
can occur at any age, from many causes. Among the causes are excessive insulin
produced in the body (hyperinsulinemia), inborn errors of metabolism, medications and poisons, alcohol, hormone deficiencies, prolonged starvation, alterations of metabolism associated with infection, and organ failure. Research in healthy adult show that mental efficiency decline slightly but measurably as blood glucose fall below the lower reference value in many people (Crye et al., 2009).

2.2.4 Glucose test profiles

Clinical laboratory investigation plays a great role in the diagnosis of either hyperglycaemic or hypoglycaemic status of an individual. Laboratory diagnostic profile tools that forms part of ones glucose status diagnosis are: fasting blood glucose (FBG), random blood glucose (RBG), oral glucose screening test (OGST) and oral glucose tolerance test (OGTT).

The success of FBG requires that an individual abstains or refrains from food of any kind overnight or any other period except water; and for purposes of confirming, fasting should take a minimum of 8 hours overnight. It is a reliable simple test preferred by many health workers. It is not affected by age or activities surrounding the patient and may be repeated after a while to further verify results (Cowie et al., 2006).

RBG which is a non-fasting test has been used in community based screening since it is the most convenient way to reach out to large numbers of people. Measurement of random blood glucose has the advantage that it can be undertaken at any time of the day, does not require a veni-puncture. In the early detection of diabetes type 2, RBG has been found to have a higher sensitivity (75%) than either FBG (50%) and glycosylated
haemoglobin (HbA1c) (16.7%). However, RBG has a lower specificity (88%) in the
detection of type 2 diabetes mellitus than either either FBG (95%) and glycosylated
haemoglobin (HbA1c) (98.9%) (Harris et al., 2003).

Screening glucose challenge test (sometimes called the O'Sullivan test) is performed
under the two approaches recommended for the screening of GDM. Since there is no
worldwide agreement on the best way to screen for GDM, universal and selective
screening approaches have been adopted (Hillier et al., 2008). Universal screening
involves giving a drinking a solution containing 50 grams of glucose to non-fasting
pregnant woman and blood glucose levels determined 1 hour later. Women with a 1-hour
glucose level > 7.8 mmol/L are referred for a diagnostic OGTT (Tracy et al., 2005).
Selective screening was developed by categorizing pregnant women into three groups
((low-, intermediate-, and high-risk) based on a complex scoring system using weighted
risk factors: age, BMI before pregnancy, and race (Naylor et al., 1997).

OGTT is the gold standard for the diagnosis of diabetes, insulin resistance, and
sometimes reactive hypoglycemia or rarer disorders of carbohydrate metabolism
according to WHO and ADA (CDC, 2004). OGTT has also been used to evaluate
clinically useful measures of beta-cell function to assess insulin secretion, in comparison
with the gold standard method of the hyperglycemic clamp (Hyper-C) (Fida et al., 2008).
Usually the OGTT is performed in the morning as glucose tolerance can exhibit a diurnal
rhythm with a significant decrease in the afternoon (ADA, 2007).
Two hours postprandial blood sugar measures blood glucose exactly 2 hours after eating a meal which acts as a glucose challenge to the body's metabolism. By this point blood sugar has usually gone back down in healthy people, but it may still be elevated in people with diabetes (Goldstein et al., 2004). Thus, it serves as a test of whether a person may have diabetes, or of whether a person who has diabetes is successfully controlling their blood sugar. A fasting blood sugar (FBS) is done before the 2 hr PPG (Temelkova-Kurktschiev et al., 2000).

2.3 Kidney diseases

Diseases of the kidney are diverse, but individuals with kidney disease frequently display characteristic clinical features. Common clinical conditions involving the kidney include the nephritic and nephrotic syndromes, renal (kidney) cysts, acute kidney injury, chronic kidney disease, urinary tract infection, nephrolithiasis, and urinary tract obstruction. Various cancers of the kidney exist; the most common adult renal cancer is renal cell carcinoma (Kapoor and Chan, 2001).

Nephrotic syndrome is classically characterized by six main abnormalities of which proteinuria of greater than 3.5 g/24 hr is the cornerstone. The cardinal manifestations of the nephrotic syndrome include edema, hypoalbuminemia, hyperlipidemia, and lipiduria (Nandish et al., 2006). Nephrotic syndrome affects both adults and children although the condition, in many cases, appears in childhood between ages of 1½ and 5 years. It seems to affect boys more often than girls. Exactly what causes it is often unknown (Eddy and Symons, 2003).
Polycystic kidney disease (PKD) also known as polycystic kidney syndrome is a cystic genetic disorder of the kidneys. It characterized by the presence of multiple cysts (hence, "polycystic") in both kidneys. The cysts are numerous and are fluid-filled resulting in massive enlargement of the kidneys (Yode et al., 2002).

Acute renal failure (ARF) is the rapid cessation of renal excretory function within a time frame of hours or days, accompanied by a rise in serum urea and creatinine, and accumulation of nitrogenous waste products in a patient whose renal function was previously normal. It is usually, but not always, accompanied by a fall in urine output. The condition is potentially reversible, and in routine clinical practice, measurement of serum creatinine is used to follow the changes in glomerular filtration rate (GFR) (Schrier et al., 2004). In Africa, use of traditional medicine (traditional folk remedies) has been associated with the increase of acute renal failure and hepatic damage. It has been estimated that one-third of cases of acute renal failure in Africa are caused by folk medicines (Swanepoel et al., 2003). Current studies have associated acute renal failure as a severe complication of malaria caused by plasmodium falciprum although its rare occurrence has been reported in plasmodium vivax. Prevalence of ARF in malaria all over the world has been reported as 0.57% to 60%. (Mehta et al., 2001).

Chronic renal failure (CRF), also known as chronic kidney failure (CKF), is a progressive loss in renal function over a period of months or years. All individuals with a glomerular filtration rate (GFR) <60 mL/min/1.73 m² for 3 months or more are classified as having
chronic renal failure, irrespective of the presence or absence of kidney damage. The most common causes of CRF are diabetes mellitus, hypertension, and glomerulonephritis. Together, these cause approximately 75% of all adult cases. Chronic renal failure may be identified when it leads to one of its recognized complications, such as cardiovascular disease, anemia or pericarditis. In addition to this, blood creatinine is increased being an indication of falling GFR and as a result a decreased capability of the kidneys to excrete waste products (Barsoum, 2002).

Prevention of end-stage renal disease (ESRD) requires knowledge of modifiable risk factors responsible for initiation and promotion of renal insufficiency. Alcohol consumption is a plausible risk factor for ESRD. More recently, alcohol use has been associated with immunoglobulin A nephropathy and renal papillary necrosis. Alcohol consumption may potentiate the nephrotoxicity of lead and antiinflammatory drugs (Pemeger et al., 1995). Patients with postinfectious glomerulonephritis who consume alcohol may be at increased risk of progression to chronic renal failure compared with their non-drinking counterparts Home-distilled whiskey ("moonshine") may be particularly nephrotoxic because of its high lead content (Thomas et al., 1999).

2.3.1 Renal test profile

Clinical laboratory investigation plays a great role in the diagnosis of kidney pathological disorders status of an individual. Laboratory diagnostic profile tools that forms part of ones kidney status diagnosis are:-blood urea (or blood urea nitrogen), creatinine and creatinine clearance and electrolytes (sodium, potassium and chloride).
The major pathway of nitrogen excretion in human is as urea. Blood urea nitrogen (BUN) is used as first line investigation of glomerular function. However, over 60% of glomerular must be destroyed before blood urea nitrogen concentration significantly rises. Low blood urea nitrogen is observed in a condition such as pregnancy the commonest cause in young women, over enthusiastic intravenous infusion, inappropriate antidiuretic hormone (ADH) secretion and all these are caused by an increase in glomerular filtrate rate (GFR). In children low BUN is due to decreased synthesis where amino acids are used for protein anabolism during growth, low protein intake, very severe liver disease, and inborn errors of urea cycle (Meyer and Hostetter, 2007).

Creatinine is the degradation product of creatine. The formation of creatinine is reasonably constant, and has a direct relationship to the body muscle mass. Creatinine is freely filtered by the glomeruli but is not reabsorbed to any appreciable extent if at all under normal circumstances along the tubules of the nephron. A small but significant amount of creatine is also excreted by active tubular secretion, which increases with increasing plasma creatinine concentration. The formation and excretion of creatinine which is usually constant makes it one of the best renal function parameter (Campens, 1997; Swanepoel, 1999). By virtue of its relative independence of such factors as, diet (protein intake), hydration and protein metabolism, the plasma creatinine is significantly more reliable screening test for renal function than blood urea nitrogen. In relation to sex men have a higher normal creatinine than women whilst children due to their little body mass have lower normal creatinine (Jones et al., 1998). Creatinine clearance is a more
widely used method in the evaluation of GFR (Lindegaard et al., 1991; Montgomery et al., 2000). In the management of renal diseases and monitoring of chemotherapy treatment for cancer patients, creatinine clearance test is the first choice test (Rossini et al., 2009).

Sodium is the predominant extracellular cation and cannot freely cross the cell membrane. Its homeostasis is vital to the normal physiologic function of cells. The blood sodium concentration and osmolality are closely controlled by water homeostasis, which is mediated by thirst, ADH, and the kidneys. It is found in either decreased [hyponatremia] or elevated [hypernatremia] form in pathological states. Hyponatremia and hypernatremia represent disorders of water balance. Impaired renal water excretion and ADH play an important role in hyponatremia, while excess water loss leads to hypernatremia (Madias and Adrogue, 2005).

Potassium is a major intracellular cation. At least 95% of the body's potassium is found inside cells, with the remainder in the blood. Potassium is essential for many body functions, including muscle and nerve activity. In pathological conditions it is either reduced [hypokalemia] or elevated [hyperkalemia]. Hypokalemia is perhaps the most common electrolyte abnormality encountered in clinical practice. When defined as a value of less than the lower limit of reference range of a given population, hypokalemia is found in over 20 percent of hospitalized patients. One of the most significant causes of hypokalemia is insufficient intake of potassium. Furthermore, loss of potassium can also occur due to excessive loss of fluid (vomiting, perspiration and diarrhea). Certain
medications such as some laxatives and thiazide diuretics (furosemide and hydrochlorothiazide) increase the risk of hypokalemia.

Chloride is the major extracellular anion and represents the largest fraction of the plasma total inorganic anion concentration of approximately 154 mmol/L. Chloride is therefore significantly involved in the maintenance of water distribution, osmotic pressure and anion-cation balance in the extracellular fluid compartment. It is found in either decreased [hypochloremia] or elevated [hyperchloremia] form in pathological states (Reid et al., 2003).

Biocarbonate is important in the maintenance of acid-base homeostasis which is a vital function of the living organism. Deviations of systemic acidity in either direction can impose adverse consequences and when severe can threaten life itself. Acid-base disorders frequently are encountered in the outpatient and especially in the inpatient setting. Effective management of acid-base disturbances, commonly a challenging task, rests with accurate diagnosis, sound understanding of the underlying pathophysiology and impact on organ function, and familiarity with treatment and attendant complications (Adrogue and Madias, 1998). Clinical acid-base disorders are conventionally defined from the vantage point of their impact on the carbonic acid-bicarbonate buffer system. This approach is justified by the abundance of this buffer pair in body fluids; its physiologic preeminence; and the validity of the isohydric principle in the living organism, which specifies that all the other buffer systems are in equilibrium with the carbonic acid-bicarbonate buffer pair (Madias and Adrogue, 1995).
2.4 Liver diseases and test profile

The liver is subject to damage by a wide variety of diseases. Viruses, bacterial infections, toxins, and several forms of cancer are a few of the possible causes of liver disease. Some diseases cause very sudden and severe liver damage and others are slow and insidious. If the disease progresses slowly, symptoms of disease may not appear for many months, but the liver may be slowly destroyed. A normal liver has a lot of excess capacity. By the time signs appear in some slow moving diseases, 3/4 of the liver may already have been destroyed. On the other hand, many liver problems are mild or short lived, and the liver is very good at healing and regenerating itself once the cause of the disease is under control (Tessier et al., 2002).

Liver disease (also called hepatic disease) is a broad term describing any single number of diseases affecting the liver. Many are accompanied by jaundice caused by increased levels of bilirubin in the system. The bilirubin results from the breakup of the hemoglobin of dead red blood cells; normally, the liver removes bilirubin from the blood and excretes it through bile. Some of the liver diseases includes; hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, cirrhosis, cystic Fibrosis, cancer, wilson’s disease, gilbert’s disease and glycogen storage disease type II (Ryder, and Beckingham, 2001).

Hepatitis is an inflammation of the liver characterized by the presence of inflammatory cells in the tissue of the organ. The condition can be self-limiting (healing on its own) or can progress to fibrosis (scarring) and cirrhosis. Hepatitis may occur with limited or no symptoms, but often leads to jaundice, anorexia (poor appetite) and malaise (Armstrong
et al., 2000). Hepatitis is acute when it lasts less than six months and chronic when it persists longer. A group of viruses known as the hepatitis viruses (hepatitis A,B,C and E) cause most cases of hepatitis worldwide, but it can also be due to toxins (notably alcohol, certain medications, some industrial organic solvent and plants), other infections and autoimmune diseases (Marc et al., 2009).

Research established a firm connection between heavy alcohol consumption and liver disease. Studies show that the amount of alcohol consumed and the duration of that consumption are closely associated with cirrhosis. Often, as people continue to drink heavily, they progress from fatty liver to hepatitis to cirrhosis. The disorders can also occur together, however, and liver biopsies can show signs of all three in some people (Mandayam et al., 2004).

Non alcoholic fatty liver disease (NAFLD) refers to a group of conditions where there is accumulation of excess fat in the liver of people who drink little or no alcohol. This is a chronic liver disease that affects a high proportion of the world’s population. Insulin resistance and oxidative stress play a critical role in the pathogenesis. The clinical implications of NAFLD are derived mostly from its common occurrence in the general population as well as its potential to progress to cirrhosis and liver failure (Paul and Keith, 2002).

Cirrhosis is a potentially life-threatening condition of the liver that refers to scarring of the liver which results in abnormal liver function as a consequence of chronic (long-term)
liver injury. This scarring replaces healthy tissue and prevents the liver from working normally. Cirrhosis usually develops after years of liver inflammation. When chronic diseases cause the liver to become permanently injured and scarred, the condition is called Cirrhosis. Cirrhosis harms the structure of the liver and blocks the flow of blood. The loss of normal liver tissue slows the processing of nutrients, hormones, drugs, and toxins by the liver. Also, the production of proteins and other substances made by the liver is suppressed (Rockey, 2005).

Evidence of chronic liver disease is found in 25% of patients with cystic fibrosis (CF) and is the cause of liver decompensation in 2–3%. Studies suggest that approximately 20–25% of CF patients will develop liver disease but only 6–8% of these will have established cirrhosis, majority of which will present in the first 20 years of life. The marked variation in the presence and severity of disease may be due to modifier genes (Corbett et al., 2004). Most cases are detected on routine screening and only a small proportion present with variceal bleeding, ascites or persistent jaundice. Abnormalities of liver function tests have a low sensitivity and specificity and the presence of established cirrhosis will be diagnosed on imaging (Lamireau et al., 2004).

Liver cancer can refer to a variety of malignant diseases that are either primary or secondary to the liver. Primary liver cancers, which originate in the liver, include hepatocellular carcinoma (HCC), cholangiocarcinoma, hepatoblastoma, fibrolamellar carcinoma, angiosarcoma, and several other rarer forms. The liver is also a frequent site
for secondary tumors that have metastasized, most frequently from colorectal, breast, or gastric cancers (Llovet et al., 2005).

Wilson's disease is an autosomal-recessive disorder caused by mutation in the ATP7B gene, with resultant impairment of biliary excretion of copper. Subsequent copper accumulation, first in the liver but ultimately in the brain and other tissues, produces classical clinical manifestations that may include hepatic, neurological, psychiatric, ophthalmological, and other derangements (Walshe, 2000). A prevalence rate of 30 cases per million (or one per 30,000) and a birth incidence rate of one per 30,000 to 40,000 are often quoted (Olivarez et al., 2001).

Gilbert's syndrome is a condition where the liver does not process bilirubin very well. It is sometimes called Gilbert's disease although it does not cause 'disease' as such. The liver itself is normal and the condition is usually harmless. The condition is named after the doctor who first described it in 1901. An enzyme in liver cells called uridine-diphosphate glucuronosyltransferase (UGT) helps the liver cells to process the bilirubin. People with Gilbert's syndrome have a reduced level of this enzyme and so a backlog of bilirubin can build up in the bloodstream. (Bancrof et al., 1998).

Glycogen storage disease type II (also called Pompe disease or acid maltase deficiency) is an autosomal recessive metabolic disorder which damages muscle and nerve cells throughout the body. It is caused by an accumulation of glycogen in the lysosome due to deficiency of the lysosomal acid alpha-glucosidase enzyme. It is the only glycogen
storage disease with a defect in lysosomal metabolism, and the first glycogen storage
disease to be identified, in 1932. The build-up of glycogen causes progressive muscle
weakness (myopathy) throughout the body and affects various body tissues, particularly
in the heart, skeletal muscles, liver and nervous system (Chien et al., 2009).

2.4.1 Liver function tests

Liver function tests (LFTs or LFs) are groups of clinical biochemistry laboratory blood
assays designed to give information about the state of a patient’s liver. Most liver diseases
cause only mild symptoms initially, but it is vital that these diseases be detected early.
Hepatic (liver) involvement in some diseases can be of crucial importance. Some tests are
associated with functionality (e.g., albumin); some with cellular integrity (e.g.,
transaminase) and some with conditions linked to the biliary tract (gamma-glutamyl
transferase and alkaline phosphatase). A complete liver function tests profile includes:-
total protein, albumin, alkaline phosphatase, alanine transaminase, aspartate
transaminase, gamma-glutamyltransferase, total bilirubin and direct bilirubin (Shivaraj,
et.al., 2009).

Serum total protein, also called plasma total protein or total protein, is a biochemical test
for measuring the total amount of protein in blood plasma or serum. Protein in the plasma
is made up of albumin and globulin. The globulin in turn is made up of α1, α2, β, and γ
globulins. All plasma proteins are high molecular weight polypeptides. Simple proteins
contain only amino acids, whilst complex proteins contain additional non amino acids
materials such as heme, vitamins derivatives, lipids or carbohydrates. Protein plays a
central role in cell function eg as enzyme and cell structure (Preejith, et al., 2003). Most proteins are synthesized in the liver [hepatocytes], those of the complement system are synthesized both in the liver and macrophages. Immunoglobulins are the exception and are derived only from the B cells of the immune systems. The main functions of the plasma proteins includes; inflammatory response and control of infection, transportation of hormones, vitamins, lipids, bilirubin, calcium trace metals and some drugs, control of extra-cellular fluid distribution. (Lovrien and Matulis, 1995).

Albumin with a molecular weight of 65000 daltons is synthesized in the liver, and can be measured cheaply and easily. It is the main constituent of total protein; the remaining fraction is called globulin (including the immunoglobulins). It has a normal plasma half life of about 20 days. Its major function is the transportation and storage of wide variety of low molecular weight substances such as cortisol, sex hormones, calcium and host of drugs. Low albumin level (hypoalbuminaemia) is a hallmark of protein malnutrition, malabsorption, parasitism, malignancy or the “tea and toast” syndrome of an elderly person living alone (Suzuki et al., 2006).

Alkaline phosphatase is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is an enzyme found throughout the body. Like all enzymes, it is needed in small amounts, to trigger specific chemical reactions. When it is present in large amounts, it may signify
bone or liver disease or a tumour. Medical testing of alkaline phosphatase is concerned with the enzyme that is found in the liver, bone, placenta, and intestine (Schiele et al., 1998).

ALT is a cytoplasmic enzyme found in high concentration in the liver and to a lesser extent in skeletal muscles, kidney and heart. ALT is a member of transaminase family of enzymes, it acts in the transfer of amino groups in various cell reactions. A rise in plasma ALT activities is an indicator of damage to cytoplasmic membrane in the cell. Liver cell contain more ALT and AST and in most conditions damage to the cytoplasmic and mitochondria membranes leads to a relatively greater increase in plasma AST activity than that of ALT. However ALT is confined to the cytoplasm and its concentration is greater than that of AST (Dufour et al., 2000).

Aspartate transaminase also called aspartate aminotransferase (AST) is similar to ALT in that it is another enzyme associated with liver parenchymal cells. AST is a member of transaminase family of enzymes, it acts in the transfer of amino groups in various cell reactions It is a cytoplasmic enzyme like ALT found in the liver, heart, skeletal muscles, kidney, pancreas, erythrocytes, lungs and brain tissues (Dufour et al., 2000). When disease or injury affects these tissues, the cells are destroyed and AST is released into the bloodstream. The amount of AST is directly related to the number of cells affected by the disease or injury, but the level of elevation depends on the length of time the blood is tested after the injury (Rochling, 2001).
GGT enzyme transfers gamma-glutamyl functional groups to an acceptor that may be an amino acid, a peptide or water (forming glutamate) across the cellular membrane. It is found in many tissues, including the kidneys, bile duct, pancreas, spleen, heart, brain, and seminal vesicles the most notable one being the liver, and has significance in medicine as a diagnostic marker. Elevated serum GGT activity can be found in diseases of the liver, biliary system, and pancreas. GGT is elevated by large quantities of alcohol ingestion. Isolated elevation or disproportionate elevation compared to other liver enzymes (such as ALP or ALT) may indicate alcohol abuse or alcoholic liver disease. It may indicate excess alcohol consumption up to 3 or 4 weeks prior to the test (Ruttmann et al., 2005).

Bilirubin is a breakdown product of heme (a part of hemoglobin in red blood cells). The liver is responsible for clearing the blood of bilirubin. Bilirubin is taken up into hepatocytes, conjugated (modified to make it water-soluble), and secreted into the bile, which is excreted into the intestine. Increased total bilirubin causes jaundice. Jaundice (also known as icterus) is a yellowish pigmentation of the skin, the conjunctival membranes over the sclerae (whites of the eyes), and other mucous membranes caused by hyperbilirubinemia (increased levels of bilirubin in the blood). For the diagnostic purpose bilirubin is divided into three ie total bilirubin (indirect + direct bilirubin), unconjugated (indirect) bilirubin and conjugated (direct) bilirubin (Baranano et al., 2002).

2.5 Phosphorus metabolism and related disorders
Phosphorus is an abundant element in the body. At least 85% is found in the bone whilst the other 15% is found mostly combined with lipids proteins, carbohydrates and other
organic substances. Most phosphorus in the extracellular fluid is inorganic. Major organs involved in phosphorus homeostasis are small intestines, kidneys and the skeleton, which acts as a storage reservoir. Phosphorus is present virtually in all foods. Consequently dietary deficiencies do not occur (Ferrari et al., 2005). Pathological conditions related to phosphorus are hypophosphotaemia (reduced phosphorus levels) and hyperphosphotaemia (elevated phosphorus levels). Hypophosphotaemia is a common condition in critically ill patients and its aetiology multifactorial. Phosphate plays an important role in the glycolysis and the production of high energy compounds such as Adenosine tri-phosphate (ATP). Evidence of its depletion in critically ill patients may manifest itself as impairment in respiratory, cardiovascular, neuromuscular and haematological function. Osteomalacia (adults) and rickets (children) are caused by an inadequate supply of vitamin D leading to inadequate mineralisation of bone matrix are directly related to hypophosphotaemia. Hyperphosphatemia is an electrolyte disturbance in which there is an abnormally elevated level of phosphate in the blood. Often, calcium levels are lowered (hypocalcemia) due to precipitation of phosphate with the calcium in tissues. Chronic renal failure is a cause of hyperphosphotaemia whereby kidneys are not working well (French and Bellomo, 2004).

2.6 Calcium metabolism and related disorders

Calcium is a mineral that helps build strong bones and teeth. Calcium and phosphorus in the mineral phase of the hard tissue ie bone and teeth co-exist in a relatively fixed proportions. The three main regulators of calcium and phosphorus homeostasis are parathyroid hormone, vitamin D and calcitonin each exert independent effects on both
ions. Intracellularly, calcium is the prime inorganic messenger for regulation of cell functions. Calcium is needed throughout an individual’s life and the amount required changes over time. A lot of calcium is required during childhood years to build strong bone, a bit less during the middle years to keep bones strong, and much later in life to prevent bone loss (Henry and Shapiro, 2003).

Pathological conditions associated with calcium levels in blood are hypercalcaemia and hypocalcaemia. These are determined in relation to the reference ranges of calcium in a particular population. Hypercalcemia is a condition of elevated levels of calcium in plasma and it is a common metabolic abnormality frequently related to primary hyperparathyroidism and cancer. Hypocalcemia is a common condition characterized by a net loss of calcium from extracellular fluid in greater quantities than can be replaced by the intestine or bone. Hypocalcemia is caused by a number of clinical entities eg a parathyroid hormone [PTH] deficiency/malfunction, a Vitamin D deficiency, or unusually high magnesium levels hypermagnesaemia, or low magnesium levels hypomagnesaemia (Holick and Garabedian, 2006).

2.7 Magnesium metabolism and related disorders

Magnesium is one of the major intracellular cation. It is an essential factor in many important enzymatic reactions, either as an integral part of a metalloenzyme or as an activator. The best defined manifestation of magnesium deficiency is impairment of neuromuscular function e.g., hyperirritability, tetany, convulsions and electrocardiographic changes. Conditions associated with hypomagnesaemia (reduced
magnesium blood levels) include chronic alcoholism, childhood malnutrition, lactation, malabsorption, acute pancreatitis, hypoparathyroidism, aldosteronism and prolonged intravenous feeding (Lu and Nightingale, 2000). Increased levels of magnesium (hypermagnesaemia) have been observed in dehydration, severe diabetic acidosis and Addison's disease. Conditions that interfere with glomerular filtration e.g., renal failure, result in retention of magnesium and hence elevation of plasma levels (Roffi et al., 1994).

2.8 Uric acid metabolism and related disorders

Uric Acid is the end product of purine metabolism in primates including man. It is formed from Xanthine by the action of Xanthine Oxidase. In most other mammals it further broken down to a soluble compound, allantoin, and it is because of poor solubility of uric Acid that man is more prone to clinical guot and kidney damage. Most of the uric acid formation occurs in the liver, which has a high activity of Xanthine Oxidase, as does the intestinal mucosa. An average adult excretes approximately 0.4 – 0.8g of uric acid in urine every 24 hours. Urinary excretion is slightly lower in males than in females, and this may contribute to the higher incidence of hyperuricaemia in men (Vitart et al., 2008). Hyperuricaemia is much more frequent and clinically more significant than hypouricaemia. Renal failure, Ketoacidosis, lactate excess and the use of diuretics will cause hyperuricaemia (Sachs et al., 2009). Gout is a disorder of purine metabolism or renal excretion of uric acid characteried by (1) hyperuricaemia (2) precipitation of monosodium urate as deposits throughout the body except for the central nervous system, but with a special predilection for joint, bone and subcutaneous tissue recurrent clinical attacks of arthritis and nephropathy (Weaver, 2008). Gout affects around 1–2% of the
Western population at some point in their lifetimes, and is becoming more common (Schlesinger, 2010).

2.9 Lipid profile

For the diagnosis and management of lipids pathological disorders, cholesterol and triglycerides forms the base of investigation. Cholesterol is a waxy steroid metabolite found in the cell membranes and transported in the blood plasma of all animals. It is an essential structural component of mammalian cell membranes, where it is required to establish proper membrane permeability and fluidity. In addition, cholesterol is an important component for the manufacture of bile acids, steroid hormones, and Vitamin D (Cleeman and Lenfant, 1998). Since cholesterol it is insoluble in body water, it is transported within lipoproteins. In addition to providing a soluble means for transporting cholesterol through the blood, lipoproteins have cell-targeting signals that direct the lipids they carry to certain tissues. For this reason, there are several types of lipoproteins within blood identified in order of increasing density: chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). The more cholesterol and less protein a lipoprotein has the less dense it is. Among these five types of lipoproteins, LDL and HDL are the most abundant and mostly considered in clinical practice. The cholesterol within all the various lipoproteins is identical. However, the different lipoproteins contain apolipoproteins, which serve as ligands for specific receptors on cell membranes. In this way, the lipoprotein particles are molecular addresses that determine the start- and endpoints for cholesterol transport (Lewis and Rader, 2005).
LDL cholesterol typically makes up 60–70 percent of the total plasma cholesterol. The main function of LDL is to transport cholesterol from the liver to tissues that incorporate it into cell membranes. High levels of LDL cholesterol as seen in hypercholesterolemia have been related to be the main cause of atherosclerosis (condition caused by the deposition of cholesterol in the blood vessels thereby forming atherosclerotic plaque). Studies have shown a strong correlation between elevated blood levels of LDL cholesterol and atherosclerosis (Anthony, 2005).

HDL cholesterol normally makes up 20–30 percent of the total serum cholesterol. HDL carries old cholesterol that has been discarded by cells back to the liver for recycling or excretion. HDL-cholesterol levels are inversely correlated with risk for CHD (Cui et al., 2001). Some evidence indicates that HDL protects against the development of atherosclerosis, although a low HDL level often reflects the presence of other atherogenic factors such as overweight and obesity, physical inactivity, cigarette smoking, type 2 diabetes, certain drugs (beta-blockers, anabolic steroids) and genetic factors. Epidemiological studies have shown that high concentrations of HDL have protective value against cardiovascular diseases such as ischemic stroke and myocardial infarction (Hausenloy and Yellon, 2008).

Triglyceride is an ester derived from glycerol and three fatty acids. Most natural lipids, from either animal or plant sources, are triglycerides. These lipids, the triglycerides, occur in body cells as oily droplets. They circulate in the water-based blood serum encased in a covering of water-soluble protein. In the human body, high levels of
triglycerides in the bloodstream have been linked to atherosclerosis (hardening of the arteries), and, by extension, the risk of heart disease and stroke. However, the relative negative impact of raised levels of triglycerides compared to that of LDL:HDL ratios is as yet unknown. The risk can be partly accounted for by a strong inverse relationship between triglyceride level and HDL-cholesterol level (Holvoet et al., 2003).

2.10 Fertility hormonal profile

Fertility hormonal profile analysis plays an important role in the diagnosis and management of various pathological disorders that contribute to infertility in an individual. Infertility is defined as the absence of pregnancy following 12 months of unprotected intercourse. Infertility may be caused by ovulatory dysfunction, blocked fallopian tubes, male factor infertility or unexplained causes. Ovulatory dysfunction can be caused by hypothalamic causes, endocrinopathies (hyperprolactinemia, thyroid dysfunction) or ovarian causes (polycystic ovarian syndrome, ovarian failure). Studies have shown that in about one in five infertile couples the problem is solely on the infertility in the male partner (Brugh and Lipshultz, 2004). Infertility can be primary, in women who have never conceived, or secondary, in women who have previously conceived. To investigate primary and secondary infertility that affect both male and female the following diagnostic parameters are carried out: testosterone (TESTO), prolactin (PRL), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol 2 (EII) and progesterone (PRG).
Testosterone, a steroid hormone derived from cholesterol, occurs more abundantly in circulation among men, than women. Testosterone is synthesized in the testes among males, the ovaries among females, and possibly the adrenal glands in both sexes, though the physiological mechanism of testosterone synthesis and excretion in women is not completely understood. On average, an adult human male body produces about ten times more testosterone than an adult human female body, but females are more sensitive to the hormone. In females testosterone increases the libido which is expressed by clitoral engorgement. The levels are increased at mid-cycle where it plays a great role during ovulation (Davis and Tran, 2001). Adult testosterone effects are more clearly demonstrable in males than in females, but are likely important to both sexes. Some of these effects may decline as testosterone levels decrease in the later decades of adult life. During the aging process, testosterone levels diminish gradually in both sexes and without an abrupt decline during menopause (Lobo, 2001). In both sexes, decline in testosterone levels leads to bone density loss and decline in muscle mass, lower coital frequency and loss of sexual desire (Wilson, 2001).

Prolactin is a protein hormone produced in the anterior pituitary by the pituitary mammatropic cells. Its secretion is controlled by an inhibitory influence from the hypothalamus that is thought to be dopamine. Prolactin has many effects including regulating lactation and stimulating proliferation of oligodendrocyte precursor cells. It stimulates the mammary glands to produce milk (lactation). Increased serum concentrations of prolactin during pregnancy cause enlargement of the mammary glands of the breasts and prepare the production of milk. However, the high levels of
progesterone during pregnancy suppress the production of milk. Milk production normally starts when the levels of progesterone fall by the end of pregnancy and a suckling stimulus is present. Prolactin provides the body with sexual gratification after sexual acts. The hormone counteracts the effect of dopamine, which is responsible for sexual arousal. Unusually high amounts are suspected to be responsible for impotence and loss of libido (see hyperprolactinemia symptoms) (Gerlo et al., 2006).

Follicle-stimulating hormone is a hormone found in humans and other animals. It is synthesized and secreted by gonadotrophs of the anterior pituitary gland. FSH regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and Luteinizing hormone (LH) act synergistically in reproduction. In males, FSH stimulates maturation of seminiferous tubules and spermatogenesis. In females, FSH initiates follicular growth, specifically affecting granulosa cells (Fowler et al., 2003).

Luteinizing hormone is produced by the anterior pituitary gland. Its structure is similar to that of the other glycoprotein hormones, follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (HCG). In females, an acute rise of LH called the LH surge triggers ovulation and development of the corpus luteum. In males, where LH had also been called interstitial cell-stimulating hormone (ICSH), it stimulates Leydig cell production of testosterone. (Pincus et al., 1997)

Estradiol is the predominant sex hormone present in females. It is also present in males, being produced as an active metabolic product of testosterone. It represents the major estrogen in humans. Estradiol has not only a critical impact on reproductive and sexual
functioning, but also affects other organs including the bones. In plasma, estradiol is largely bound to sex hormone-binding globulin, also to albumin. In female, estradiol acts as a growth hormone for tissue of the reproductive organs, supporting the lining of the vagina, the cervical glands, the endometrium, and the lining of the fallopian tubes. The development of secondary sex characteristics in women is driven by estrogens, to be specific, estradiol. Thus, estradiol enhances breast development, and is responsible for changes in the body shape, affecting bones, joints, fat. The effect of estradiol (and estrogens) upon male reproduction is complex. Estradiol is produced in the Sertoli cells of the testes. There is evidence that estradiol functions to prevent apoptosis of male sperm cells. Several studies have noted that sperm counts have been declining in many parts of the world and it has been postulated that this may be related to estrogen exposure in the environment. Suppression of estradiol production in a subpopulation of subfertile men may improve the semen analysis (Raman and Schlegel, 2002).

Progesterone also known as P4 (pregn-4-ene-3,20-dione) is a C-21 steroid hormone involved in the female menstrual cycle, pregnancy (supports gestation) and embryogenesis of humans and other species. Progesterone is produced in the ovaries (to be specific, after ovulation in the corpus luteum), the adrenal glands (near the kidney), and, during pregnancy, in the placenta. In women, progesterone levels are relatively low during the preovulatory phase of the menstrual cycle, rise after ovulation, and are elevated during the luteal phase. Progesterone is sometimes called the "hormone of pregnancy", and it has many roles relating to the development of the fetus. If pregnancy does not occur, progesterone levels will decrease, leading, in the human, to menstruation
During implantation and gestation, progesterone appears to decrease the maternal immune response to allow for the acceptance of the pregnancy. Progesterone decreases contractility of the uterine smooth muscle. In addition, progesterone inhibits lactation during pregnancy. The fall in progesterone levels following delivery is one of the triggers for milk production. A drop in progesterone levels is possibly one step that facilitates the onset of labor. Progesterone levels are relatively low in children and postmenopausal women. Adult males have levels similar to those in women during the follicular phase of the menstrual cycle. Since most progesterone in males is created during testicular production of testosterone, and most in females by the ovaries, the shutting down (whether by natural or chemical means), or removal, of those inevitably causes a considerable reduction in progesterone levels (Dennerstein et al., 2003).

2.11 Cardiac diseases

The following are compounding risk factors that are associated with cardiovascular diseases. These risk factors include those that cannot be changed, those that can be changed and those that are thought to be contributing to heart disease. Increasing age is a factor that can not be changed. Heart diseases tend to develop gradually over the course of one's life. Most of the consequences of heart disease are evident in old ages eg approximately 80% of people who die from heart attack are 65 years and above. Male gender is another factor that can not be changed. Men are prone to heart disease than women. It is thought that women are somewhat more protected from heart disease than men because of their natural production of the hormone estrogen during their fertile years.
years. Heredity is known to be a risk factor that can be a cause of cardiovascular disease and cannot be changed. Children born in families with history of heart disease are said to have a genetic predisposition to develop heart disease as they grow and develop throughout their lives (Gouva et al., 2004).

There are four cardiovascular risk factors that are influenced, in large part by our lifestyle choices. These risk factors includes:- Cigarette or tobacco consumption, physical inactivity, high blood lipid levels, and high blood pressure. Other risk factors that contribute to heart disease are diabetes, obesity and individual response to stress. Other cardiovascular complications are: coronary heart disease, hypertension, stroke, congenital heart disease and rheumatic heart disease (Wingo et al., 2000).

2.11.1 Cardiac function tests

To investigate the above mention pathological complications of the cardiac system, the following parameters constitutes cardiac profile are analyzed in the clinical laboratory:

Creatine kinase or creatine phosphokinase, creatine kinase isoenzymes ie CK BB (CK-1), CK MB (CK-2) and CK MM (CK-3), lactate dehydrogenase, aspartate aminotransferase, hydroxybutyrate dehydrogenase, myoglobin and troponin.

Creatine kinase also known as creatine phosphokinase is an enzyme expressed by various tissues and cell types. CK catalyses the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). Clinically, creatine kinase is assayed in blood tests as a marker of myocardial infarction.
"cytosolic" CK enzymes consist of two subunits, which can be either \( B \) (brain type) or \( M \) (muscle type). CK has three different isoenzymes; CK-MM, CK-BB and CK-MB (Schlattner et al., 2006). Elevation of CK is an indication of damage to muscle. It is therefore indicative of injury, rhabdomyolysis, myocardial infarction, myositis and myocarditis. Troponin measurement has largely replaced this in many hospitals, although some centers still rely on CK-MB (Wallimann et al., 1994).

Lactate dehydrogenase is an enzyme present in a wide variety of organisms, including plants and animals. Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD\(^+\). It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased. In medicine, LDH is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis (Butt et al., 2002).

**2.12 Pancreas function and tests profile**

Pancreas is a gland organ in the digestive and endocrine system of human. The part of the pancreas with endocrine function is made up of approximately a million cell clusters called islets of Langerhans. Four main cell types exist in the islets. They are relatively
difficult to distinguish using standard staining techniques, but they can be classified by their secretion: $\alpha$ cells secrete glucagon (increase glucose in blood), $\beta$ cells secrete insulin (decrease glucose in blood), $\delta$ cells secrete somatostatin (regulates/stops $\alpha$ and $\beta$ cells), and PP cells secrete pancreatic polypeptide. The pancreas as an exocrine gland helps out the digestive system. It secretes pancreatic juice that contains digestive enzymes that pass to the small intestine. These enzymes help to further break down the carbohydrates, proteins, and lipids (fats) in the chime. Alpha-Amylase is one of the diagnostic parameter performed in the investigation of pancreatic disorders (Todoriv et al., 2006). Amylase is present in a number of organs and tissues. The greatest concentration is present in the pancreas, where the enzyme is synthesized by the acinar cells and the secreted into the intestinal tract by way of the pancreatic-duct system. The salivary glands also secrete a potent amylase to initiate hydrolysis of starches while the food is still in the mouth and oesophagus. Diseases resulting in elevation of plasma alpha-amylase include: acute pancreatitis, parotitis, alcoholism, renal insufficiency and viral diseases such as hepatitis, AIDS and abdominal typhoid (Perry et al., 2007).

2.13 Thyroid gland hormones and disorders

The major thyroid hormone secreted by the thyroid gland is thyroxine, also called T4 because it contains four iodine atoms. To exert its effects, T4 is converted to triiodothyronine (T3) by the removal of an iodine atom. This occurs mainly in the liver and in certain tissues where T3 acts, such as in the brain. The amount of T4 produced by the thyroid gland is controlled by another hormone, which is made in the pituitary gland located at the base of the brain, called thyroid stimulating hormone (abbreviated TSH).
The amount of TSH that the pituitary sends into the bloodstream depends on the amount of T4 that the pituitary senses. If the pituitary senses very little T4, then it produces more TSH to stimulate the thyroid gland to produce more T4. Once the T4 in the bloodstream goes above a certain level, the pituitary’s production of TSH is shut off (Warren et al., 2004).

Thyroid hormones circulate in the blood in two forms: 1) bound to proteins that prevent them from entering the various tissues that need the hormones and 2) free T4, which does enter the various target tissues to exert its effects. The free fraction is the most important to determine how the thyroid is functioning, and tests to measure this are called the Free T4 (FT4) and free T3 (FT3). Disorders of the thyroid gland fall into the following categories: hyperthyroidism, hypothyroidism, initial hyperthyroidism followed by hypothyroidism, cancers and non-cancerous nodules (Laurberg et al., 1998).

Hyperthyroidism, or overactive thyroid, is the overproduction of the thyroid hormones T3 and T4, and is most commonly caused by the development of Graves' disease, an autoimmune disease in which antibodies are produced which stimulate the thyroid to secrete excessive quantities of thyroid hormones. The disease can result in the formation of a toxic goiter as a result of thyroid growth in response to a lack of negative feedback mechanisms. Another type of hyperthyroidism is characterized by one or more nodules or lumps in the thyroid that may gradually grow and increase their activity so that the total output of thyroid hormone into the blood is greater than normal. This condition is known as toxic nodular or multinodular goiter (Krohn et al., 2000).
Hypothyroidism is the underproduction of the thyroid hormones $T_3$ and $T_4$. Hypothyroid disorders may occur as a result of congenital thyroid abnormalities (see congenital hypothyroidism), autoimmune disorders such as Hashimoto's thyroiditis, iodine deficiency, especially in poorer countries, or the removal of the thyroid following surgery to treat severe hyperthyroidism (Sherman, 2001).

Initial hyperthyroidism followed by hypothyroidism is the overproduction of $T_3$ and $T_4$ followed by the underproduction of $T_3$ and $T_4$. There are two types: Hashimoto's thyroiditis and postpartum thyroiditis. Hashimoto's thyroiditis is an autoimmune disorder whereby the body's own immune system reacts with the thyroid tissues. At the beginning, the gland is overactive, and then becomes underactive as the gland is destroyed resulting in too little thyroid hormone production or hypothyroidism (Rose et al., 2002).

Cancers do occur in the thyroid gland and are more common in females occurring in the third, fourth and fifth decade of life. In most cases, the thyroid cancer presents as a painless mass in the neck. It is very unusual for the thyroid cancers to present with symptoms, unless it has been neglected. Diagnosis is made using a needle biopsy and various radiological studies (Muhammad et al., 2001).

Studies show that the prevalence of thyroid dysfunction and thyroid disease is higher in patients with DM than in the general population. Thus, screening for thyroid disorders in individuals with diabetes might be recommended. However, there is no consensus as to best markers for this screening. Thyroid autoantibodies are more frequent in patients with
DM than in the general population. Higher prevalence rates have been reported in Type 1 DM than in Type 2 DM (Warren et al., 2004).

Breast cancer is a hormone-dependent neoplasm. Conflicting results regarding the clinical correlation between breast cancer and thyroid diseases have been reported in the literature. Studies have shown that thyroid diseases are common among women with breast cancer, whereas other reports have not confirmed such an association of breast cancer with thyroid diseases. Almost every form of thyroid disease, including nodular hyperplasia, hyperthyroidism and thyroid cancer, has been identified in association with breast cancer. These findings have led to the investigation of the relationship between breast cancer and autoimmune thyroid diseases (AITDs) (Gogas et al., 2001).

2.14 Cancer

Cancer (malignant neoplasm) is a class of diseases in which a group of cells display uncontrolled growth, invasion that intrudes upon and destroys adjacent tissues, and sometimes metastasis, or spreading to other locations in the body via lymph or blood. These three malignant properties of cancers differentiate them from benign tumors, which do not invade or metastasize (Anand et al., 2008). Researchers divide the causes of cancer into two groups: those with an environmental cause and those with a hereditary genetic cause. Common environmental factors leading to cancer include: tobacco, diet and obesity, infections, radiation, lack of physical activity, and environmental pollutants. These environmental factors cause or enhance abnormalities in the genetic material of cells (Gorlov et al., 2007). Cell reproduction is an extremely complex process that is
normally tightly regulated by several classes of genes, including oncogenes and tumor suppressor genes. Hereditary or acquired abnormalities in these regulatory genes can lead to the development of cancer. A small percentage of cancers, approximately five to ten percent, are entirely hereditary (Paola et al., 2002).

The presence of cancer can be suspected on the basis of symptoms, or findings on radiology. Definitive diagnosis of cancer, however, requires the microscopic examination of a biopsy specimen. Most cancers can be treated. Possible treatments include chemotherapy, radiotherapy and surgery. The prognosis is influenced by the type of cancer and the extent of disease. While cancer can affect people of all ages, and a few types of cancer are more common in children, the overall risk of developing cancer increases with age. Cancer is a leading cause of death. In 2007 cancer caused about 13% of all human deaths worldwide (7.9 million), and the number of cases is rising as more people live to old age (WHO, 2011). There are lots of different kinds of cancers. Some of the most common include breast cancer, brain cancer, leukemia, testicular cancer, mesothelioma, and lung cancer (Desai et al., 2001).

2.14.1 Tumour markers (Cancer Markers)

Tumor markers are antigens produced by tumor cells or by other cells within the body in response to cancer or certain benign conditions. These markers may be detected within exfoliated or distributed cells, or as circulating agents within the peripheral blood or plasma. Other surrogate biological specimens, typically bodily fluids (e.g., urine, saliva, sputum, cerebrospinal fluid, or effusions) may also carry tumor markers (Sturgeon,
2002). Tumor markers are usually constituents of healthy cells that are produced in greater abundance by cancerous cells; however, normal cells may also produce them in response to the malignancy. In addition, tumor growth may cause an obstruction and/or cellular breakdown of normal tissue either of which could lead to increased levels of an analyte that could serve as a surrogate marker of malignancy. Some of the tumor markers in highest use in clinical practice includes: cancer antigen 125 (CA 125), cancer antigen 15-3 (CA 15-3), cancer antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA) and total prostatic specific antigen (TPSA) (Tsao et al., 2006).

CA 125 is a surface antigen associated with epithelial ovarian cancer, and to date CA125 is the most sensitive marker for residual epithelial ovarian cancer. CA125 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis. CA125 is the only marker that has been accepted for clinical use in ovarian cancer (Rustin et al., 2004).

CA 15-3 is a serum cancer antigen whose principal utility is in monitoring therapy; used in the management of patients with breast cancer. It has value for evaluating patients for breast-cancer recurrence, assessing prognosis, and checking for residual disease following surgery. Cancers of the ovary, lung, and prostate may also raise CA 15-3 levels. The literature indicates elevated levels of CA 15-3 may be associated with noncancerous conditions, such as benign breast or ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis (Goldhirsch et al., 2003). Breast cancer is by far the
most common cancer affecting women worldwide with approximately one million new cases diagnosed each year (Peto et al., 2000).

CA 19-9 is a glycosylated protein produced by adenocarcinomas of the pancreas, stomach, gall-bladder, colon, ovary and lung. Levels of CA 19-9 have also been identified in patients with hepatocellular and bile duct cancer. In individuals who have pancreatic cancer, the literature indicates that higher levels of CA 19-9 tend to be associated with more advanced disease (Rosty and Goggins, 2002). CA 19-9 levels correlate well with the stage of disease and are associated with ability to successfully provide surgical intervention (Neoptolemos et al., 2004).

Carcinoembryonic antigen (CEA) is a glycoprotein involved in cell adhesion. It is normally produced during fetal development, but the production of CEA stops before birth. Therefore, it is not usually present in the blood of healthy adults, although levels are raised in heavy smokers. (CEA) is present on virtually all colorectal tumors (Strate and Syngal, 2005). Colorectal cancer (CRC) is the third most common cancer, worldwide with an estimated one million new cases and half a million deaths each year (Desch et al., 2005).

Prostate-specific antigen (PSA) is a protein produced by the cells of the prostate gland. PSA is present in small quantities in the serum of men with healthy prostates, but is often elevated in the presence of prostate cancer and in other prostate disorders. A blood test to measure PSA is considered the most effective test currently available for the early
detection of prostate cancer. Rising levels of PSA over time are associated with both localized and metastatic prostate cancer (Hugosson et al., 2004). Prostate cancer tends to develop in men over the age of fifty and although it is one of the most prevalent types of cancer in men, many never have symptoms, undergo no therapy, and eventually die of other causes. This is because cancer of the prostate is, in most cases, slow-growing, symptom-free, and since men with the condition are older they often die of causes unrelated to the prostate cancer, such as heart/circulatory disease, pneumonia, other unconnected cancers, or old age (Hsing and Chokkalingam, 2006).

2.15 Quality control assessment
The dependence of modern medicine on laboratory services has increased enormously during the last 50 years. Rapid development in this field presents a challenge for standard setting and quality control (Heuck, 1998). The advent of modern analytical instruments has prompted the need for using different methods to control the quality of analytes. In a modern clinical chemistry laboratory quality control is an important tool to maintain the high predictive values of the methods in use (Ohman, 1997). Most qualitative analytical procedures involve several operations or steps, and each operation is subject to some degree of inaccuracy or imprecision or to the possibility of a mistake. The following factors have been so far identified to affect the reliability and reproducibility of laboratory results; personnel, environment, specimens, laboratory materials, test methods, equipment’s, reading and reporting. For perfection in the analysis to be achieved, these errors should be minimised to the most practical level (Katayama, 1997). It is therefore essential to build a reliable quality control system in order to report excellent results
assured of quality and have a proper documentation of all the work carried out on the quality control materials (Krishnan et. al., 1999).

This important aspect of laboratory medicine has been neglected in most laboratories in provincial and district hospitals and this has grossly compromised the quality of results (KACP, 2000). In Kenyatta National Hospital Clinical Chemistry laboratory, internal quality control is fully practised. To ensure the establishment of quality reference values, an internal quality control material was incorporated throughout the analytical process of the current study.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site
The study was conducted in the eleven counties representing nine regions of Kenya including, Kiambu and Nyeri (Central region), Mombasa (Coast region), Meru and Machakos (Eastern region), Nairobi (Nairobi region), Garissa (North Eastern region), Kisumu (Nyanza region), Uasin Gishu (North Rift Valley region), Kajiado (South Rift Valley region) and Kakamega (Western region). The main analytical centre was the Department of Clinical Chemistry, Kenyatta National Hospital.

3.2 Study population
The target population was divided into two categories. Category 1 involved study population recruited from the nine counties for the establishment of reference ranges for routine biochemical parameters. Category 2 involved study population recruited from the Nairobi Metropolitan region for the establishment of reference ranges for special parameters which include fasting lipid profile (FLP) and fasting blood glucose (FBG), oral glucose tolerance test, creatinine clearance, tumour makers and hormones. Prior to the recruitment of the study population in both categories, sensitization exercise was performed through public lectures and advertisements in various institutions which included churches, mosques, medical colleges, universities and hospitals. All subjects who fulfilled the inclusion criteria were recruited in the study.
3.2.1 Inclusion criteria
Subjects recruited in the study were Kenyan citizens between 18-60 years of age, normotensive (systolic blood pressure of $115 \pm 15$ mmHg and diastolic blood pressure of $75 \pm 15$ mmHg), non-alcoholic, non-smokers and non-tobacco users. Subjects involved in the establishment of reference ranges of lipid profile, fasting blood glucose and oral glucose tolerance test fasted 8-10 hours prior to blood collection in the morning. A questionnaire was administered to consenting study subjects to gather some sociodemographic data (Appendix 1).

3.2.2 Exclusion criteria
Subjects whose blood specimens tested positive results for HIV, HBsAg and VDRL were excluded from the study. Also excluded from the study were subjects who were obese, hypertensive (systolic blood pressure $> 139$ mmHg and diastolic blood pressure $> 90$ mmHg), pregnant, involved in any excessive exercise, under any medication and taking any contraceptives (female subjects).

3.3 Study design
This was a random cross sectional prospective study involving 6795 healthy male and female subjects. The study was undertaken between May 2008 and November 2010.

3.3.1 Sample size
Sample size determination was based on the target population which was divided into two categories. Category 1 was based on people in each county attending the recruitment
centres which were the prayer houses with a population size of between 2000 and 4000 people. In this category a population size of 4000 people was used to determine the sample size per each region. Category 2 was based on staff and students in the health institutions with a population of between 8000 and 10,000 people. In this category a population size of 10,000 people was used to determine the sample size. The following formula and sample size determination table by Bartlett et al. 2001), was used to determine the minimum number of study subject involved in each category.

\[ n_0 = \left( t^2 \right) \left( p \right) \left( q \right) / \left( d^2 \right) \]

where \( n_0 \) = minimum sample size required

\( t = 1.96 \)

\( (p)(q) = \) estimate of variance = 0.25

\( d = \) acceptable margin error for proportion being estimated = 0.05

Since the data for the study was categorical the sample size for each category was:

Category 1 = 351 (study subjects in each county), therefore in nine regions = 3159;
Category 2 = 370

3.4 Ethical approval

This study was approved by Kenyatta National Hospital Research and Ethical Committee and the study approval reference number was P 342/11/2007 (Appendix 2)

3.5 Blood collection for routine biochemical parameters

Using a 10 mL syringe, 6 mL of venous blood was collected from those volunteers who consented. Blood collected was divided into two volumes: 4 mL was put in a plain
vacuitainer tubes and the remaining 2 mL was put in a fluoride vacuitainer tubes. Gentle swirling of the vacuitainer tubes for one minute was performed to mix the blood in the fluoride vacuitainer tubes. All the specimen tubes were labeled correctly with the subject’s name and the study number. The two specimens collected in plain vacuitainer tubes and fluoride vacuitainer tube were centrifuged using a speed of 3000 g for five minutes. Separation of serum and plasma was done using a pasteur pipette for each specimen and transferred into specific vials labelled S for serum and P for plasma and each vial had subject’s identification details. Specimens were arranged in a coolbox awaiting transportation to the main analytical center.

3.5.1 Specimen collection for fasting lipid profile and fasting blood glucose at the main analytical centre (knh)

This study involved 580 study subjects drawn from staff and students of Kenyatta National Hospital, Kenyatta University, Jomo Kenyatta University of Agriculture Science and Technology and Kenya Medical Training College. Instructions were given to the study subject to avoid any other meal after super. A 5 mL of blood was drawn from the study subject and distributed into two tubes. A 3 mL aliquot was put in a plain vacuitainer tube for lipid profile analysis and the remaining 2 mL were put in a fluoride vacuitainer tube for glucose analysis. Gentle swirling of the bottles for one minute was performed to mix the blood in the fluoride bottle. All the specimen bottles were labeled correctly with the subject’s name and the study number. The two specimens collected in plain vacuitainer tube and fluoride tube were centrifuged at 3000 g for five minutes. Separation of serum and plasma was done using a pasteur pipette for each specimen and
transferred into specific vials labelled S for serum and P for plasma and each vial had subject’s identification details. Specimens were stored at -20°C awaiting analysis.

3.5.2 Specimen collection for oral glucose tolerance test (OGTT) at the main analytical centre

Blood and urine specimens were collected from 321 volunteer study subjects who were drawn from staff and students of Kenyatta National Hospital, Kenyatta University, Jomo Kenyatta University of Agriculture Science and Technology, and Kenya Medical Training College. Instructions were given to the study subject to avoid any other meal after super. The procedure for oral glucose tolerance test involved testing the fasting blood glucose using capillary blood obtained by a finger prick. The subject was requested to submit a random urine specimen, which was used to investigate any presence of glycosuria. Only those whose urine gave negative qualitative results of glycosuria were recruited in the study. Each subject consumed a glucose solution that was prepared by dissolving 75 grams of pure glucose in 250 mL of water. A period of 5 minutes was allowed for the consumption of the glucose solution. Citric acid was provided to prevent nausea. Blood glucose level was tested by using capillary blood obtained through a finger prick after every 30 minutes for two hours and a half. Subjects were required to void urine after every one hour during the period of the procedure. The results were recorded in OGTT chart.

3.5.3 Blood collection for tumour markers and hormonal profiles

Blood was collected from 582 volunteer study subjects who were drawn from staff and students community of Kenyatta National Hospital. Kenyatta University, Jomo Kenyatta
University of Agriculture Science and Technology, and Kenya Medical Training College. Using a 5 mL syringe, 4 mL of venous blood was collected and put in a plain bottle labeled within the subject’s identification details. It was allowed to clot at room temperature for 1 hr and then centrifuged at 3000 g for five minutes. Separation of serum was done using a pasteur pipette for each specimen and transferred into specific vials labelled with subject’s identification details. Specimens were stored at -20°C awaiting analysis.

3.5.4 Collection of blood and urine for creatinine clearance analysis

A pre-prepared 2 litres urine container with 10 mL of concentrated hydrochloric acid was given to each study subject. Instructions were given to discard the first morning urine voided and thereafter to collect all the urine voided for the next 24 hours. The method of urine collection involved urinating in a clean container and then transferring the urine carefully into the 2 litres pre-prepared urine container. 4 mL of venous blood specimen was drawn from the study subject using a 5 mL syringe and put in a vacuatainer plain tube, within the urine collection period. All the specimen bottles were labelled correctly with the study subject name and the study number. Weight for the study subject was taken using a weighing balance during the submission of the 24 hr urine specimen to the analytical centre.

The following formula was used for the calculation of the creatinine clearance:

\[
\frac{(U \times V)}{(1440 \text{ min.} \times S)}
\]

Where: \( U \) = Concentration of creatinine in urine in umol/L

\( V = 24 \text{ hours urine volume in mL} \)
1440 min. = Period of urine collection in minutes

\[ S = \text{Concentration of creatinine in serum in } \mu\text{mol/L} \]

The following formula was used for the calculation of the estimated creatinine clearance (Ecrcl)

\[ \text{Ecrcl} = \frac{(140-\text{age}) \times \text{weight (kg)} \times \text{constant (male }=1.23, \text{ female }=1.04)}{\text{SCr (umol/L)}} \]

Blood and urine specimens were collected from 265 volunteer study subjects who were drawn from staff and student community of Kenyatta National Hospital, Kenyatta University, Jomo Kenyatta University of Agriculture Science and Technology, and Kenya Medical Training College.

### 3.6 Specimen analysis

#### 3.6.1 Routine biochemical parameters

The collected serum was analyzed for the following analytes: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, \( \gamma \)-glutamyl transaminase, random glucose, chloride, albumin, bicarbonates, total bilirubin, direct bilirubin, creatinine, potassium, sodium, blood urea nitrogen, total protein, uric acid, phosphorus, amylase and magnesium. The serum was used for the screening of HIV, hepatitis and syphilis. The plasma separated from the fluoride bottle was used for glucose analysis, since fluoride is an anticoagulant known for enzyme inhibition thereby stopping any process of glycolysis taking place in the blood cells. The fluoride anticoagulant does not interfere with glucose estimation reaction. 24 hour urine specimen was measured, the volume noted and a sample analyzed for creatinine levels.
3.6.2 Specimen analysis for special biochemical parameters

The serum collected was analyzed for the following analytes: total fasting cholesterol, high density lipoprotein, low density lipoprotein, fasting triglycerides, testosterone, prolactin, follicle stimulating hormone, luteinizing hormone, estradiol II, progesterone, CA 125, CA 15-3, CA 19-9, CEA and TPSA.

3.7 Equipments used for analysis

The machine used for the sample analysis was Clinical Chemistry Autoanalyzer Olympus 640 whilst Olympus 400 system ((Olympus Diagnostica GmbH, Hamburg, Germany) was used as a backup machine in case of any mechanical breakdown of the former. The Olympus is a discrete, Random Access Clinical Chemistry analyzer capable of performing a wide range of chemical tests in a single run. Mini Vidas (Biomerieux, Lyon, France) is a closed system machine which was used to analysis tumour makers and hormones. The Hitachi spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan) was used for instrument comparison and the glucometer (Accu-Chek glucose meter from Roche Diagnostics GmbH, Mannheim, Germany) for oral glucose tolerance test analysis.

3.7.1 Reagent preparation

All reagents for the auto analyzer machine were commercially prepared to fit the required volumes and concentration. Reagents used in Olympus auto analyzer machine were in specific containers referred to as “reagent cartridges”. The reagent cartridges were bar-coded for the identification by the machine. User defined chemistry programme was used
for those reagents, which were not bar corded. Reagents strips and solid phase receptacles were used in Mini Vidas machine for the analysis of tumour makers and hormones. Reagents used in the spectrophotometer were commercially prepared and the parameter analysis was performed manually. Biosensor strip was used in the glucometer for glucose analysis. Spectrophotometer used reagent kits and the analysis was carried out manually.

3.7.2 Calibration of the test
To ensure that the values recovered from the patient sample assayed were both accurate and precise, the machine performed a calibration procedure for the parameters. The purpose of the calibration procedure was to determine the relationship between measured absorbance (or in case of ion selective electrodes, voltage potential) to known concentration of these same analytes contained in calibrator solutions (such as Olympus multi-calibrator for analyzed parameters or calibration standards 1 and 2 for electrolytes). Calibration factors were installed once the relationship was achieved. Tumour makers and hormones were calibrated using specific calibrators.

3.7.3 Quality control (QC) materials
The assayed multisera normal was used for the quality control of the analytical work during the study period. The QC multisera was supplied in lyophilised form and was reconstituted as per the manufacturer’s preparation guide. For internal quality control assessment, the prepared QC multisera was analysed daily or any other time samples for the study were being analysed.
3.8 Analytical methods for routine biochemical parameters

3.8.1 Amylase (EC 3.2.1.1)

Amylase reagent was used to measure the concentration of amylase by a kinetic colour method using Olympus Autoanalyzer. In the reaction, 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG₃) substrate reacted with amylase in the serum to release 2-chloro-4-nitrophenol (CNP) from the substrate which was directly proportional to the concentration of amylase in the sample. 3 μl of sample was reacted with 300 μl of reagent and the change in absorbance was monitored at 340nm, due to reduction of NAD. This change was directly proportional to the concentration of AMY in the sample and was used to calculate and express concentration in U/L. The reaction took place at 37°C for three and half minutes.

**Principle of the reaction**

\[ \text{CNPG}_3 + \text{H}_2\text{O} \xrightarrow{\text{amylase}} \text{CNP} + \text{maltotriose} \]

3.8.2 Bicarbonate

Bicarbonate reagent was used to measure the concentration of bicarbonate in the sample by an enzymatic rate method. In the assay reaction, \( \text{HCO}_3^- \) reacts with phosphoenolpyruvate (PEP) a reaction catalyzed by Phosphoenolpyruvate Carboxylase (PEPC) to form oxaloacetate and hydrogen phosphate (\( \text{H}_2\text{PO}_4^- \)). The oxaloacetate is then reduced to malate in the presence of Malate dehydrogenase (MDH) with the concurrent oxidation of β-Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to β-Nicotinamide Adenine Dinucleotide (oxidized form) (NAD). Magnesium ions were
required in this reaction to act as a co-factor. 2 μl of sample was reacted with 200 μl of reagent and the change in absorbance was monitored at 340nm, due to reduction of NAD. This change was directly proportional to the concentration of HCO₃⁻ in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37°C for three minutes.

**Principle of the reaction**

\[
\text{Phosphoenolpyruvate} \quad \text{HCO}_3^- \xrightarrow{\text{PEPC}} \text{oxaloacetate} \quad H_2PO_4^- \\
\text{Oxaloacetate} \quad \text{NADH} \quad \text{H}^+ \xrightarrow{\text{MDH}} \text{Malate} \quad \text{NAD}^+
\]

3.8.3 Calcium

Calcium had two methods of analysis namely (i) o-Cresolphthalein (oCPC) and (ii) Arsenazo III (2,2'-[1,8-Dihydroxy-3,6- disulphonaphthylene-2,7-bisazo] bisbenzenearsonic acid) which were used in this study.

(i) **o-Cresolphthalein method**

This was an end point reaction for calcium estimation. Calcium ions reacted with o-CPC-complexone in an alkaline medium in the presence of hydroxyquinoline to form a purple coloured complexone which was directly proportional to the calcium concentration in the sample. 3μl of sample was reacted with 100 μl of reagent and the change in absorbance was monitored at 575nm. This change was directly proportional to the concentration of Calcium in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37°C for one minute.

**Principle of the reaction**

\[
\text{Ca}^{2+} \quad \text{o-CPC} \quad \xrightarrow{8\text{-Hydroxyquinoline}} \quad \text{Ca- o-CPC complex}^{2+} \text{ (purple)}
\]
(ii) **Arseazo III method**

This was an end point reaction for calcium estimation. Calcium ions reacted with Arseazo III in an acidic medium to form a purple coloured complex which was directly proportional to calcium concentration in the sample. 1μl of sample was reacted with 100 μl of reagent and the change in absorbance was monitored at 650nm. This change was directly proportional to the concentration of Calcium in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37°C for two minutes.

**Principle of the reaction**

\[
\text{Ca}^{2+} + \text{Arseazo III} \quad \text{pH} 6.5 \quad \rightarrow \quad \text{Ca}^{2+} - \text{Arseazo III complex (purple)}
\]

### 3.8.4 Creatine kinase (EC 2.7.3.2)

This was an enzymatic kinetic UV method for quantitative determination of creatine kinase. CK reversibly catalyzed the transfer of a phosphate group from creatine phosphate to adenosine diphosphate (ADP), to give creatine and adenosine triphosphate (ATP) as products. The ATP formed was used to produce glucose -6- phosphate and ADP from glucose. This reaction was catalyzed by hexokinase (HK) which required magnesium ions for maximum activity. The glucose -6-phosphate (G-6-P) was oxidized by the action of the enzyme glucose-6- phosphate dehydrogenase (G6P-DH) with simultaneous reduction of the coenzyme NADP to give NADPH and 6-phosphogluconate. 3 μl of sample was reacted with 300 μl of reagent and the change in absorbance, due to reduction of NAD was monitored at 340nm. This change was directly
proportional to the concentration of CK in the sample and was used to calculate and express concentration in U/L. The reaction took place at 37°C for three and half minutes.

**Principle of the reaction**

\[
\begin{align*}
\text{CK} + \text{ADP} & \rightarrow \text{Creatine} + \text{ATP} \\
\text{ATP} + \text{glucose} & \rightarrow \text{ADP} + \text{G-6-P} \\
\text{G-6-P} + \text{NADP}^+ & \rightarrow 6\text{-Pphosphogluconate} + \text{NADPH} + \text{H}^+
\end{align*}
\]

3.8.5 Lactate dehydrogenase (EC 1.1.1.27)

This was an enzymatic kinetic UV test for the quantitative determination of LDH. In the reaction, LDH catalyzed the oxidation of lactate to pyruvate coupled with the reduction of NAD\(^+\) to NADH. 2 μl of sample was reacted with 40 μl of reagent and the change in absorbance due to reduction of NAD was monitored at 340nm. This change was directly proportional to the concentration of LDH in the sample and was used to calculate and express concentration in U/L. The reaction took place at 37°C for three and half minutes.

**Principle of the reaction**

\[
\begin{align*}
\text{Lactate} + \text{NAD}^+ & \rightarrow \text{Pyruvate} + \text{NADH} + \text{H}^+
\end{align*}
\]

3.8.6 Magnesium

This was an end point reaction method for the determination of MG. In the reaction, MG formed a coloured complex with xylidyl blue in a strong alkaline medium. 2μl of sample was reacted with 200 μl of reagent and the change in absorbance was monitored at 546nm. This change was directly proportional to the concentration of MG in the sample and was used to calculate and express concentration in mmol/L. The reaction took place...
at 37°C for two minutes. Purple colour produced was directly proportional to the concentration of MG in the sample.

**Principle of the reaction**

\[
\text{MG}^{2+} + \text{Xylidyl blue} \quad \xrightarrow{\text{pH}=11.4} \quad \text{Purple colour}
\]

### 3.8.7 Glucose

**(i) Hexokinase method**

Glucose reagent was used to measure the concentration of glucose by a timed end point reaction method. In the reaction, hexokinase catalysed the transfer of phosphate group from adenosine triphosphate (ATP) to glucose to form adenosine diphosphate (ADP) and glucose-6-phosphate. The glucose-6-phosphate was then oxidized to 6-phosphogluconate with the concomitant reduction of β-nicotinamide adenine dinucleotide (NAD) to reduced β-nicotinamide adenine dinucleotide (β-NADH) by the catalytic action of glucose-6-phosphate dehydrogenase (G6PDH). 3 µl of sample was reacted with 300 µl of reagent and the change in absorbance was monitored at 340nm, due to reduction of NAD. 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°C for three and half minutes.

**Principle of the reaction**

\[
\text{Glucose} + \text{ATP} \quad \xrightarrow{\text{HK}} \quad \text{Glucose-6-phosphate} + \text{ADP}
\]

\[
\text{Glucose-6-phophatase} + \text{NAD}^+ \quad \xrightarrow{\text{G6P-DH}} \quad 6\text{- phosphogluconate} + \text{NADH} + \text{H}^+
\]

**(ii) Glucose oxidase method**
Glucose reagent was used to measure the concentration of glucose 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 ($\text{H}_2\text{O}_2$). The $\text{H}_2\text{O}_2$ formed reacted with phenol and 4-aminophenazone under the catalysis of peroxidase (POD) to form a red–violet quinoneimine which was directly proportional to the concentration of glucose in the sample. 2µl of sample was reacted with 300 µl of reagent and the change in absorbance was monitored at 500nm. 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°C for three and half minutes.

**Principle of the reaction**

\[
\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GoD}} \text{Gluconic acid} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4\text{H}_2\text{O}
\]

3.8.8 Phosphorus

Phosphorus reagent was used to measure the phosphorus concentration by a timed end point method. In the reaction, inorganic phosphorus reacted with ammonium molybdate in an acidic solution to form a coloured phosphomolybdate complex. 4µl of sample was reacted with 268 µl of reagent and the change in absorbance was monitored at 340nm. This change was directly proportional to the concentration of PHOS in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37°C for three minutes.

**Principle of the reaction**

70
3.8.9 Total protein

Total protein reagent was used to measure the concentration of total protein by a timed endpoint biuret method. In the reaction, the peptide bonds in the protein sample binds to cupric ions in an alkaline medium to form a peptide/copper complex. 6μl of sample was reacted with 300 μl of reagent and the change in absorbance was monitored at 560nm. 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 37°C for four minutes.

**Principle of the reaction**

\[
\text{Protein} + \text{Cu}^{2+} + \text{OH}^{-} \rightarrow \text{Protein-Copper complex.}
\]

3.8.10 Uric acid

Uric acid reagent was used to measure the concentration of the uric acid concentration by a timed end point 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-dinchloro-2-hydroxybenzene sulfonate (DCHBS) in a reaction catalysed by peroxidase to produce a coloured product. 4μl of sample was reacted with 100 μl of reagent and the change in absorbance was monitored at 520nm. 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 37°C for three and half minutes.

**Principle of the reaction**

\[
\begin{align*}
\text{Uric acid} &+ \text{O}_2 + \text{H}_2\text{O} & \xrightarrow{\text{Uricase}} & \text{Allantoin} + \text{H}_2\text{O}_2 + \text{CO}_2 \\
\text{H}_2\text{O}_2 &+ 4 \text{AAP} + \text{DCHBS} & \xrightarrow{\text{Peroxidase}} & \text{Quinoneimine} + \text{H}_2\text{O}.
\end{align*}
\]

3.8.11 Electrolytes (sodium, potassium and chloride)

(i) Ion selective electrode method

The Olympus system determined sodium, potassium and chloride serum levels by measuring electrolytes ion activity in solution. The measurement was made by specific ion selective electrode. 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 was made by measuring potentials developed at the face of specific ion-selective electrode.

**Principle of the reaction**

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

- Sodium E = constant + (slope) (log [Na⁺])
- Potassium E = Constant + (slope) (log [K⁺])
- Chloride E = constant + (slope) (log [Na⁺])
(ii) **Potassium estimation (Colorimetric method)**

Potassium ions in a protein–free alkaline medium react with sodium tetraphenylboron to produce a finely dispersed turbid suspension of potassium tetraphenylboron complexion. 5μl of sample was reacted with 50 μl of reagent and the change in absorbance was monitored at 578nm. This change was directly proportional to the concentration of POT in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37°C for four minutes.

(iii) **Sodium estimation (Colorimetric method)**

This technique involved the use of a single to use liquid reagent that contained a sodium selective macrocyclic compound known as a chromogenic cryptahemispherand. This chromogenic cryptahemispherand compound was composed of highly selective sodium ionophore that was covalently linked to an ionizable chromophoric group. At the constant pH (alkalinic) maintained by the buffered reagent, this chromophoric group existed in an equilibrium of its protonated and unprotonated forms which had distinctly different absorption spectra. Complexation of sodium ion by the chromogenic ionophore induces a change in the basicity of the chromophoric group and hence a change in the ration of its two spectrally different forms. 2μl of sample was reacted with 130 μl of reagent and the change in absorbance was monitored at 500nm. This change was directly proportional to the concentration of SOD in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37°C for four minutes.
3.8.12 Albumin

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 sample was reacted with 300 μl of reagent and the change in absorbance was monitored at 600nm. 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°C for one and half minutes.

** Principle of the reaction **

\[ \text{Albumin} + \text{BCG} \rightarrow \text{Albumin/BCG complex} \]

3.8.13 Blood urea nitrogen

BUN was analyzed using two methods: (i) Berthelot Reaction and (ii) Glutamate Dehydrogenase (GLUDH).

(i) Berthelot reaction

In the first step of reaction, urea was hydrolyzed with urease to form ammonia and carbon dioxide. In the second step of the reaction, ammonia was reacted with phenol and hypochlorite in alkaline medium to form a green indophenol complex which was 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 600nm. 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°C for one minute.

** Principle of the reaction **

\[ \text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_3 + \text{CO}_2 \]
NH₄⁺ + 5 NaOCl + 2 phenol → Indophenol + 5 NaCl + 5H₂O

(ii) Glutamate dehydrogenase

BUN reagent was used to measure the concentration of urea by an enzymatic rate method. In the reaction urea was hydrolyzed by urease to ammonia and carbon dioxide. Glutamate dehydrogenase (GLDH) catalysed the condensation of ammonia and α-Ketoglutarate to glutamate with the concomitant oxidation of reduced β-nicotinamide adenine dinucleotide (NADH) to oxidized β-nicotinamide adenine dinucleotide (NAD). 3 µl of sample was reacted with 300 µl of reagent and the change in absorbance was monitored at 340nm, due to NADH oxidation. 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°C for one minute.

Urea + H₂O ⇌ Urease 2NH₃ + CO₂

NH₃ + α-Ketoglutarate + NADH + H⁺ ⇌ GLDH glutamate + NAD⁺ + H₂O

3.8.14 Creatinine

The creatinine reagent was used to measure the creatinine concentration by a modified rate Jaffe method. In the reaction, creatinine combined with picrate in an alkaline solution to form a creatinine – picrate complexone. 20 µl of sample was reacted with 220 µl of reagent and the change in absorbance was monitored at 520nm. This change was directly proportional to the concentration of CREAT in the sample and was used to calculate and express concentration in µmmol/L. The reaction took place at 37°C for two minutes.
3.8.15 Alkaline phosphatase (EC 3.1.3.1)

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-nitrophenylphosphate 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 this change was directly proportional to the activity of ALP. The activity was calculated and expressed in U/L. The reaction took place at 37°C for three minutes.

Principle of the reaction

\[
\text{p-Nitrophenylphosphate} + \text{H}_2\text{O}^{\text{PH} 10.3, \text{Mg}} \rightarrow \text{p-nitrophenol} + \text{Phosphate (colorless) (yellow)}
\]

3.8.16 Alanine aminotransferase (EC 2.6.1.2)

The ALT reagent was used to measure alanine aminotransferase in the sample by an enzymatic kinetic UV rate method. 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 that was required 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 this change was directly proportional to the activity of ALT. The activity was calculated and expressed in U/L. The reaction took place at 37⁰C for three minutes.

**Principle of the reaction**

\[
\text{L-Alanine} + \alpha\text{-Ketoglutarate} \overset{\text{ALT}}{\longrightarrow} \text{Pyruvate} + \text{L-Glutamate}
\]

\[
\text{Pyruvate} + \text{NADH} + \text{H}^+ \overset{\text{LDH}}{\longrightarrow} \text{Lactate} + \text{NAD}^+
\]

### 3.8.17 Aspartate aminotransferase (EC 2.6.1.1)

AST reagent was used to measure aspartate aminotransferase activity by an enzymatic kinetic UV rate method. In the reaction aspartate aminotransferase catalysed the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) 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 this change was directly proportional to the activity of AST. The activity was calculated and expressed in U/L. The reaction took place at 37⁰C for three minutes.

**Principle of the reaction**

\[
\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \overset{\text{AST}}{\longrightarrow} \text{Oxaloacetate} + \text{L-Glutamate}
\]

\[
\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \overset{\text{MDH}}{\longrightarrow} \text{Malate} + \text{NAD}^+
\]
3.8.18 Gamma glutamyl transferase (EC 2.3.2.2)

GGT reagent was used to measure γ-glutamyl transferase activity by an enzymatic kinetic UV rate method. In the reaction, γ-glutamyl transferase catalysed 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 405nm. The activity was calculated and expressed in U/L. The reaction took place at 37°C for three minutes.

Principle of the reaction

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine \( \xrightarrow{\text{GGT}} \) L- γ-

glutamylglycylglycine + 5-amino-2-nitrobenzoate

3.8.19 Total bilirubin

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 578nm. 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°C for two minutes. Nb/ A separate sample blank were performed (set) to reduce endogenous serum interference.

Principle of the reaction

Bilirubin + DPD + caffeine \( \xrightarrow{\text{}} \) Azobilirubin
3.8.20 Direct bilirubin

A stabilized diazonium salt (3,5-dichlorophenyldiazonium 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 546nm. 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°C for two minutes.

Principle of the reaction

\[ \text{Bilirubin} + \text{DPD} \xrightarrow{H^+} \text{Azobilirubin (purple colour)} \]

3.8.21 Triglycerides

This was based on a series of coupled enzymatic reactions. Triglyceride in the sample was hydrolyzed by lipase to give glycerol and fatty acids. The glycerol was phosphorylated by ATP 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_{2}O_{2}) and dihydroxyacetone phosphate. The hydrogen peroxide formed reacts with 4- aminoantipyrine (4-AAP) (aminaphenazone) and 3,5-dichloro-2-hydroxybenzesulfonic 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°C for two minutes.
Principle of the reaction

\[
\begin{align*}
\text{TG} + 3\text{H}_2\text{O} & \xrightarrow{\text{Lipase}} \text{glycerol} + 3 \text{fatty acids} \\
\text{Glycerol} + \text{ATP} & \xrightarrow{\text{GK', Mg}^{2+}} \text{glycerol-3-phosphate} + \text{ADP} \\
\text{glycerol-3-phosphate} + \text{O}_2 & \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + \text{H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 + 4\text{AAP} + \text{DHBS} & \xrightarrow{\text{POD}} \text{quinoneimine (red) dye} + \text{HCL} + 2\text{H}_2\text{O}
\end{align*}
\]

3.8.22 Total cholesterol

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°C for two minutes.

Principle of the reaction

\[
\begin{align*}
\text{Cholesterol ester} + \text{H}_2\text{O} & \xrightarrow{\text{CHE}} \text{cholesterol} + \text{fatty acids} \\
\text{Cholesterol} + \text{O}_2 & \xrightarrow{\text{CHO}} \text{cholestene-3-one} + \text{H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 + 4\text{AAP} + \text{Phenol} & \xrightarrow{\text{POD}} \text{quinoneimine (red) dye} + 4\text{H}_2\text{O}
\end{align*}
\]

3.8.23 HDL-Cholesterol

Anti human –β-lipoprotein antibody binds to lipoproteins other than HDL (LDL, VLDL, and chylomicrons). The antigen antibody complexes formed block enzyme reactions. HDLC was quantified by the presence of an enzyme chromogen system. 2.5 µl of sample was reacted with 250 µl of reagent and the change in absorbance was monitored at 540 nm.
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°C for two minutes.

**Principle of the reaction**

LDL, VLDL and chylomicrons → anti-human-b-lipoprotein antibody → antigen-antibody complexes

\[ \text{HDLC} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{CHO and CHE} \rightarrow \text{cholestene-3-one + fatty acid + H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + 4\text{-AAP} + \text{Phenol} \rightarrow \text{POD} \rightarrow \text{Quinoneimine (red) dye + 4H}_2\text{O} \]

3.8.24 LDL – Cholesterol

A protecting agent protected LDL from enzymatic reactions. All non-LDLC lipoproteins (HDL, VLDL, CM) were broken down by reaction with cholesterol esterase (CHE) and cholesterol oxidase (CHO). H$_2$O$_2$ 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 LDLC and the catalase inactivated by sodium azide. LDL 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 LDL in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37°C for two minutes.

**Principle of the reaction**

\[ 2\text{LDLC} + 2\text{H}_2\text{O} + 2\text{O}_2 \rightarrow \text{CHO and CHE} \rightarrow 2\text{cholestene-3-one + 2fatty acid + 2H}_2\text{O}_2 \]
3.9 Analytical methods for special biochemical parameters (hormones and tumour markers)

Mini-Vidas Machine: Hormones and tumour makers were analyzed using a Mini-Vidas machine. Every analyte had a specific master lot data entry card which contained all the specifications i.e analyte identification, factory master calibration curve, control data, and lot number of the reagent.

The strip: The strip consisted of 10 wells covered with a labeled foil seal. The label comprised a bar code which 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-(T4, T3, FT4, FT3, TSH, LH, PRG, FSH, PRL, TPSA, CA 19-9, CA125, CEA, CA 15-3) immunoglobulins (mouse) or specific polyclonal anti- (EST, TESTO) immunoglobulins (rabbit). Each SPR was identified by specific analyte code.

Analytical Principle: The assay principle combined an enzyme immunoassay sandwich method with a final fluorescent detection (enzyme linked fluorescent assay (ELFA))

Analytical method: The SPR served as the solid phase as well as the pipetting device for the assay. Reagents for the assay were ready for use and pre-dispersed in the 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- (T4, T3, FT4, FT3, TSH, LH, PRG, FSH, PRL, TPSA, CA 19-9, CA125, CEA, CA 15-3, EST, TESTO) immunoglobulins (conjugate). The sample/conjugate mixture was cycled in and out of SPR several times to increase reaction speed. The antigens binds 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-methyl-umbelliferyl phosphate) was cycled in and out of the SPR. The conjugate enzyme catalyzed the hydrolysis of the substrate into a fluorescent product (4-methyl-umbelliferone). The intensity of the fluorescence was directly proportional to the antigen present in the sample. At the end of the assay, results were automatically calculated by the machine in relation to the calibration curve stored in memory and then printed out.

3.10 Evaluation of the performance of manual and automated methods

A Manual Spectrophotometer and Olympus Autoanalyzers were used to analyse TP, ALB, T-BILI and ALP, respectively. Detailed methods for the analysis of TP, ALB, T-BILI and ALP, respectively using Olympus Autoanalyzer are indicated in subsection 3.8 while the methods used to analyze the same analytes using the Manual method are indicated in sub-subsections 3.10.1-3.10.4 below as per the procedures provided in the reagent kits. The principle of reaction for each assay was the same for both techniques.
3.10.1 Total protein by manual method

Total protein reagent was used to measure the concentration of total protein by a timed endpoint biuret method. In the reaction, the peptide bonds in the protein sample binds to cupric ions in an alkaline medium to form a peptide/copper complex. 0.1ml of sample was reacted with 5 ml of reagent and the change in absorbance was monitored at 560nm. 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 room temperature for 15 minutes. The standard and control were set the same as the test sample.

**Principle of the reaction**

\[
\text{Protein} + \text{Cu}^{2+} + \text{OH}^- \rightleftharpoons \text{Protein-Copper complex.}
\]

3.10.2 Albumin by manual method

Albumin reagent was used to measure albumin concentration by a timed endpoint method. Albumin combined with bromocresol green to form a coloured product. 0.01ml of sample was reacted with 3 ml of reagent and the change in absorbance was monitored at 600nm. 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 room temperature for 5 minutes. The standard and control were set the same way as the test sample.

**Principle of the reaction**

\[
\text{Albumin} + \text{BCG} \rightleftharpoons \text{Albumin / BCG complex}
\]
3.10.3 Total bilirubin by manual method

A stabilized diazonium salt (3, 5-dichlorophenyldiazonium tetrafluoroborate (DPD), reacted with conjugated bilirubin directly and with unconjugated bilirubin in the presence of an accelerator (caffeine) to form azobilirubin (purple). 0.2 ml of sample was reacted with 2 ml of reagent and the change in absorbance was monitored at 578nm. 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 room temperature for 15 minutes. A separate sample blank was performed (set) to reduce endogenous serum interference. The standard and control were set the same way as the test sample.

Principle of the reaction

\[
\text{Bilirubin} + \text{DPD} + \text{caffeine} \rightarrow \text{Azobilirubin}
\]

3.10.4 Alkaline phosphatase by manual method

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-nitrophenylphosphate to the yellow colored product, p-nitrophenol and phosphate. The reaction occurred at an alkaline pH of 10.3. 0.05 ml of the sample was reacted with 3 ml of the reagent. The change in absorbance was monitored at 405 nm and this change was directly proportional to the activity of ALP. The activity was calculated and expressed in U/L. The reaction took place at 37°C for three minutes. The standard and control were set the same way as the test sample.
Principle of the reaction

\[ \text{p-Nitrophenylphosphate} + \text{H}_2\text{O} \xrightarrow{\text{PH 10.3, Mg}} \text{p-Nitrophenol} + \text{Phosphate (colorless)} \]  
(yellow)

3.11 Screening for human immunodeficiency virus (HIV)

Immunochromatographic reagent strip (Determine HIV-1/2, Tokyo, Japan) was used for screening of HIV 1 and 2. 50 μL of the sample 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. Positive results were indicated by the appearance of two red bars each on the control window and on the patient window. Negative results were indicated by the appearance of only one red bar at the control window.

3.12 Screening for hepatitis b surface antigen (HBsAg)

The HbsAg one step hepatitis B Surface Antigen Test Strip (HBsAg, Beijing, China) was used for the screening of HbsAg. 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.
3.13 Screening for venereal disease research laboratory (VDRL)

The syphilis ultra rapid test which is a qualitative membrane strip based immunoassay (Treponema Pallidum Strip, Beijing, China) was used for the screening of *Treponema pallidum* which is the causative agent of the venereal disease, syphilis. 50 uL of the serum sample was placed on the sample pad followed by 1 drop of buffer. The result was read after 10 minutes. Positive result was indicated by the appearance of two red lines, one on the control region and the other on the test region. Negative result was indicated by the appearance of one red line on the control region.

3.14 Statistical methods used in the establishment of reference ranges

In order to produce unbiased national reference ranges for the adult Kenyan population, the data from 6795 study subjects was statistically treated using the following steps; (1) Partitioning of reference values, (2) Inspection of data distribution, (3) Detection and handling of outliers, (4) Determination of reference limits (5) Selection of statistical method

3.15 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 forty biochemical analytes. The reference ranges produced was used for comparison with the adult reference ranges for other populations as given in literature. The data was also used to categorize the study subjects in four age categories: 18-28, 29-39, 40-50 and 51-61
years. By categorizing it was possible to get the effect of sex and age on the reference ranges.

3.16 Inspection of data distribution

Histograms and box plot for the analytes were prepared by the use of computer. 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, and this represented 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

3.16.1 Identification and handling abnormal values

The identification of abnormal values (outliers) was done by the visual inspection of the histograms. According to Solberg (1987), there is no other statistical test for the identification of 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 for the construction of the reference ranges.
3.16.2 Determination of reference limits

By definition, reference limit is a descriptive of reference distribution that tells us something about the observed variation of values in the selected set of reference individuals. In this study, the lower and upper reference limits of each analyte was obtained by the formula: mean ± 1.96 multiplied by standard deviation (x ±1.96SD). All the values in between and including the two reference limits gives the reference range (interval) of the analytes. 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 which were cut off in both tails of the reference distribution. The confidence interval of the percentiles which showed the limits within which the true percentiles were located with a specified degree of confidence, for each analyte was also determined.

3.16.3 Selection of statistical method

The cleaned data obtained from the randomly selected individuals was subjected to normality distribution testing using 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 was obtained using the following formula:

\[ x - 1.960SD, x + 1.960 SD \] where \( x = \text{Mean} \) and \( SD = \text{standard deviation} \).

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 comparison of means. The tests were conducted at 95% confidence interval and
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

RESULTS

4.1 Study population characteristics

A total of 6795 study subjects were recruited for the study. Blood specimens for 27 (0.4\% ) participants were not analyzed for biochemical parameters due to their positive results for HIV (19), HBsAg (6) and VDRL (2). The remaining study participants had female and male representation of 3379 (49.9\%) and 3389 (50.1\%), respectively.

The distribution of the study participants for routine biochemical parameters across the counties (Population Category 1) were as follows:- Nairobi = 436 (male (223); female (213), Central = 457 (male(227); female (230), Eastern = 429 (male (220); female (209), North Eastern = 440 (male (221); female (219), Coast = 441 (male (218); female (222), Nyanza = 433 (male (219); female (214), Western = 431 (male (204); female (227), North Rift = 444 (male (215); female (229) and South Rift = 439 (male (213); female (226). Total population category 1 was 3950 (male (1960) and female (1989).

Distribution of study participants for special biochemical parameters from Nairobi metropolitan region (Population Category 2) were as follows:- FLP and FBG: male(260); female (320), OGTT: male(142); female (179), CrCl: male(106); female (159), Tumour makers (CA125, CA19-9,CA 15-3, CEA): male (233); female (242), TPSA: male (127), Thyroid hormones: male (230); female (230), Fertility hormones (FSH, LH, EII, PRG, PRL, TESTO): male (221); female (270), TESTO: male only (99). Total population category 2 was 2818 (male (1418) and female (1400).
4.2 Internal quality control (IQC) for the studied parameters

An internal quality control serum for specific parameter was included in each analytical session through out the study period. The analytical sessions (within the bracket) were as follows: routine biochemical parameters (111), thyroid hormones (24), fertility hormones (34), testosterone (41), tumour makers (36), TPSA (30) lipid profile (54) and OGTT (45).

Quality control results for the analyzed parameters were within the specific assigned QC range (Table 1) of target value ± 2 standard deviations (SD).
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<th>Study QC report</th>
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</tr>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>GGT (U/L)</td>
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</tr>
<tr>
<td>D-BILI (μmol/L)</td>
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</tr>
<tr>
<td>T-BILI (μmol/L)</td>
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</tr>
<tr>
<td>TP (g/L)</td>
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</tr>
<tr>
<td>BUN (mmol/L)</td>
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</tr>
<tr>
<td>CL (mmol/L)</td>
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</tr>
<tr>
<td>CREAT (μmol/L)</td>
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<tr>
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</tr>
<tr>
<td>POT (mmol/L)</td>
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<td>MG (mmol/L)</td>
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<td>TSH (μU/mL)</td>
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<td>EII (pg/mL)</td>
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<td>CA 15-3 (U/L)</td>
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<td>24.3-37.3</td>
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<td>CEA (U/L)</td>
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<td>TPSA (ng/mL)</td>
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<td>*GLU (mmol/L)</td>
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<td>**GLU (mmol/L)</td>
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<td>LDH (U/L)</td>
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<tr>
<td>UA ((μmol/L)</td>
<td>111</td>
<td>284-346</td>
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*GLU = for glucose quality control results using OLYMPUS AU 640 autoanalyer
**GLU = for glucose quality control results using ACCUCHEK glucometer
SD = standard deviation and CV = Coefficient of variation

4.3 Established national reference ranges for routine biochemical parameters for the studied healthy adult Kenyans

Difference between male and female population was determined in order to construct separate or combined reference ranges of the studied parameters. Means’ difference between male and female for CREAT, GGT, LDH and UA (p < 0.000) were statistically significant therefore separate reference ranges were established. Combined reference ranges for the following routine biochemical parameters were constructed since gender differences were not statistically significant: ALB (p=0.206), ALP (p=0.822), ALT (p=0.461), AST (p=0.206), AMY (p=0.822), HCO₃⁻ (p=0.136), CAL (p=0.671), CL (p=0.584), CPK (p=0.721), DBILI (p=0.358), GLU (p=0.130), MG (p=0.419), PHOS (p=0.177), POT (p=0.310), PROT (p=0.458), SOD (p=0.598), TBILI (p=0.539) and BUN (p=0.108)(Table 2).
Table 2: National reference ranges for routine biochemical parameters for the studied healthy adult Kenyans

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<th>Parameter (unit)</th>
<th>Sex</th>
<th>N</th>
<th>x(SD)</th>
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<th>x+1.96SD</th>
<th>Rr</th>
<th>t-value</th>
<th>Sig*</th>
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<tbody>
<tr>
<td>ALB (g/L)</td>
<td>M</td>
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<td>40.9(5.3)</td>
<td>30.5</td>
<td>51.3</td>
<td>29-53</td>
<td>1.264</td>
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</tr>
<tr>
<td></td>
<td>F</td>
<td>1968</td>
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<td>29.5</td>
<td>52</td>
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<tr>
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<td>M/F</td>
<td>3886</td>
<td>41(5.4)</td>
<td>29</td>
<td>53</td>
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<td></td>
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<td>ALP (U/L)</td>
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<td>1935</td>
<td>153(40)</td>
<td>75</td>
<td>231</td>
<td>77-230</td>
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<tr>
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<td>77</td>
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<td>CAL (mmol/L)</td>
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### Table 1: Laboratory Values

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<th>Female (F)</th>
<th>Combined (M/F)</th>
<th>Reference Range</th>
<th>Mean</th>
<th>SD</th>
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<td>2.6</td>
<td>0.7-2.6</td>
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<td>0.7-2.6</td>
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<td>129-151</td>
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<td>1884</td>
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<td>129</td>
<td>151</td>
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<td>129</td>
<td>151</td>
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<td>TP (g/L)</td>
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</tr>
<tr>
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<td>1889</td>
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<td>82</td>
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</tr>
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<td>72(5)</td>
<td>62</td>
<td>82</td>
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<td>0-19</td>
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<td></td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>1865</td>
<td>4.4(1.4)</td>
<td>1.5</td>
<td>7.3</td>
<td>1.5-7.3</td>
<td>-1.607</td>
</tr>
<tr>
<td></td>
<td>1922</td>
<td>4.3(1.4)</td>
<td>1.6</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3787</td>
<td>4.4(1.4)</td>
<td>1.5</td>
<td>7.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA (μmol/L)</td>
<td>1942</td>
<td>383(95)</td>
<td>97</td>
<td>469</td>
<td>97-469</td>
<td>-20.16</td>
</tr>
<tr>
<td></td>
<td>1914</td>
<td>229(71)</td>
<td>90</td>
<td>368</td>
<td>90-368</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as Mean (x) ± 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, Rr = reference range; p < 0.05 is considered statistically significant by 2-tailed t-test.

### 4.4 Established age specific national reference range for the studied healthy adult Kenyans

Healthy Kenyan adult population was divided into four age categories: 18-28, 29-39, 40-50 and 51-60 years (Table 3). Estimation of the differences among age categories was done by comparing each age group with the other age group category. Post hoc testing (bonferroni) for multiple comparisons was used to test for significance difference at 95% confidence interval and p < 0.05 was considered significant. BUN means difference was found to be statistically significant between age category 18-50 years and 51-61 years (p = 0.017) (Appendix 6). Female creatinine means difference was found to be statistically significant between age category 18-28 years and 40-50 years (p = 0.042), 18-28 years
and 51-60 years (p = 0.021) (Appendix 6). The other age category in females that showed statistically significant difference was 29-39 years and 40-50 years (p = 0.039), 29-39 years and 51-61 years (p = 0.011) (Appendix 6). Male creatinine means difference was found to be statistically significant between age category 18-28 years and 40-50 years (p = 0.017), 18-28 years and 51-61 years (p = 0.039). The other age group category in males that showed statistically significant difference was 29-39 years and 40-50 years (p = 0.014), 29-39 years and 51-60 years (p = 0.012) (Appendix 6). Age group dependent reference ranges for BUN and CREAT are as shown in Table 3. Biochemical parameters ALB, ALP, AST, ALT, AMY, HCO₃⁻, CAL, CL, CPK, D-BILI, GGT, RBG, MG, PHOS, POT, TP, SOD, T-BILI and UA did not show any significant difference between the four age categories.

Table 3: Age specific national reference range for the studied healthy adult Kenyans

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Age category (yrs)</th>
<th>Sex</th>
<th>N</th>
<th>x(SD)</th>
<th>x-1.96SD</th>
<th>x+1.96SD</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CREAT (μmol/L)</td>
<td>18-28 M</td>
<td>712</td>
<td>90(18)</td>
<td>55</td>
<td>125</td>
<td>55-125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29-39 M</td>
<td>604</td>
<td>91(17)</td>
<td>58</td>
<td>124</td>
<td>58-124</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-50 M</td>
<td>319</td>
<td>94(18)</td>
<td>59</td>
<td>129</td>
<td>59-129</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51-61 M</td>
<td>206</td>
<td>96(18)</td>
<td>61</td>
<td>131</td>
<td>61-131</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18-28 F</td>
<td>603</td>
<td>82(16)</td>
<td>51</td>
<td>113</td>
<td>51-113</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29-39 F</td>
<td>728</td>
<td>83(16)</td>
<td>52</td>
<td>114</td>
<td>52-114</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-50 F</td>
<td>452</td>
<td>85(17)</td>
<td>52</td>
<td>118</td>
<td>52-118</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51-61 F</td>
<td>100</td>
<td>85(18)</td>
<td>50</td>
<td>120</td>
<td>50-120</td>
<td></td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>18-50 M/F</td>
<td>3420</td>
<td>4.1(1.23)</td>
<td>1.7</td>
<td>6.5</td>
<td>1.7-6.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51-61 M/F</td>
<td>467</td>
<td>4.6(1.33)</td>
<td>2</td>
<td>7.2</td>
<td>2-7.2</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as Mean (x) ± standard deviation (SD) for the number of subjects shown in the column labeled N; Rr = reference range.
4.5 Regional differences of the established national reference ranges for the studied routine biochemical parameters for healthy adult Kenyans

Uric acid levels for male in South Rift Valley were different from other regions. Uric acid mean and standard deviation was 306 μmol/L and 86 μmol/L respectively, translating into reference range of 137 - 475 μmol/L. The other regions had a male uric acid mean and standard deviation of 285 μmol/L and 157 μmol/L, translating into a uric acid reference range of 128 - 442 μmol/L. The male mean difference for the two groups was statistically significant (p = 0.014). Mean difference was statistically insignificant for the following parameters across the regions:-

ALB (p=0.258), ALP (p=0.117), ALT (p=0.214), AST (p=0.912), AMY (p=0.369), BUN (p=0.685) HCO₃⁻ (p=0.789), CAL (p=412), CL (0.568), CPK (0.247), CREAT (male (p=0.458), female (p=0.159), DBILI (p=0.725), GLU (p=0.133), GGT (male (p=0.182), female (p=0.361), LDH (0.425), MG (p=0.258), PHOS (p=0.341), POT (p=0.411), TP (p=0.214), SOD (p=0.231), TBILI (p=0.369) and UA (female (p=0.085).

4.6 Comparison of the established national reference ranges for routine biochemical parameters for healthy adult Kenyans with other studies quoted in literature from Germany, United State of America, North Ireland and Tanzania

Comparisons were made between the established national reference ranges for routine biochemical parameters for healthy adult Kenyans with other studies quoted in literature from Germany, United State of America, North Ireland and Tanzania. The comparisons were based on the upper and lower reference range limits. No gender differences were observed in the five geographical regions for the parameters ALB, AMY, HCO₃⁻, CAL, CL, MG, PHOS, POT, SOD and BUN. Gender differences were observed across the five geographical regions for the parameters CREAT, GGT and UA. The studied Kenyan
population established common male and female reference ranges for ALT (1-44 U/L) and CPK (26-262 U/L) whilst Germany, United State of America, North Ireland and Tanzania have separate reference ranges for male and female for these two parameters (Table 8). Tanzanian population have reference range gender differences for D-BILI (male: 0.9-8.4 μmol/L, female: 0.7-5.8 μmol/L) and T-BILI (male: 6-42 μmol/L, female: 5-31 μmol/L) as opposed to Germany, United State of America, North Ireland and Kenya (Table 4).
Table 4: Comparison of established national reference ranges for routine biochemical parameters for healthy adult Kenyans with other studies quoted in literature from Germany, United State of America, North Ireland and Tanzania

<table>
<thead>
<tr>
<th>Parameter(unit)</th>
<th>Sex</th>
<th>KE</th>
<th>GER</th>
<th>USA</th>
<th>NIR</th>
<th>TZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALB (g/L)</td>
<td>M/F</td>
<td>29-53</td>
<td>34-48</td>
<td>35-50</td>
<td>38-44</td>
<td>36-50</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>M</td>
<td>73-224</td>
<td>0-117</td>
<td>32-92</td>
<td>98-279</td>
<td>46-158</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>M</td>
<td>1-44</td>
<td>0-50</td>
<td>10-40</td>
<td>0-40</td>
<td>9-55</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0-35</td>
<td>7-35</td>
<td>0-31</td>
<td>7-45</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>M</td>
<td>3-45</td>
<td>0-50</td>
<td>10-42</td>
<td>37</td>
<td>15-53</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0-35</td>
<td>31</td>
<td>14-35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMY (U/L)</td>
<td>M/F</td>
<td>0-111</td>
<td>0-220</td>
<td>22-80</td>
<td>0-95</td>
<td>43-164</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>M/F</td>
<td>13-24</td>
<td>22-29</td>
<td>19-30</td>
<td>20-29</td>
<td>19-30</td>
</tr>
<tr>
<td>CAL (mmol/L)</td>
<td>M/F</td>
<td>1.9-2.7</td>
<td>2.2-2.6</td>
<td>2.1-2.6</td>
<td>2.02-2.6</td>
<td>2.01-2.5</td>
</tr>
<tr>
<td>CL (mmol/L)</td>
<td>M/F</td>
<td>93-110</td>
<td>96-111</td>
<td>98-108</td>
<td>98-107</td>
<td>98-107</td>
</tr>
<tr>
<td>CPK (U/L)</td>
<td>M</td>
<td>26-262</td>
<td>0-190</td>
<td>38-174</td>
<td>24-195</td>
<td>77-787</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0-167</td>
<td>96-140</td>
<td>24-170</td>
<td>57-307</td>
<td>57-307</td>
</tr>
<tr>
<td>CREAT (µmol/L)</td>
<td>M</td>
<td>61-127</td>
<td>0-120</td>
<td>53-115</td>
<td>53-97</td>
<td>48-96</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>52-118</td>
<td>0-103</td>
<td>53-115</td>
<td>44-80</td>
<td>40-81</td>
</tr>
<tr>
<td>D-BILI (µmol/L)</td>
<td>M</td>
<td>0.1-5.9</td>
<td>0-5.1</td>
<td>0-3.4</td>
<td>0-4.3</td>
<td>0.9-8.4</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0-42</td>
<td>0-45</td>
<td>5-24</td>
<td>7-32</td>
<td>7-52</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>M</td>
<td>3-47</td>
<td>0-64</td>
<td>8-37</td>
<td>11-50</td>
<td>9-121</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0-42</td>
<td>0-45</td>
<td>5-24</td>
<td>7-32</td>
<td>7-52</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>M</td>
<td>216-466</td>
<td>0-225</td>
<td>266-500</td>
<td>230-460</td>
<td>127-264</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>86-532</td>
<td>0-215</td>
<td>66-87</td>
<td>66-87</td>
<td>66-85</td>
</tr>
<tr>
<td>MG (mmol/L)</td>
<td>M/F</td>
<td>0.6-1.6</td>
<td>0.7-1.05</td>
<td>0.7-1.15</td>
<td>0.7-0.9</td>
<td>0.7-1.00</td>
</tr>
<tr>
<td>PHOS (mmol/L)</td>
<td>M/F</td>
<td>0.7-2.6</td>
<td>0.9-1.5</td>
<td>0.8-1.5</td>
<td>0.8-1.6</td>
<td>0.7-1.5</td>
</tr>
<tr>
<td>POT (mmol/L)</td>
<td>M/F</td>
<td>3.2-5.2</td>
<td>3.6-4.5</td>
<td>3.6-5</td>
<td>3.8-5.5</td>
<td>3.8-5.5</td>
</tr>
<tr>
<td>RBG (mmol/L)</td>
<td>M/F</td>
<td>2.4-7</td>
<td>3.1-6.4</td>
<td>2.7-8.3</td>
<td>3.9-5.8</td>
<td>2.9-5.3</td>
</tr>
<tr>
<td>TP (g/L)</td>
<td>M/F</td>
<td>62-82</td>
<td>66-87</td>
<td>64-83</td>
<td>66-87</td>
<td>66-85</td>
</tr>
<tr>
<td>SOD (mmol/L)</td>
<td>M/F</td>
<td>129-151</td>
<td>136-145</td>
<td>135-145</td>
<td>134-143</td>
<td>134-143</td>
</tr>
<tr>
<td>T-BILI (µmol/L)</td>
<td>M</td>
<td>0-19</td>
<td>0-17</td>
<td>1.7-22.2</td>
<td>0-17</td>
<td>6-42</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5-31</td>
<td>2.5-6.4</td>
<td>1.7-8.3</td>
<td>1.5-5</td>
<td>1.5-5</td>
</tr>
</tbody>
</table>

100
<table>
<thead>
<tr>
<th>UA (µmol/L)</th>
<th>M</th>
<th>97-469</th>
<th>0-420</th>
<th>155-428</th>
<th>202-416</th>
<th>196-459</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>90-368</td>
<td>0-340</td>
<td>120-360</td>
<td>142-339</td>
<td>148-360</td>
<td></td>
</tr>
</tbody>
</table>

KE = Kenya, GER = Germany (Heil et al., 2002), USA = United States of America (NCCLS, 1994), NIR = Northern Ireland (Gardner et al., 1980) and TZ = Tanzania (Elmar et al., 2008)

4.7 Established reference ranges for special biochemical parameters for the studied healthy adult Kenyans

Difference between male and female population was determined inorder to construct separate or combined reference ranges of the studied parameters. Means difference between male and female for the studied special biochemical parameters TSH (p = 0.078), T3 (p = 0.823), T4 (p = 0.473), FT3 (p = 0.347), FT4 (p = 0.309), CA 125 (p = 0.365), CA 19-9 (p = 0.877), CA 15-3 (p = 0.277) and CEA (p = 0.917) (Table 9), were not statistically significant and therefore a combined reference range was established. IPSA was male oriented parameter whose reference ranges among the various age groups was statistically significant (p < 0.001) (Table 10). Means difference between male and female for the studied fertility hormones FSH, LH, EII, PRG, PRL and TESTO (p < 0.001) were statistically significant and therefore separate reference ranges were established (Table 5&6).

4.7.1 Thyroid stimulating hormone

TSH test results for 460 adults were used to construct TSH reference range. Male and female TSH concentration mean difference was not statistically significant (p = 0.078). Mean and standard deviation (SD) were 4 µU/mL and 1.98 µU/mL, respectively.
Therefore the reference range of TSH established in this study for the adult Kenyans is 0.5 – 8 µU/mL (Table 9).

4.7.2 Triiodothyronine

T3 test results for 460 adults were used to construct T3 reference range. Male and female T3 concentration mean difference was not statistically significant (p = 0.823). Mean and standard deviation (SD) were 1.76 nmol/L and 0.53 nmol/L, respectively. Therefore the reference range of T3 established in this study for adult Kenyans is 0.6 – 2.9 nmol/L (Table 5).

4.7.3 Thyroxine or tetraiodothyronine

T4 test results for 460 adults were used to construct T4 reference range. Male and female T4 concentration mean difference was not statistically significant (p = 0.473). Mean and standard deviation (SD) were 89 nmol/L and 21.4 nmol/L, respectively. Therefore the reference range of T4 established in this study for adult Kenyans is 42 – 132 nmol/L (Table 5).

4.7.4 Free triiodothyronine

FT3 test results for 460 adults were used to construct FT3 reference range. Male and female FT3 concentration mean difference was not statistically significant (p = 0.347). Mean and standard deviation (SD) were 4.8 pmol/L and 1.3 pmol/L, respectively. Therefore the reference range of FT3 established in this study for adult Kenyans is 3 – 9 pmol/L (Table 5).
4.7.5 Free tetraiodothyronine

FT4 test results for 460 adults were used to construct FT4 reference range. Male and female FT4 concentration mean difference was not statistically significant (p = 0.309). Mean and standard deviation (SD) were 13.5 pmol/L and 4 pmol/L, respectively. Therefore the reference range of FT4 established in this study for adult Kenyans is 4 – 24 pmol/L (Table 5).

4.7.6 CA 125

CA 125 test results for 469 adults were used to construct CA 125 reference range. Male and female CA 125 concentration mean difference was not statistically significant (p = 0.366). Mean and standard deviation (SD) were 7.8 U/L and 6.6 U/L, respectively. Therefore the reference range of CA 125 established in this study for adult Kenyans is 0 – 23 U/L (Table 5).

4.7.7 CA 15-3

CA 15-3 test results for 469 adults were used to construct CA 15-3 reference range. Male and female CA 15-3 concentration mean difference was not statistically significant (p = 0.277). Mean and standard deviation (SD) were 7.2 U/L and 6 U/L, respectively. Therefore the reference range of CA 15-3 established in this study for adult Kenyans is 0 – 19 U/L (Table 5).
4.7.8 CA 19-9

CA 19-9 test results for 469 adults were used to construct CA 19-9 reference range. Male and female CA 19-9 concentration mean difference was not statistically significant ($p = 0.877$). Mean and standard deviation (SD) were 6.1 U/L and 5 U/L, respectively. Therefore the reference range of CA 19-9 established in this study for adult Kenyans is 0 – 16 U/L (Table 5).

4.7.9 CEA

CEA test results for 469 adults were used to construct CEA reference range. Male and female CEA concentration mean difference was not statistically significant ($p = 0.917$). Mean and standard deviation (SD) were 1.5 U/L and 0.96 U/L, respectively. Therefore the reference range of CEA established in this study for adult Kenyans is 0 – 3.5 U/L (Table 5).
Table 5: Reference ranges for thyroid function tests and tumour markers for the studied healthy adult Kenyans

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Sex</th>
<th>N</th>
<th>x(SD)</th>
<th>x-1.96SD</th>
<th>x+1.96SD</th>
<th>Rr</th>
<th>t-value</th>
<th>Sig*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH (μU/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>230</td>
<td>3.86(2)</td>
<td>0.1</td>
<td>8.02</td>
<td>0.2 - 8</td>
<td>-1.764</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>230</td>
<td>4.1(2)</td>
<td>0.1</td>
<td>7.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>460</td>
<td>4.03(1.98)</td>
<td>0.2</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3 (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>230</td>
<td>1.8(0.6)</td>
<td>0.6</td>
<td>3</td>
<td>0.7 - 3</td>
<td>0.224</td>
<td>0.823</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>230</td>
<td>1.8(0.5)</td>
<td>0.8</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>460</td>
<td>1.76(0.53)</td>
<td>0.7</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>230</td>
<td>1.8(0.6)</td>
<td>0.6</td>
<td>3</td>
<td>0.7 - 3</td>
<td>0.224</td>
<td>0.823</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>230</td>
<td>1.8(0.5)</td>
<td>0.8</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>460</td>
<td>1.76(0.53)</td>
<td>0.7</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT3 (pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>230</td>
<td>4.8(1.2)</td>
<td>2.4</td>
<td>7.2</td>
<td>3 - 7.4</td>
<td>0.942</td>
<td>0.347</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>230</td>
<td>4.9(1.3)</td>
<td>2.3</td>
<td>7.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>460</td>
<td>4.8(1.3)</td>
<td>2.4</td>
<td>7.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT4 (pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>230</td>
<td>13.3(3.8)</td>
<td>5.8</td>
<td>21</td>
<td>5.7 - 21</td>
<td>1.011</td>
<td>0.309</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>230</td>
<td>13.7(4.2)</td>
<td>5.5</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>460</td>
<td>13.5(4)</td>
<td>5.7</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA 125 (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>233</td>
<td>8.1(6.8)</td>
<td>0</td>
<td>22</td>
<td>0 - 23</td>
<td>-0.906</td>
<td>0.365</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>236</td>
<td>7.7(6.4)</td>
<td>0</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>469</td>
<td>7.9(6.6)</td>
<td>0</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA15-3 (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>233</td>
<td>6.9(5.4)</td>
<td>0</td>
<td>18</td>
<td>0 - 20</td>
<td>1.088</td>
<td>0.277</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>236</td>
<td>7.5(6.4)</td>
<td>0</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>469</td>
<td>7.2(6)</td>
<td>0</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA 19-9 (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>233</td>
<td>6.1(5.4)</td>
<td>0</td>
<td>17</td>
<td>0 - 16</td>
<td>-0.155</td>
<td>0.108</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>236</td>
<td>6.01(4.6)</td>
<td>0</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>469</td>
<td>6.1(5)</td>
<td>0</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEA (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>233</td>
<td>1.5(0.96)</td>
<td>0</td>
<td>3.5</td>
<td>0 - 3.5</td>
<td>-0.105</td>
<td>0.917</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>236</td>
<td>1.49(0.96)</td>
<td>0</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>469</td>
<td>1.5(0.96)</td>
<td>0</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as Mean (x) ± 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, Rr = reference range; p < 0.05 is considered statistically significant by 2-tailed t-test.

4.7.10 TPSA

Among the studied tumour markers, TPSA was the only male oriented tumour marker. TPSA test results for 340 adult males 18-28 years (85), 29-39 years (81), 40-50 years
(77) and 51-61 years (97) were used to construct TPSA reference ranges. Multiple means comparison test of the four male age categories showed statistically significant differences between all the age group categories (p = 0.001) as shown in Appendix 2. Therefore TPSA reference ranges were constructed for each specific age category as shown in Table 6.

**Table 6: Established reference ranges for TPSA for the studied healthy adult male Kenyans**

<table>
<thead>
<tr>
<th>Age group (yrs)</th>
<th>N</th>
<th>x</th>
<th>x(SD)</th>
<th>x-1.96SD</th>
<th>x+1.96SD</th>
<th>Rr (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-28</td>
<td>85</td>
<td>0.73</td>
<td>0.73(0.33)</td>
<td>0.1</td>
<td>1.4</td>
<td>0.1-1.4</td>
</tr>
<tr>
<td>29-39</td>
<td>81</td>
<td>1.3</td>
<td>1.3(0.64)a</td>
<td>0.1</td>
<td>2.6</td>
<td>0.1-2.6</td>
</tr>
<tr>
<td>40-50</td>
<td>77</td>
<td>2.3</td>
<td>2.3(0.61)bd</td>
<td>1.1</td>
<td>3.5</td>
<td>1.1-3.5</td>
</tr>
<tr>
<td>51-61</td>
<td>97</td>
<td>3.7</td>
<td>3.7(1.33)ef</td>
<td>1.1</td>
<td>6.3</td>
<td>1.1-6.3</td>
</tr>
</tbody>
</table>

Results are expressed as Mean (x) ± standard deviation (SD) for the number of subjects shown in the column labeled N; Rr = reference range. a p<0.05 indicates significance differences between age category 18-28 and 29-39 years; b p<0.05 indicates significance differences between age category 18-28 and 40-50 years; c p<0.05 indicates significance differences between age category 18-28 and 51-61 years; d p<0.05 indicates significant differences between age category 29-39 and 40-50 years; e p<0.05 indicates significant differences between age category 29-39 and 51-61 years; f p<0.05 indicates significant differences between age category 40-50 and 51-61 years.

4.7.11 Establishment of fertility hormones reference ranges for healthy adult Kenyans

Fertility hormones (TESTO, LH, FSH, EII, PRG) reference ranges for male and female were determined separately since they are dependent on the menstrual cycle phases in females. Therefore, no gender differences were determined for these fertility hormones. PRL reference range was constructed independently since it is not influenced by the menstrual cycle phases in female.
4.7.11.1 Established male fertility hormones reference ranges for the studied healthy adult Kenyans

4.7.11.1.1 Male testosterone

Testosterone test results for 320 adult males 18-28 years (104), 29-39 years (78), 40-50 years (92) and 51-61 years (46) were used to construct TESTO reference ranges. Multiple means comparison of the four male age categories showed no statistical significant differences between age category 18-28 years and 29-30 years (p = 0.562), whilst as statistically significant difference was observed in the other age categories as shown in Appendix 3. Therefore TESTO reference ranges was constructed for age group 18-39 years, 40-50 years and 51-61 years as shown in Table 7.

4.7.11.1.2 Male follicle stimulating hormone

Male FSH test results for 221 adult males were used to construct FSH reference range. Mean and standard deviation (SD) were 4.1 μU/mL and 1.74 μU/mL, respectively. Therefore FSH reference range of adult Kenyan male established in this is 0.7 – 7.5 μU/mL (Table 7).

4.7.11.1.3 Male luteinizing hormone

Male LH test results for 221 adult males were used to construct LH reference range. Mean and standard deviation (SD) were 3.14 μU/mL and 1.31 μU/mL, respectively. Therefore LH reference range of adult Kenyan male established in this study is 0.5 – 5.7 μU/mL (Table 7).
4.7.11.1.4 Male estradiol II

Male EII test results for 221 adult males were used to construct EII reference range. Mean and standard deviation (SD) were 10.76 pg/mL and 7.22 pg/mL, respectively. Therefore EII reference range of adult Kenyan male established in this study is 0 – 25 pg/mL (Table 7).

4.7.11.1.5 Male progesterone

Male PRG test results for 221 adult males were used to construct PRG reference range. Mean and standard deviation (SD) were 0.57 ng/mL and 0.28 ng/mL respectively. Therefore PRG reference range of adult Kenyan male established in this study is 0.12 – 1.02 ng/mL (Table 7).

4.7.11.1.6 Male prolactin

Male and female PRL concentration mean difference was statistically significant (p = 0.001). PRL results for 221 male adults were used to construct male PRL reference range. Mean and standard deviation (SD) for the studied male population was 8.07 ng/ml and 4.57 ng/mL, respectively. Therefore reference range for PRL established in this study for adult Kenyan male is 0-17 ng/mL (Table 7).
Table 7: Age specific male fertility hormones reference ranges for the studied healthy adult Kenyans

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Age group (yrs)</th>
<th>N</th>
<th>x(SD)</th>
<th>x-1.96SD</th>
<th>x+1.96SD</th>
<th>Rr</th>
</tr>
</thead>
<tbody>
<tr>
<td>TESTO (ng/mL)</td>
<td>18-28</td>
<td>85</td>
<td>8.13(2.2)</td>
<td>3.5</td>
<td>12.7</td>
<td>3.5-12.7</td>
</tr>
<tr>
<td></td>
<td>29-39</td>
<td>97</td>
<td>8.08(2.3)</td>
<td>3.5</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>92</td>
<td>4.67(1.11)</td>
<td>2.5</td>
<td>6.9</td>
<td>2.5-6.9</td>
</tr>
<tr>
<td></td>
<td>51-60</td>
<td>46</td>
<td>3.71(0.97)</td>
<td>1.8</td>
<td>5.6</td>
<td>1.8-5.6</td>
</tr>
<tr>
<td>FSH (μU/mL)</td>
<td>18-60</td>
<td>221</td>
<td>4.1(1.74)</td>
<td>0.7</td>
<td>7.5</td>
<td>0.7-7.5</td>
</tr>
<tr>
<td>LH (μU/mL)</td>
<td>18-60</td>
<td>221</td>
<td>3.14(1.31)</td>
<td>0.5</td>
<td>5.7</td>
<td>0.5-5.7</td>
</tr>
<tr>
<td>E2 (pg/mL)</td>
<td>18-60</td>
<td>221</td>
<td>10.76(7.22)</td>
<td>0</td>
<td>25</td>
<td>0-25</td>
</tr>
<tr>
<td>PRG (ng/mL)</td>
<td>18-60</td>
<td>221</td>
<td>0.57(0.28 )</td>
<td>0.12</td>
<td>1.02</td>
<td>0.12-1.02</td>
</tr>
<tr>
<td>PRL (ng/mL)</td>
<td>18-60</td>
<td>221</td>
<td>8.07(4.57)</td>
<td>0</td>
<td>17</td>
<td>0-17</td>
</tr>
</tbody>
</table>

Results are expressed as Mean (x) ± standard deviation (SD) for the number of subjects shown in the column labeled N; Rr = reference range. Age category 18-28 years and 29-39 years for TESTO had similar Mean (x) ± SD and were therefore combined to produce one reference range.

4.7.11.2 Establishment of female fertility hormones reference ranges for the studied adult Kenyans

In the establishment of female fertility hormones, menstrual cycle phases (follicular, ovulation, luteal), menstrual bleeding period and menopause period were considered in the studied adult female Kenyans.

4.7.11.2.1 Female testosterone

Female TESTO reference ranges were constructed using 270 (follicular phase (79), ovulation phase (75), luteal phase (78), menopause (38)) test results. Female TESTO levels in follicular phase, luteal phase and menopause were not statistically significant (follicular phase/luteal phase (p = 1.000), follicular phase/menopause (p = 0.439) and luteal phase/menopause (p = 0.287) (Table 8). Therefore, same reference range was
constructed for follicular phase, luteal phase and menopause. Mean and standard deviation (SD) were 0.75 ng/mL and 0.64 ng/mL, respectively. Therefore the reference range of female TESTO established in this study for follicular phase, luteal phase and menopause is 0 – 2 ng/mL (Table 8).

Ovulation phase TESTO levels was statistically different from the other phases with a mean and standard deviation of 2.02 ng/mL and 0.66 ng/mL, respectively. Therefore the reference range of female TESTO established in this study for ovulation phase is 0.7 – 3.3 ng/mL (Table 8).

4.7.11.2.2 Female follicle stimulating hormone

Female FSH reference ranges were constructed using 270 follicular phase (79), ovulation phase (75), luteal phase (78), menopause (28)} test results. Multiple comparision of the means difference for the phases using post hoc test (Tukey HSD) was found to be statistically significant (p < 0.001). Therefore, reference ranges were constructed for each specific phase, luteal phase and menopause. Mean and standard deviation (SD) were follicular phase (10.14 µU/mL and 3.1 µU/mL), ovulation phase (12.62 µU/mL and 3.79 µU/mL), luteal phase (4.96 µU/mL and 1.27 µU/mL) and menopause (49.5 µU/mL and 20 µU/mL) respectively. Therefore the reference range of female FSH established in this study are: - follicular phase (4 - 16 µU/mL), ovulation phase (5 -20 µU/mL), luteal phase (10 - 89 µU/mL) and menopause (10 - 89 µU/mL) respectively (Table 8).
4.7.11.2.3 Female luteinizing hormone

Female LH reference ranges were constructed using 270 (follicular phase (79), ovulation phase (75), luteal phase (78), menopause (38)) test results. Multiple comparison of the means difference for the phases using post hoc test (Tukey HSD) was found to be statistically significant (p < 0.001). Therefore, reference ranges were constructed for each specific phase. Mean and standard deviation (SD) were: follicular phase (4.63 μIU/mL and 2 μU/mL), ovulation phase (44.73 μU/mL and 19.44 μU/mL), luteal phase (3.59 μU/mL and 1.61 μU/mL) and menopause (19.5 μU/mL and 6.6 μU/mL) respectively. Therefore the reference range of female LH established in this study are follicular phase (0.7 – 8.6 μU/mL), ovulation phase (7 – 83 μU/mL), luteal phase (0.4 – 6.8 μU/mL) and menopause (7 – 32 μU/mL) (Table 8).

4.7.11.2.4 Female estradiol II

Female EII reference ranges were constructed using 270 (follicular phase (79), ovulation phase (75), luteal phase (78), menopause (38)) test results. Comparison of the means difference by Tukey HSD was found to be statistically significant (p = 0.001). Therefore, reference ranges were constructed for each specific phase. Mean and standard deviation (SD) were: follicular phase (100.05 pg/mL and 51.04 pg/mL), ovulation phase (300.88 pg/mL and 84.9 pg/mL), luteal phase (214 pg/mL and 70.3 pg/mL) and menopause (15.8 pg/mL and 10 pg/mL), respectively. Therefore the reference range of female EII established in this study are follicular phase (0 – 200 pg/mL), ovulation phase (134 – 467 pg/mL), luteal phase (75 – 351 pg/mL) and menopause are (0 – 35 pg/mL), respectively (Table 8).
4.7.11.2.5 Female progesterone

Female PRG reference ranges were constructed using 270 \{follicular phase (79), ovulation phase (75), luteal phase (78), menopause (38)\} test results. Comparision of the means difference by Tukey HSD was found to be statistically significant \(p < 0.001\). Therefore, reference ranges were constructed for each specific phase. Mean and standard deviation (SD) were follicular phase (0.38 ng/mL and 0.17 ng/mL), ovulation phase (2.52 ng/mL and 5 ng/mL), luteal phase (3.73 ng/mL and 4.48 ng/mL) and menopause (0.18 ng/mL and 0.1 ng/mL), respectively. Therefore the reference range of female FSH established in this study are follicular phase (0.04 – 0.72 ng/mL), ovulation phase (0 - 5 ng/mL), luteal phase (0 – 18ng/mL) and menopause are (0 - 0.38 ng/mL), respectively (Table 8).

4.7.11.2.6 Female prolactin

Female PRL reference ranges were constructed for both menstruating and menopausal females. Results for 232 female adults were used to construct PRL reference range for menstruating group. Mean and standard deviation (SD) was 12.37 ng/mL and 5.47 ng/mL, respectively. Lower and upper reference limits were 1.7 ng/m/L and 23 ng/mL, respectively. Therefore PRL reference range for menstruating group established in this study is 1.7 - 23 ng/mL.

Results for 38 female adults were used to construct PRL reference range for menopausal female group. Mean and standard deviation (SD) was 18.3 ng/mL and 5.5ng/mL, respectively. Therefore PRL reference range for menopausal female group established in this study is 8 – 29 ng/mL (Table 8).

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4.7.11.2.7 Menstrual bleeding period

A total of 229 menstrual female subjects were categorized in three age groups: 18-28 yrs (97), 29-39 yrs (55) and 40-50 yrs (77). Comparison of the means difference by Tukey HSD was found to be statistically insignificant (p = 0.759). Therefore, reference range was constructed for the whole female group. Mean and standard deviation (SD) was 3 days and 0.81 days, respectively. Therefore Menstrual bleeding period reference range for menstrual female group established in this study is 2–5 days (Table 8).

Table 8: Established female fertility hormones reference ranges for the studied adult Kenyans

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>N</th>
<th>Fertility cycle</th>
<th>x(SD)</th>
<th>x-1.96SD</th>
<th>x+1.96SD</th>
<th>Rr</th>
</tr>
</thead>
<tbody>
<tr>
<td>TESTO (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>79</td>
<td>0.75(0.64)</td>
<td>0</td>
<td>2.05</td>
<td>0 - 2</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td>78</td>
<td>0.73(0.66)</td>
<td>0</td>
<td>2.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fol/Lut</td>
<td>157</td>
<td>0.79(0.66)</td>
<td>0</td>
<td>2.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>75</td>
<td>2.02(0.66)</td>
<td>0.7</td>
<td>3.3</td>
<td>0.7 - 3.3</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td>75</td>
<td>4.96(1.3)</td>
<td>2.5</td>
<td>7</td>
<td>2.5 - 7</td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>78</td>
<td>4.96(1.3)</td>
<td>2.5</td>
<td>7</td>
<td>2.5 - 7</td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>38</td>
<td>49.5(20)</td>
<td>10</td>
<td>89</td>
<td>10 - 89</td>
<td></td>
</tr>
<tr>
<td>FSH (µU/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>79</td>
<td>10.14(1.3)</td>
<td>4</td>
<td>16</td>
<td>4 - 16</td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>75</td>
<td>12.62(3.8)</td>
<td>5</td>
<td>20</td>
<td>5 - 20</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td>78</td>
<td>4.96(1.3)</td>
<td>2.5</td>
<td>7</td>
<td>2.5 - 7</td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>38</td>
<td>49.5(20)</td>
<td>10</td>
<td>89</td>
<td>10 - 89</td>
<td></td>
</tr>
<tr>
<td>LH (µU/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>79</td>
<td>4.63(2)</td>
<td>0.7</td>
<td>8.6</td>
<td>0.7 - 8.6</td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>75</td>
<td>44.7(19.4)</td>
<td>7</td>
<td>83</td>
<td>7 - 83</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td>78</td>
<td>3.59(1.61)</td>
<td>0.4</td>
<td>6.8</td>
<td>0.4 - 6.8</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td>38</td>
<td>19.5(6.6)</td>
<td>7</td>
<td>32</td>
<td>7 - 32</td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>38</td>
<td>19.5(6.6)</td>
<td>7</td>
<td>32</td>
<td>7 - 32</td>
<td></td>
</tr>
<tr>
<td>E2I (pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>79</td>
<td>100(51)</td>
<td>0</td>
<td>200</td>
<td>0 - 200</td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>75</td>
<td>301(85)</td>
<td>134</td>
<td>467</td>
<td>134 - 467</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td>78</td>
<td>214(70)</td>
<td>75</td>
<td>351</td>
<td>75-351</td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>38</td>
<td>16(10)</td>
<td>0</td>
<td>35</td>
<td>0 - 35</td>
<td></td>
</tr>
<tr>
<td>PRG (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>79</td>
<td>0.38(0.17)</td>
<td>0.04</td>
<td>0.72</td>
<td>0.04-0.72</td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>75</td>
<td>2.52(5)</td>
<td>0</td>
<td>5</td>
<td>0 - 5</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td>78</td>
<td>3.73(4.5)</td>
<td>0</td>
<td>18</td>
<td>0 - 18</td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>38</td>
<td>0.18(0.1)</td>
<td>0</td>
<td>0.38</td>
<td>0 - 0.38</td>
<td></td>
</tr>
<tr>
<td>PRL (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menstrual</td>
<td>232</td>
<td>12.37(5.47)</td>
<td>1.7</td>
<td>23</td>
<td>1.7-23</td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>38</td>
<td>18.35(5.5)</td>
<td>8</td>
<td>29</td>
<td>8 - 29</td>
<td></td>
</tr>
<tr>
<td>Bleeding period (day)</td>
<td>229</td>
<td>3.00(0.81)</td>
<td>1.5</td>
<td>4.5</td>
<td>2 - 5</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as Mean (x) ± standard deviation (SD) for the number of subjects shown in the column labeled N; Rr = reference range. In the Follicular and Luteal stages of the fertility cycle, the levels of TESTO were similar and were therefore combined to produce one reference range.

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4.8 Establishment of glomerular filtration rate of the adult Kenyans

The female study participants involved in the establishment of glomerular filtration rate of the adult Kenyans, had a mean age and weight of 34.5 years and 69.5 kg, respectively. On the other hand the male study participants had a mean age and height of 33.1 years and 64.1 kg, respectively. Mean difference for all the studied parameters were statistically significant for both sexes with p-value less than 0.05 as shown in Table 9. Sex related reference ranges for serum creatinine, measured creatinine clearance, estimated creatinine clearance, urine volume and urine creatinine were constructed using mean ± 1.96SD. Same sex mean difference was found to be statistically significant for established Mcrel and Ecrcel for both male (p = 0.021) and female (p = 0.001) as shown in Table 10. Study participants were categorized in four groups; that is, group 1(18-28), group 2 (29-39), group 3 (40-50) and group 4 (51-61) years, for each gender and the mean standard deviation of each group was determined as shown in Table 11. A relationship between age and creatinine clearance was established where creatinine clearance decreases as age advances in both sexes. Creatinine clearance significantly (p=0.001) declined at a rate of 0.46 mL/min per year for males from ages 18 to 60 years for measured creatinine clearance. Estimated creatinine clearance of the same male study participants had a significant (p=0.001) decline of 0.29 mL/min per year. On the other hand, creatinine clearance significantly (p=0.001) declined at a rate of 0.39 mL/min per year for females from ages 18 to 60 years for measured creatinine clearance. Estimated creatinine clearance of the same female study participants had a significant (p=0.001) decline of 0.2 mL/min per year
Table 9: Sex related reference ranges for serum creatinine, measured creatinine clearance, estimated creatinine clearance, urine volume and urine creatinine

<table>
<thead>
<tr>
<th>Parameter/unit</th>
<th>Sex</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Rr</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCr (μmo/L)</td>
<td>M</td>
<td>106</td>
<td>97.9 ± 15.2</td>
<td>68-128</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>159</td>
<td>90.5 ± 15.7</td>
<td>60-122</td>
<td></td>
</tr>
<tr>
<td>Mcrcl (mL/min)</td>
<td>M</td>
<td>106</td>
<td>81.3 ± 14.8</td>
<td>52-110</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>159</td>
<td>71.6 ± 10.8</td>
<td>50-92</td>
<td></td>
</tr>
<tr>
<td>Ecrc (mL/min)</td>
<td>M</td>
<td>106</td>
<td>85.6 ± 16.4</td>
<td>54-118</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>159</td>
<td>81.7 ± 12.4</td>
<td>58-106</td>
<td></td>
</tr>
<tr>
<td>UV (mL)</td>
<td>M</td>
<td>106</td>
<td>1588 ± 364</td>
<td>875-2301</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>159</td>
<td>1447 ± 329</td>
<td>802-2092</td>
<td></td>
</tr>
<tr>
<td>UCr (μmol/L)</td>
<td>M</td>
<td>106</td>
<td>6994 ± 1738</td>
<td>3588-10,400</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>159</td>
<td>6574 ± 1690</td>
<td>3262-9886</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation (SD). The reference range (Rr) for each analyte was calculated as the minimum and maximum value of 95% of the study subjects using the formula mean (X) ± 1.96 standard deviation (sd), N = number of study subjects.

Table 10: Same sex differences for Mcrcl and Ecrc

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Sex</th>
<th>Number</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mcrcl (mL/min)</td>
<td>M</td>
<td>106</td>
<td>81.3 ± 14.8</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>159</td>
<td>85.6 ± 16.4</td>
<td></td>
</tr>
<tr>
<td>Ecrc (mL/min)</td>
<td>M</td>
<td>106</td>
<td>71.6 ± 10.7</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>159</td>
<td>81.7 ± 12.4</td>
<td></td>
</tr>
</tbody>
</table>

Mcrcl = measured creatinine clearance, Ecrc= estimated creatinine clearance

Table 11: Relationship between age and creatinine clearance

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Sex</th>
<th>Number</th>
<th>Mcrcl (mL/min)</th>
<th>Ecrc (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-28</td>
<td>M</td>
<td>38</td>
<td>89.1±14.6</td>
<td>91.3±19.6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>54</td>
<td>77.1±9.9</td>
<td>81.1±11.8</td>
</tr>
<tr>
<td>29-39</td>
<td>M</td>
<td>40</td>
<td>80.0±12.4</td>
<td>85.5±13.9</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>47</td>
<td>72.9±9.8</td>
<td>79.9±10.7</td>
</tr>
<tr>
<td>40-50</td>
<td>M</td>
<td>23</td>
<td>77.2±9.2</td>
<td>80.8±10.6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>49</td>
<td>66.7±9.3</td>
<td>76.2±11.1</td>
</tr>
<tr>
<td>51-61</td>
<td>M</td>
<td>5</td>
<td>69.8±7.0</td>
<td>78.8±11.4</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9</td>
<td>60.9±8.7</td>
<td>72.7±12.5</td>
</tr>
</tbody>
</table>

Mcrcl = measured creatinine clearance, Ecrc= estimated creatinine clearance

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4.9 Establishment of reference ranges for fasting lipids, fasting glucose and oral glucose tolerance test (OGTT) for adult Kenyans

The number of results for study subjects used in the statistical analysis of fasting venous plasma glucose (FPG), fasting lipid profile (FLP) and oral glucose tolerance test (OGTT) were 567,554 and 303 respectively. Study participants had a mean age of 32.9 years. Gender difference for each analyte was determined to assess whether combined or gender – specific reference ranges should be established. Difference between male and female participants is presented in Table 16. The results for both male and female for the studied analytes were not statistically significant with p>0.05. Therefore results for males and females for each analyte were used to construct common reference range. Reference ranges for the studied analytes are presented in Table 12.

For the establishment of OGTT curve the reference range upper limits for each half hour of the two hour testing period using capillary whole blood were: 0 hr (5.4 mmol/L), 0.5 hrs (8.9 mmol/L), 1 hr (9.8 mmol/L), 1.5 hrs (8.1 mmol/L) and 2 hrs (7.2 mmol/L) (Figure 1). Qualitative urine test for glucose and ketones were negative at 0 hour,1 hour and 2 hours analytical period.
Table 12: Sex difference and established reference ranges for fasting lipid, fasting blood glucose and oral glucose tolerance test for healthy adult Kenyans

<table>
<thead>
<tr>
<th>Analyte(unit)</th>
<th>Sex</th>
<th>N</th>
<th>x(SD)</th>
<th>p-value</th>
<th>x-1.96SD</th>
<th>x+1.96SD</th>
<th>Rr</th>
</tr>
</thead>
<tbody>
<tr>
<td>*FPG (mmol/L)</td>
<td>M</td>
<td>284</td>
<td>4.1(0.8)</td>
<td>0.124</td>
<td>2.1</td>
<td>5.7</td>
<td>2.1-5.7</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>283</td>
<td>4.2(0.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>M</td>
<td>277</td>
<td>4.7(1.0)</td>
<td>0.201</td>
<td>2.9</td>
<td>6.4</td>
<td>2.9-6.4</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>277</td>
<td>4.6(0.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>M</td>
<td>277</td>
<td>1.4(0.5)</td>
<td>0.703</td>
<td>0.4</td>
<td>2.4</td>
<td>0.4-2.4</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>277</td>
<td>1.4(0.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>M</td>
<td>277</td>
<td>1.6(0.3)</td>
<td>0.527</td>
<td>1.1</td>
<td>2.1</td>
<td>1.1-2.1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>277</td>
<td>1.6(0.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>M</td>
<td>277</td>
<td>2.8(0.8)</td>
<td>0.418</td>
<td>1.1</td>
<td>4.3</td>
<td>1.1-4.3</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>277</td>
<td>2.7(0.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC/HDL-C ratio</td>
<td>M</td>
<td>277</td>
<td>3.2(1.1)</td>
<td>0.354</td>
<td>0</td>
<td>7</td>
<td>0.0-7.0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>277</td>
<td>3.3(1.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OGGT (0 hr)</td>
<td>M</td>
<td>134</td>
<td>4.3(0.6)</td>
<td>0.123</td>
<td>3.2</td>
<td>5.4</td>
<td>3.2-5.4</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>F</td>
<td>169</td>
<td>4.3(0.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OGGT (0.5 hrs)</td>
<td>M</td>
<td>134</td>
<td>6.8(1.1)</td>
<td>0.477</td>
<td>4.7</td>
<td>8.9</td>
<td>4.7-8.9</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>F</td>
<td>169</td>
<td>6.8(1.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OGGT (1 hrs)</td>
<td>M</td>
<td>134</td>
<td>7.0(1.5)</td>
<td>0.415</td>
<td>4.4</td>
<td>9.8</td>
<td>4.4-9.8</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>F</td>
<td>169</td>
<td>7.1(1.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OGGT (1.5 hrs)</td>
<td>M</td>
<td>134</td>
<td>6.1(1.2)</td>
<td>0.596</td>
<td>4</td>
<td>8.1</td>
<td>4.0-8.1</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>F</td>
<td>169</td>
<td>6.0(1.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OGGT (2 hrs)</td>
<td>M</td>
<td>134</td>
<td>5.1(0.9)</td>
<td>0.639</td>
<td>3.4</td>
<td>7.2</td>
<td>3.4-7.2</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>F</td>
<td>169</td>
<td>5.3(1.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*= Fasting glucose using plasma specimen, N = gender, x = mean, SD = standard deviation and

Rr = reference range
Figure 1: Seventy five (75) gms oral glucose tolerance test curve for healthy adult Kenyan population.
4.10 Comparison of established fasting profiles and oral glucose tolerance test (OGTT) reference ranges for adult Kenyans with some literature quoted reference ranges

Fasting profiles which includes lipid profile, fasting blood glucose and OGTT were categorized under special biochemical parameters in this study. The studied fasting profiles and OGTT reference ranges were compared with values from Uganda, Nigeria, Kuwait, USA and Germany. No gender differences were observed in the six geographical regions. Male and female have the same fasting lipid profile and fasting blood glucose reference ranges. This study produced a lower fasting blood glucose (FBG) reference range lower limit of 2.1 mmol/L compared to the USA one of 4.1 mmol/L (Table 13). German population has the highest FBG upper reference range limit of 6.4 mmol/L compared to the value for this study of 5.7 mmol/L. The studied Kenyan population has the highest total cholesterol (6.4 mmol/L) upper reference range limit, whilst Uganda, USA and Germany populations have the lowest (5.2 mmol/L) (Table 13). Triglycerides upper reference range limit of the studied Kenyan population of 2.2 mmol/L is similar to that of the German population (2.3 mmol/L), whilst Ugandan population has the highest (4 mmol/L) (Table 13). HDLC upper reference range limit is similar in Kenyan studied population (2.1 mmol/L), Ugandan population (2 mmol/L), Nigerian population (2 mmol/L) and Kuwaitis population (1.9 mmol/L) (Table 13). Among populations being compared, Kenyan population produced the highest LDLC upper reference range limit (4.4 mmol/L). OGTT (0 hr) upper reference range limit is similar for Kenyan population (5.4 mmol/L) and German population (5.5 mmol/L) whilst Ugandan population has the highest (7.6 mmol/L) among the compared groups (Table 13). OGTT (2hr) comparision was done between the Kenyan population and the American population whereby the later
had the highest upper reference range limit (7.8 mmol/L). This study produced a full OGTT profile which was missing in the other five populations (Table 13).

Table 13: Comparison of established fasting profiles and oral glucose tolerance test (OGTT) reference ranges for the adult Kenyans with some literature quoted reference ranges

<table>
<thead>
<tr>
<th>Analyte (unit)</th>
<th>Sex</th>
<th>KE</th>
<th>UG</th>
<th>NG</th>
<th>KW</th>
<th>USA</th>
<th>GER</th>
</tr>
</thead>
<tbody>
<tr>
<td>*FPG (mmol/l)</td>
<td>M/F</td>
<td>2.1-5.7</td>
<td>-</td>
<td>-</td>
<td>3.8-6</td>
<td>4.1-5.9</td>
<td>3.1-6.4</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>M/F</td>
<td>2.9-6.4</td>
<td>2.7-5.2</td>
<td>3.4-5.5</td>
<td>3.3-5.4</td>
<td>&lt;5.2</td>
<td>&lt;5.2</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>M/F</td>
<td>0.4-2.4</td>
<td>0.6-4</td>
<td>0.6-1.6</td>
<td>0.3-2.1</td>
<td>&lt;2.6</td>
<td>&lt;2.3</td>
</tr>
<tr>
<td>HDLc (mmol/l)</td>
<td>M/F</td>
<td>1.1-2.1</td>
<td>0.6-2</td>
<td>0.9-2</td>
<td>0.7-1.9</td>
<td>&gt;1.6</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>LDLc (mmol/l)</td>
<td>M/F</td>
<td>1.1-4.3</td>
<td>1.3-3.6</td>
<td>1.7-3.5</td>
<td>2.6-3</td>
<td>2.6-3.3</td>
<td>&lt;4</td>
</tr>
<tr>
<td>OGTT (0hr) (mmol/l)</td>
<td>M/F</td>
<td>3.2-5.4</td>
<td>3.1-7.6</td>
<td>-</td>
<td>-</td>
<td>&lt;6.1</td>
<td>3.3-5.5</td>
</tr>
<tr>
<td>OGTT (0.5 hr) (mmol/l)</td>
<td>M/F</td>
<td>4.7-8.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>OGTT (1 hr) (mmol/l)</td>
<td>M/F</td>
<td>4.4-9.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>OGTT (1.5 hr) (mmol/l)</td>
<td>M/F</td>
<td>4.8-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>OGTT (2 hr) (mmol/l)</td>
<td>M/F</td>
<td>3.4-7.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;7.8</td>
<td></td>
</tr>
</tbody>
</table>

KE = Kenya, UG = Uganda (Bimenya et al., 2006), NG = Nigeria (Owiredu et al., 2009), KW = Kuwait (Olusi, et al., 2002), USA = United States of America (ADA, 1997) and GER = Germany (Heil et al., 2002).

4.11 Comparison of established adult Kenyan reference ranges for tumour markers, thyroid hormones and fertility hormones with some literature quoted reference ranges

There is no gender difference for the five tumour markers i.e. CA 125, CA19-9, CA15-3, CEA and TPSA in the three regions targeted for comparision. CA 125 and CA 153 upper reference range limits (42 IU/ML and 37 IU/ML respectively) for the studied Kenyan population is higher than that of the compared regions (Table 14). The upper reference
range limit for CEA for the Kenyan population (3.4 IU/ML) is the lowest among the three regions being compared (Table 19). Age group 18-28 yrs and 29-39 yrs have different TPSA reference ranges for the Kenyan population whilst the French and German populations have common reference range for the two age groups (Table 14). Thyroid hormones reference ranges for the Kenyan, French and German adult population have no gender differences. Upper reference range limit for T4 (130 nmol/L) and FT4 (24 pmol/L) are similar for Kenyan and French adult population (Table 15).

Current study established separate PRL reference ranges for menstrual (1.7-23 ng/ml) and menopausal (8 - 29 ng/ml) women groups whilst French (5-35 ng/ml) and German (0-30 ng/ml) produced the same for these two women groups. Adult Kenyan and German male have similar PRL upper reference range limit (17ng/ml). Reference ranges for FSH, LH, E2 and PRG were different in all the adult populations being compared (Table 16).

There were gender differences in TESTO levels for the adult population in the three regions being compared. French and German female TESTO reference ranges (French: 0.1-0.9 ng/ml and German: 0.06-0.82 ng/ml) are common across all adult women age groups. Current study established different Kenyan adult female TESTO reference ranges for follicular phase/luteal phase/menopausal phase (0-2 ng/ml) and ovulation phase (0.7-3.3 ng/ml). The adult male TESTO reference ranges were different in the three populations being compared. French and German adult male TESTO reference ranges (French: 3-10.6 ng/ml and German: 2.84-8 ng/ml) are common across the adult male population. Current study established age group based male TESTO reference ranges.
which were: 18-39 yrs (3.5-12.7 ng/ml), 40-50 yrs (2.5-6.9 ng/ml) and 51-61 yrs (1.8-5.6 ng/ml). Kenyan adult male age group based TESTO reference ranges are different from the established French and German male TESTO reference ranges quoted in literature (Table 16).

Table 14: Comparison of established Kenyan adult reference ranges for tumour markers with some reference ranges quoted in literature

<table>
<thead>
<tr>
<th>Parameter(units)</th>
<th>Gender</th>
<th>Kenyan</th>
<th>French</th>
<th>German</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA125(U/ML)</td>
<td>M/F</td>
<td>11-42</td>
<td>5-35</td>
<td>0-35</td>
</tr>
<tr>
<td>CA15.3(U/ML)</td>
<td>M/F</td>
<td>9-37</td>
<td>0-30</td>
<td>0-22</td>
</tr>
<tr>
<td>CA 19.9(U/ML)</td>
<td>M/F</td>
<td>11-35</td>
<td>0-27</td>
<td>&lt;37</td>
</tr>
<tr>
<td>CEA(nM/ML)</td>
<td>M/F</td>
<td>0.4-3</td>
<td>0-5</td>
<td>&lt;4.6</td>
</tr>
<tr>
<td>PSA(nM/ML)</td>
<td>M (18-28 YRS)</td>
<td>0.1-1.4</td>
<td>0.2-1.7</td>
<td>0.1-3</td>
</tr>
<tr>
<td></td>
<td>M (29-39 YRS)</td>
<td>0.1-2.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>M (40-50 YRS)</td>
<td>1.1-3.5</td>
<td>0.3-2.2</td>
<td>0-2</td>
</tr>
<tr>
<td></td>
<td>M (51-61 YRS)</td>
<td>1.1-6.3</td>
<td>0.3-3.4</td>
<td>0-3</td>
</tr>
</tbody>
</table>

French (Vidas 30418, 2004) German (Heil et al., 2002)

Table 15: Comparison of established Kenyan adult reference ranges for thyroid hormones with some reference ranges quoted in literature

<table>
<thead>
<tr>
<th>Parameter(units)</th>
<th>Gender</th>
<th>Kenyan</th>
<th>French</th>
<th>German</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH (mU/ML)</td>
<td>M/F</td>
<td>0.2-8</td>
<td>0.3-7</td>
<td>0.3-4.2</td>
</tr>
<tr>
<td>T3(nMol/L)</td>
<td>M/F</td>
<td>0.5-2.8</td>
<td>0.9-2.3</td>
<td>1.3-3.1</td>
</tr>
<tr>
<td>T4(nMol/L)</td>
<td>M/F</td>
<td>42-130</td>
<td>50-130</td>
<td>66-181</td>
</tr>
<tr>
<td>FT3(pMol/L)</td>
<td>M/F</td>
<td>2.4-7.2</td>
<td>4-8.3</td>
<td>4-7.8</td>
</tr>
<tr>
<td>FT4(pMol/L)</td>
<td>M/F</td>
<td>6-24</td>
<td>8-24</td>
<td>12-22</td>
</tr>
</tbody>
</table>

French (Vidas 30418, 2004) German (Heil et al., 2002)
Table 16: Comparison of established Kenyan adult reference ranges for fertility hormones with some reference ranges quoted in literature

<table>
<thead>
<tr>
<th>Parameter(units)</th>
<th>gender</th>
<th>Kenyan</th>
<th>French</th>
<th>German</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRL(ng/mL)</td>
<td>menstrual</td>
<td>1.7-23</td>
<td>5-35</td>
<td>0-30</td>
</tr>
<tr>
<td></td>
<td>menopausal</td>
<td>8 - 29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0-17</td>
<td>3-25</td>
<td>0-17</td>
</tr>
<tr>
<td>FSH (μU/mL)</td>
<td>Follicular</td>
<td>4-16</td>
<td>2.9-12</td>
<td>3.5-12.5</td>
</tr>
<tr>
<td></td>
<td>Ovulation</td>
<td>5-20</td>
<td>6.3-24</td>
<td>4.7-21.5</td>
</tr>
<tr>
<td></td>
<td>Luteal</td>
<td>2.5-7</td>
<td>1.5-7</td>
<td>1.7-7.7</td>
</tr>
<tr>
<td></td>
<td>Menopause</td>
<td>10 – 89</td>
<td>17-95</td>
<td>26-135</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0.7-7.5</td>
<td>1.7-12</td>
<td>1.5-12.4</td>
</tr>
<tr>
<td>LH(μU/mL)</td>
<td>Follicular</td>
<td>0.7-8.6</td>
<td>1.5-8</td>
<td>2.4-12.6</td>
</tr>
<tr>
<td></td>
<td>Ovulation</td>
<td>7-83</td>
<td>9.6-80</td>
<td>14-96</td>
</tr>
<tr>
<td></td>
<td>Luteal</td>
<td>0.4-6.8</td>
<td>0.2-6.5</td>
<td>1-11.4</td>
</tr>
<tr>
<td></td>
<td>Menopause</td>
<td>7-32</td>
<td>8-33</td>
<td>7.7-59</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0.5-5.7</td>
<td>1.1-7</td>
<td>1.7-8.6</td>
</tr>
<tr>
<td>E2(pg/mL)</td>
<td>Follicular</td>
<td>0-200</td>
<td>18-147</td>
<td>25-195</td>
</tr>
<tr>
<td></td>
<td>Ovulation</td>
<td>134-467</td>
<td>93-575</td>
<td>66-410</td>
</tr>
<tr>
<td></td>
<td>Luteal</td>
<td>75-351</td>
<td>43-214</td>
<td>40-260</td>
</tr>
<tr>
<td></td>
<td>Menopause</td>
<td>0-35</td>
<td>0-58</td>
<td>10-40</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0-25</td>
<td>0-62</td>
<td>11-44</td>
</tr>
<tr>
<td>PRG (ng/mL)</td>
<td>Follicular</td>
<td>0.04-0.72</td>
<td>0.25-0.54</td>
<td>0-1.6</td>
</tr>
<tr>
<td></td>
<td>Ovulation</td>
<td>0-5</td>
<td>0.25-6.22</td>
<td>0-16</td>
</tr>
<tr>
<td></td>
<td>Luteal</td>
<td>0-18</td>
<td>1.5-20</td>
<td>1.1-21</td>
</tr>
<tr>
<td></td>
<td>Menopause</td>
<td>0-0.38</td>
<td>0-0.41</td>
<td>0-1.4</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0.12-1.02</td>
<td>0.25-0.56</td>
<td>0-1.7</td>
</tr>
<tr>
<td>TESTO(ng/mL)</td>
<td>Fol/Lut/Mp</td>
<td>0-2</td>
<td>0.1-0.9</td>
<td>0.06-0.82</td>
</tr>
<tr>
<td></td>
<td>ovulation</td>
<td>0.7-3.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Male(18-39 yrs)</td>
<td>3.5-12.7</td>
<td>3-10.6</td>
<td>2.84-8.00</td>
</tr>
<tr>
<td></td>
<td>Male(40-50yrs)</td>
<td>2.5-6.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Male(51-61yrs)</td>
<td>1.8-5.6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

French (Vidas 30418, 2004) German (Heil et al., 2002)
4.12 Comparison of established Kenyan adult reference ranges for creatinine clearance with some reference ranges quoted in literature

Established Kenyan adult reference ranges for creatinine clearance was compared with American and German adult populations. Kenyan and German comparison was done using the measured creatinine clearance (Mcrcl) reference ranges whilst Kenyan and American comparison was done using estimated creatinine clearance (Ecrl) reference range. Kenyan population has separate male (52-110 mL/min) and female (50-92 mL/min) reference ranges. German population has a common measured creatinine clearance (Mcrcl) (66-143 mL/min) reference ranges which is higher than either Kenyan male or female measured creatinine clearance reference ranges. Kenya and American adult population have separate male and female estimated creatinine clearance. Estimated creatinine clearance for the American adult population (male: 57-122 mL/min, female: 60-119 mL/min) are higher than Kenyan adult population (male: 54-118 mL/min, female: 58-106 mL/min). Ecrl for the American adult population are higher than that of the Kenyan adult population (Table 17).

Table 17: Comparison of established Kenyan adult reference ranges for creatinine clearance with some reference ranges quoted in literature

<table>
<thead>
<tr>
<th>Parameter(units)</th>
<th>gender</th>
<th>Kenyan</th>
<th>American</th>
<th>German</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mcrcl (ml/min)</td>
<td>M</td>
<td>52-110</td>
<td>-</td>
<td>66-143</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>50-92</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ecrl (ml/min)</td>
<td>M</td>
<td>54-118</td>
<td>57-122</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>58-106</td>
<td>60-119</td>
<td>-</td>
</tr>
</tbody>
</table>
4.13 Comparison of the performance of an automated (Olympus 640 AU) in determining the levels of blood urea nitrogen, calcium, glucose, potassium and sodium using different reagents in serum of referent subjects

The following analytes have more than one analytical method: Blood Urea Nitrogen (Berthelot Reaction, Glutamate Dehydrogenase), Calcium (o-Cresolphthalein, Arsenazo III), Glucose (Gluose oxidase, Hexokinase), Potassium (direct ISE, indirect ISE, calorimetric) and Sodium (direct ISE, indirect ISE, calorimetric). The means difference of the analytical methods for each analyte were statistically compared using paired samples T-test. Testing was done at 95% confidence interval and a “p” value less than or equal to 0.05 was considered statistically significant.

4.13.1 Performance of both the berthelot reaction and glutamate dehydrogenase methods in determining the level of blood urea nitrogen in referent subjects

One hundred and eighty six serum specimens were used for BUN estimation using berthelot and glutamate dehydrogenase methods. The mean and standard deviation of BUN using the Berthelot method was 3.97 mmol/L and 1.08 mmol/L, respectively while that obtained using the Glutamate dehydrogenase method was 3.95 mmol/L and 1.17 mmol/L, respectively. The means difference for these two methods was statistically insignificant (t = 0.24, 95% CI (Lower = -0.1279, Upper = 0.1634), p = 0.810) (Table 18).

4.13.2 Performance of both the o–cresolphthalein (o-CPC) and arsenazo III methods in determining the level serum calcium in referent subjects

One hundred and eighty six specimens were used for Calcium estimation using o–Cresolphthalein and Arsenazo III methods. The mean and standard deviation for o–Cresolphthalein method was 2.35 mmol/L and 0.15 mmol/L, respectively. Arsenazo III
method mean and standard deviation was 2.34 mmol/L and 0.15 mmol/L, respectively. The means difference for these two methods was statistically insignificant ($t = 1.489$, 95% CI (Lower = -0.0059, Upper = 0.0425), $p = 0.138$) (Table 18).

4.13.3 Performance of both the glucose oxidase and hexokinase method in determining the level serum glucose in referent subjects

One hundred and eighty six specimens were used for Glucose estimation using Glucose oxidase and Hexokinase methods. The mean and standard deviation for Glucose oxidase method was 3.89 mmol/L and 0.98 mmol/L, respectively. Hexokinase method mean and standard deviation was 3.88 mmol/L and 0.95 mmol/L, respectively. The means difference for these two methods was statistically insignificant ($t = 0.48$, 95% CI (Lower = -0.0418, Upper = 0.0687), $p = 0.632$) (Table 18).

4.13.4 Performance of both the direct ion selective electrode (ISE) and indirect ion selective electrode in determining the level of serum potassium in referent subjects

One hundred and eighty six specimens were used for Potassium estimation using direct ion selective and indirect ion selective methods. The mean and standard deviation for direct ion selective method was 4.27 mmol/L and 0.43 mmol/L, respectively. Indirect ion selective method mean and standard deviation was 4.26 mmol/L and 0.44 mmol/L, respectively. The means difference for these two methods was statistically insignificant ($t = 0.23$, 95% CI (Lower = -0.0408, Upper = 0.0516), $p = 0.819$) (Table 18).
4.13.5 Performance of both the direct ion selective electrode and colorimetric methods in determining the level of serum potassium in referent subjects

One hundred and eighty six specimens were used for Potassium estimation using direct ion selective and Calorimetric methods. The mean and standard deviation for direct ion selective method was 4.27 mmol/L and 0.43 mmol/L, respectively. Calorimetric method mean and standard deviation was 3.9 mmol/L and 0.45 mmol/L, respectively. The means difference for these two methods was statistically significant ($t = 12.58$, 95% CI (Lower = 0.308, Upper = 0.422), $p < 0.001$) (Table 18).

4.13.6 Performance of both the indirect ion selective electrode and colorimetric methods in determining the level of serum potassium in referent subjects

One hundred and eighty six (186) specimens were used for Potassium estimation using Indirect ion selective and Calorimetric methods. The mean and standard deviation of serum potassium using the Indirect ion selective method was 4.26 mmol/L and 0.44 mmol/L, respectively while that obtained using the Calorimetric method was 3.9 mmol/L and 0.45 mmol/L, respectively. The means difference for these two methods was statistically significant ($t = 11.86$, 95% CI (Lower = 0.299, Upper = 0.419), $p < 0.001$) (Table 18).

4.13.7 Performance of both the direct ion selective electrode and indirect ion selective electrode methods in determining the level of serum sodium in referent subjects

One hundred and eighty six (186) specimens were used for Sodium estimation using direct ion selective and indirect ion selective methods. The mean and standard deviation of serum sodium using the Direct ion selective method was 140.7 mmol/L and 3.8
mmol/L, respectively while that obtained using the Indirect ion selective method was 140.6 mmol/L and 4.2 mmol/L, respectively. The means difference for these two methods was statistically insignificant ($t = 1.041$, 95% CI (Lower = -0.1492, Upper = 0.4825), $p = 0.299$) (Table 18).

4.13.8 Performance of both the direct ion selective electrode and calorimetric methods in determining the level of serum sodium in referent subjects

One hundred and eighty six specimens were used for sodium estimation using direct ion selective and calorimetric methods. The mean and standard deviation of serum sodium determined using the direct ion selective method was 140.7 mmol/L and 3.8 mmol/L, respectively while that determined using the calorimetric method was 137 mmol/L and 4.8 mmol/L, respectively. The means difference for these two methods was statistically significant ($t = 13.32$, 95% CI (Lower = 3.219, Upper = 4.339), $p < 0.001$) (Table 18).

4.13.9 Performance of both the indirect ion selective electrode and calorimetric methods in determining the level of serum sodium in referent subjects

One hundred and eighty six (186) specimens were used for Sodium estimation using indirect ion selective and calorimetric methods. The mean and standard deviation of serum sodium determined using the Indirect ion selective method was 140.6 mmol/L and 4.2 mmol/L, respectively while that obtained using the calorimetric method was 137 mmol/L and 4.8 mmol/L, respectively. The means difference for these two methods was statistically significant ($t = 14.17$, 95% CI (Lower = 3.109, Upper = 4.116), $p < 0.001$) (Table 18).
Table 18: Performance of an automated (Olympus 640 AU) in determining the levels of blood urea nitrogen (BUN), calcium (CAL), glucose (GLU), potassium (POT), and sodium (SOD) in serum of referent subjects using different reagents

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Method</th>
<th>N</th>
<th>x(SD)</th>
<th>MD</th>
<th>95% CI</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mmol/L)</td>
<td>Berthelot</td>
<td>186</td>
<td>3.97(1.08)</td>
<td>0.17</td>
<td>0.0163</td>
<td>0.24</td>
<td>0.810</td>
</tr>
<tr>
<td></td>
<td>GLDH</td>
<td></td>
<td>3.95(1.17)</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAL (mmol/L)</td>
<td>o-CPC</td>
<td>186</td>
<td>2.35(0.15)</td>
<td>0.02</td>
<td>-0.006</td>
<td>0.043</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td>Arsenazo III</td>
<td></td>
<td>2.33(0.15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLU (mmol/L)</td>
<td>GoD</td>
<td>186</td>
<td>3.89(0.98)</td>
<td>0.01</td>
<td>-0.042</td>
<td>0.069</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>HK</td>
<td></td>
<td>3.88(0.95)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POT (mmol/L)</td>
<td>Direct ISE</td>
<td>186</td>
<td>4.27(0.43)</td>
<td>0.01</td>
<td>-0.041</td>
<td>0.052</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Indirect ISE</td>
<td></td>
<td>4.26(0.43)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POT (mmol/L)</td>
<td>Direct ISE</td>
<td>186</td>
<td>4.27(0.43)</td>
<td>0.37</td>
<td>0.308</td>
<td>0.422</td>
<td>12.58</td>
</tr>
<tr>
<td></td>
<td>Calorimetric</td>
<td></td>
<td>3.90(0.45)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POT (mmol/L)</td>
<td>Indirect ISE</td>
<td>186</td>
<td>4.26(0.43)</td>
<td>0.36</td>
<td>0.299</td>
<td>0.419</td>
<td>11.86</td>
</tr>
<tr>
<td></td>
<td>Calorimetric</td>
<td></td>
<td>3.90(0.45)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (mmol/L)</td>
<td>Direct ISE</td>
<td>186</td>
<td>140.7(3.8)</td>
<td>0.1</td>
<td>-0.149</td>
<td>0.483</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>Indirect ISE</td>
<td></td>
<td>140.6(4.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (mmol/L)</td>
<td>Direct ISE</td>
<td>186</td>
<td>140.7(3.8)</td>
<td>3.8</td>
<td>3.22</td>
<td>4.33</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>Calorimetric</td>
<td></td>
<td>136.9(4.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (mmol/L)</td>
<td>Indirect ISE</td>
<td>186</td>
<td>140.6(4.2)</td>
<td>3.7</td>
<td>3.11</td>
<td>4.11</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>Calorimetric</td>
<td></td>
<td>136.9(4.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as Mean (x) ± standard deviation (SD) of the number of subjects shown in the column labeled N; GLDH = Glutamate Dehydrogenase, GoD = glucose oxidase, HK = hexokinase; L = lower, U = upper; ISE = ion selective electrode.

4.14 Comparison of the performance of an automated (Olympus 640 AU) and a manual spectrophotometer (Hitachi spectrophotometer) in determining the levels of total protein, albumin, total bilirubin, and alkaline phosphatase activity in serum of referent subjects

The instruments used for analysis comparison were an automated (Olympus 640 AU) and manual instrument (Hitachi spectrophotometer). Total protein, albumin and total bilirubin represented end point reaction methods whilst alkaline phosphatase represented
kinetic reaction method. Each parameter was analyzed using a total of 186 serum samples by the two instruments and the means difference of the results evaluated statistically using paired samples T-test. Testing was done at 95% confidence interval and a “p” value less than or equal to 0.05 was considered statistically significant.

4.14.1 Analysis of total protein levels using an auto analyzer and a manual analyser
The mean and standard deviation for total protein using the auto analyzer was 71.6 g/L and 5.2 g/L, respectively while that of the manual analyser was 67.7 g/L and 5.5 g/L, respectively. The means difference for TP using these two instruments was statistically significant ($t = 14.9$, 95% CI (Lower = 3.37, Upper = 4.4), $p < 0.001$) (Table 19).

4.14.2 Analysis of albumin levels using an auto analyzer and a manual analyser
The mean and standard deviation for albumin using the auto analyzer were 43.1 g/L and 4.4 g/L, respectively while that of the manual analyser was 39.1 g/L and 4.2 g/L, respectively. The means difference for ALB using these two instruments were statistically significant ($t = 16.4$, 95% CI (Lower = 3.5, Upper = 4.5), $p < 0.001$) (Table 19).

4.14.3 Analysis of total bilirubin levels using an auto analyzer and a manual analyser
The mean and standard deviation for T-BILI using the auto analyzer were 10.9 μmol/L and 4.6 μmol/L, respectively while that of the manual analyser was 8.6 g/L and 4.3 g/L, respectively. The means difference for TBILI using these two instruments were
statistically significant ($t = 15.8$, 95% CI (Lower = 2.0, Upper = 2.6), $p < 0.001$) (Table 19).

4.14.4 Analysis of alkaline phosphatase activity using an auto analyzer and a manual analyser

The mean and standard deviation for ALP using the auto analyzer were 154.5 U/L and 34.4 U/L, respectively while that of the manual analyser was 146.4 U/L and 34.8 U/L, respectively. The means difference for ALP using these two instruments were statistically significant ($t = 7.45$, 95% CI (Lower = 5.93, Upper = 10.2), $p < 0.001$) (Table 19).

Table 19: Performance of an automated (Olympus 640 AU) and a manual spectrophotometer (Hitachi spectrophotometer) in determining the levels of total protein, albumin, total bilirubin, and alkaline phosphatase activity in serum of referent subjects

<table>
<thead>
<tr>
<th>Parameter( unit)</th>
<th>Instrument</th>
<th>N</th>
<th>Mean (SD)</th>
<th>MD</th>
<th>95% CI</th>
<th>t-value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (g/L)</td>
<td>Automated</td>
<td>186</td>
<td>71.6(5.2)</td>
<td>3.9</td>
<td>3.4</td>
<td>4.4</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>Manual</td>
<td></td>
<td>67.7(5.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>Automated</td>
<td>186</td>
<td>43.1(4.4)</td>
<td>4.0</td>
<td>3.5</td>
<td>4.5</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>Manual</td>
<td></td>
<td>39.1(4.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBILI (μmol/L)</td>
<td>Automated</td>
<td>186</td>
<td>10.9(4.6)</td>
<td>2.3</td>
<td>2.0</td>
<td>2.6</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>Manual</td>
<td></td>
<td>8.6(4.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>Automated</td>
<td>186</td>
<td>154.5(34.4)</td>
<td>8.1</td>
<td>5.9</td>
<td>10.2</td>
<td>7.45</td>
</tr>
<tr>
<td></td>
<td>Manual</td>
<td></td>
<td>146.4(34.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = number, SD = standard deviation, MD = means difference, CI = confidence interval
CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Quality control of the analytical work

It is important to note that the analytical methods (including instruments, reagents, calibration standards, type of raw data and calculation methods) which were used in the establishment of reference ranges for the forty five biochemical parameters were in all aspects similar to those used in routine analysis of patient’s specimens. Likewise the internal quality control carried out in the establishment of reference ranges was treated the same way as when the patient’s specimens are analyzed. Otherwise, failure to include internal quality control in the analysis would have meant that the various procedural processes playing part in error creation would not have been detected and eventually rectified. The importance of quality control in the analytical work in clinical laboratories has so far been emphasized being the only way of ensuring reliability and precision (Bolann and Omenas, 1997).

The study quality control results for ALT, BUN, CAL, GLU, T3, FT3, LH, PRG, PRL, CA 19-9, CEA, TESTO, TPSA, TG and HDLC for the whole study period produced a control mean value similar to the assigned quality control mean value of each parameter. The reliability of the reference ranges for the studied parameters was further confirmed by the fact that the daily quality control results were within quality control range i.e. mean ± 2SD.
5.1.2 Establishment of national reference ranges, determination of gender difference and comparison with literature quoted reference ranges

Current study presents reference ranges for forty five biochemical parameters for Kenyan adult population which has not been reported previously in East and Central region of Africa. A major step has been achieved by this study in the establishment of reference ranges for creatinine clearance, fasting blood glucose, oral glucose tolerance test, thyroid hormones, fertility hormones and tumour makers which has never been carried out in this region according to available literature. Previous studies carried out in Kenya developed reference ranges of a few biochemical parameters for adult population in two selected locations. Kibaya *et al.*, 2008) developed reference ranges for sixteen biochemical parameters for a rural adult population in Kericho whilst Waithaka *et al.*, 2009) developed reference ranges for twelve biochemical parameters for adult population in Kenyatta National Hospital. These two studies in Kericho and Kenyatta National Hospital had an adult population of 1,541 and 1,100 respectively.

The current study has a population of 6,795 healthy adults with an equal female (3,378 (49.9%) and male (3,389 (50.1%) representation drawn from all regions of Kenya. Other studies reported elsewhere have used small number of study population. Elmar *et al.* 2008 used 301 adult study subjects from Mbeya region of Tanzania to develop reference ranges for twenty five biochemical parameters. Bimenya *et al.* 2006 used 183 adult study subjects from Kampala region of Uganda to develop reference ranges for blood glucose and lipids.
5.1.3 Establishment of reference ranges for routine biochemical parameters

All the reference ranges for routine biochemical parameters established in this study are different from literature based reference ranges established elsewhere in Germany, USA, North Ireland Republic and Tanzania. This difference is based on the upper and lower reference range limits of each biochemical parameter. Biochemical parameters reference range limits are used in making clinical decisions which can be adversely affected whenever literature based reference range limits are used. For instance, Alpha-Amylase is the only diagnostic parameter that investigations pancreatic disorders whose adult Kenyan reference range has been established in this study. Like the other enzymes, the main interest is only on elevated values since this is what signifies a pathological disorder. Compared with reference ranges from other geographical regions, the upper reference range limit for Germans and Tanzanians are higher than that of the Kenyans. On the other hand, Kenyan reference range upper limit for alpha amylase is higher than that of the Americans and the Irish. The differences in the upper reference range limit would amount to mis-interpretation of alpha amylase laboratory report of an individual whenever inappropriate reference range is used. It is evident from the findings of this study that different regions have different biochemical parameter reference ranges therefore interpretation of laboratory reports should be based on reference ranges established from healthy population the laboratory serves.

This study did not find gender differences in:- ALB, ALP, AMY, HCO₃⁻, CAL, CL, DBILI, GLU, MG, PHOS, POT, TP, SOD, TBILI and BUN, therefore, common male and female reference ranges were established for each of these parameters. Studies
carried out elsewhere in Germany, USA, North Ireland Republic and Tanzania had similar findings with the current study (Heil et al., 2002, Gardner et al., 1993, Elmar et al., 2008). ALT, AST and CPK reference ranges for studied Kenyan population showed no gender disparity as opposed to the German, American and Irish populations.

Gender disparity was evident for the parameters CREAT, GGT, LDH and UA which warranted the establishment of separate male and female reference ranges for Kenyan population. These study findings were similar to studies carried out elsewhere in Germany, USA, North Ireland Republic and Tanzania on the same biochemical parameters.

5.1.4 Establishment of reference ranges for thyroid hormones, fertility hormones, tumour markers, creatinine clearance, oral glucose tolerance test and lipid profile

Routine clinical chemistry laboratory is not able to handle all the requested parameters due to lack of facilities required for analyzing these parameters. These parameters are therefore referred to specialized laboratory for analysis. Some tests require special procedures to have been fulfilled before the specimen is taken from an individual. Referred parameters and those that require special treatment of an individual before a specimen is taken are collectively referred to as special biochemical parameters. Special biochemical parameters whose reference ranges were established in this study are thyroid hormones (TSH, T3, T4, FT3, FT4), fertility hormones (FSH, LH, EII, PRG, PRL, TESTO), tumour makers (CA 125, CA 19-9, CA 15-3, CEA, TPSA), creatinine clearance, oral glucose tolerance test and lipid profile.
5.1.5 Established thyroid hormones for the adult Kenyans

Equal number of male and female was used in the establishment of reference ranges for thyroid hormones in the current study. Common reference range for each parameter was established since there was no gender difference observed ($p > 0.05$). In the diagnosis, treatment and management of thyroid hormones disorders ie hypothyroidism and hyperthyroidism the use of proper reference range is of paramount important. Established reference ranges for adult Kenyan population were compared with literature based values quoted in the diagnostic reagent kit. Incidentally, the reagent kits currently used in the Kenyan health institutions originates either from France (bioMerieux) or Germany (Roche diagnostic).

TSH reference ranges for the three geographical regions under comparison have no gender differences. In this regard, TSH reference range is common for both male and female. TSH reference range lower limit is in agreement between the French and German populations and differs slightly from the Kenyan studied population. The implication of these lower limits of TSH is that hyperthyroidism is diagnosed at almost the same level in the three geographical regions. Marked difference is observed between the TSH upper reference range limit for the Kenyan population and German population. This difference suggests that a Kenyan individual being investigated for hypothyroidism using the German reference range will definitely be over diagnosed. TSH reference range upper limit for the Kenyan population is almost the same as that of the French population. This similarity between Adult Kenyan population and the French population for TSH reference upper range limit suggests that hypothyroidism would be diagnosed at the same level.
T3 is another important thyroid hormone used in the diagnosis of thyroid gland disorders. Gender difference was not observed; therefore a common reference range was established for the adult Kenyan population. Likewise, T3 reference range for the German and French populations which are being compared with the Kenyan population have no gender differences. Upper T3 reference range limit which is considered in the diagnosis of hyperthyroidism is higher in the Kenyan population as opposed to that of French population. On the other hand, quoted T3 reference range upper limit for the German population is higher compared with the other two populations. These differences brought about by the geographical factors suggests that there is a possibility of under diagnosing hyperthyroidism in an individual from Kenya or France using T3 reference range of the German population. Current study established T3 reference range lower limit which is the lowest among the other two compared populations. This means that a Kenyan individual being suspected of hypothyroidism will definitely be under diagnosed whenever literature based T3 lower limit is used for interpretation.

T4 is an integral part of thyroid function tests. The upper reference range limit for the Kenyan population is similar to that of the French population suggesting that hyperthyroidism would be diagnosed at the same level in these two different populations. The German population T4 reference range upper limit is higher than for the other two populations meaning that hyperthyroidism would be under diagnosed in both Kenyan and French populations in an event that German reference range is used for the T4 laboratory report interpretation. Same case applies to the diagnosis of hypothyroidism since T4 reference range lower limit for the Germans is greater than both Kenyan and French. This
differences means that hypothyroidism will be under diagnosed in Kenyan and French populations by using German reference range for interpretation of a T4 laboratory report.

FT3 reference range for adult Kenyan population is lower than the German and French adult populations. These differences suggest that both hypothyroidism and hyperthyroidism in the Kenyan population would be under diagnosed when reference is made using the compared two populations. Similarity exists between FT4 reference range upper limit of the studied Kenyan population with French population suggesting that interpretations for hyperthyroidism can be done at the same level. Over diagnosis of hyperthyroidism would be evident whenever the German adult population FT4 reference range upper limit is used to interpret the Kenyan FT4 reports. Kenyan FT4 reference range lower limit differs from that of the French population. Under diagnosis of hypothyroidism is likely if FT4 results for the Kenyan population are interpreted using French population FT4 reference range lower limit. Same situation would be witnessed if the German population FT4 reference range lower limit is used to interpret Kenyan adult population FT4 laboratory reports.

5.1.6 Established fertility hormones for the adult Kenyans

Establishment of adult Kenyan male and female reference ranges for fertility hormones in the current study was important in an effort to address infertility problems affecting the Kenyan population. Infertility pathological disorders that warranted the establishment of fertility hormones reference ranges were impotence and gynecomastia affecting the male population, amenorrhea (primary and secondary), hirsutism and virilism affecting the
female population. Separate male and female fertility hormones were established due to
gender differences. The established female fertility hormones reference ranges had in
consideration changes in hormonal patterns during the menstrual cycle. In this regard,
female established fertility hormones were divided into four phase’s i.e. follicular phase,
ovulation phase, luteal phase and menopause.

Current study established male testosterone reference ranges in relation to various age
groups. No differences that were observed between age group 18-28 yrs and 29-39 yrs
therefore a common reference range was established. Separate reference ranges were
established for age groups 40-50 yrs and 51-61 yrs due to the difference observed in this
study. Testosterone which is predominantly a male fertility hormone decreases in
advance with age. This decrease was evident in this study, considering the mean decrease
from 8.13 ng/mL in age group 18-39 yrs to 3.71 ng/mL in age group 51-61 yrs.
Investigation of hypogonadism disorders in adult Kenyan male population is achieved by
considering the testosterone reference range lower limits of each age group. Levels above
the established male testosterone reference range upper limit are not of clinical
significance since there are no known pathological disorders associated with
hypergonadism in adult males. There was no agreement between the findings of the
current study with studies carried out elsewhere in terms of age group differences. Heil et
al., 2002, established a common male testosterone reference range for German population
as opposed to Kenyan age group based testosterone reference ranges. A study by
BioMerieux group 2004, established a common male testosterone reference ranges for
French population which also differed with the age group based Kenyan population. The
German and French adult male testosterone reference ranges quoted in literature are some of the references made in the interpretation of laboratory testosterone reports in Kenyan health institutions.

Current study established female testosterone reference range based on the phases of the menstrual cycle. There was no difference in testosterone levels for follicular phase, luteal phase and menopause. Therefore a common adult female testosterone reference range was established. Ovulatory phase testosterone levels were different from menstrual and non-menstrual phases of an adult female. This difference leads to the establishment of ovulatory phase testosterone reference range. High levels of ovulatory testosterone are physiologically important to aid the ovulation process in healthy adult female. Study findings from other geographical regions did not express this physiological importance of testosterone during ovulation. Common female adult testosterone reference range was established for German and French adult female populations. The German and French adult female testosterone reference ranges quoted in literature are some of the references made in the interpretation of laboratory testosterone reports in Kenyan health institutions.

Prolactin reference ranges for the Kenyan female subjects was established for the menstrual and menopausal female groups. Differences in prolactin concentration for these groups were observed whereby it was increased in the menopausal females. In menstrual female the reference range upper limit is clinically important when dealing with the effects of hyperprolactaemia. Based on the findings of this study, a prolactin concentration of greater than 23 ng/mL in a Kenyan female of reproductive age is an
indicator of infertility. On the other hand, hypoprolacteamia which means a value of below 1.7 ng/mL would be of no clinical importance in a menstrual Kenyan female. Established prolactin reference range upper limit of Kenyan adult female population is lower than French and German adult females as quoted in literature. Using the French and German prolactin reference range to diagnose a case of hyperprolacteamia in a female Kenyan would result with under diagnosing the individual. Such individual would have all the clinical features of hyperprolacteamia with misleading laboratory results, since the literature based reference range does not represent the female Kenyan population.

Although prolactin is not a predominant male fertility hormone, hyperprolacteamia is known to cause impotence. According to the established male prolactin reference range for the Kenyan population, hyperprolacteamia is indicated by concentrations above 17 ng/mL. As in females hypoprolacteamia in males is of no clinical importance. Kenyan male population has similar prolactin reference range as male German population. This means that prolactin reference range can be used to interpret prolactin results for either male population. On the other hand the Kenyan and German male prolactin levels would be misinterpreted and under diagnosed in an event that prolactin report is interpreted using French reference range.

Current study established reference range of FSH based on the menstrual cycle phases. Kenyan female population shows a rise of FSH in the follicular and ovulation phases of the menstrual cycle leading to a major decline in the luteal phase. The increase of FSH in
the follicular phase is of clinical importance since the hormone has a responsibility of controlling the follicular phase of the menstrual cycle. Considering the FSH means for the three menstrual cycle phase ie follicular, ovulation and luteal phase respectively, there is a pattern of increase in the follicular phase then a pick in the ovulation phase and a decline in the luteal phase. This pattern corresponds with FSH physiological activity pattern within the menstrual cycle. Hypogonadism and hypergonadism would be detected at different levels of the menstrual cycle of the Kenyan adult female population based on the upper and lower reference range limits. Other populations compared with the Kenyan population also express the same physiological patterns but differing in the FSH levels of specific phase of the menstrual cycle.

Current study findings of high levels of FSH in circulation during menopause due to elimination of the negative feedback effect on the pituitary by the failure of ovarian estrogen production, is in agreement with other studies reported in literature (Burger, 1994). Despite being predominantly a female reproductive hormone, FSH plays a great role in male adult by stimulating the process of spermatogenesis. Primary hypogonadism in adult Kenyan male (eg impotence) would be detected at FSH levels less than 0.7 μIU/mL or a value greater than 7.5 μU/mL. Low level of FSH in male interferes with the process of spermatogenesis whilst high levels affects testosterone functions. Under diagnosis of primary hypogonadism would be made on male adult Kenyan when interpretation is made using either the French or the German adult male reference ranges of FSH.
It is evident from the findings of this study that the concentration of LH in a healthy female of reproductive age depends on the phases of a menstrual cycle. The LH concentration pattern produced by LH means for the three menstrual cycle is similar to that of FSH ie there is a pattern of increase in the follicular phase then a peak in the ovulation phase and a decline in the luteal phase. This pattern is in line with LH physiological activity pattern within the menstrual cycle. Hypogonadism and hypergonadism would be detected at different levels of the menstrual cycle of the adult female Kenyan population based on the upper and lower reference range limits. Despite the differences in LH references ranges of the menstrual cycle phases, other studies have produced similar patterns to that of the current study (Heil et al., 2002). Current study findings of high levels of LH in circulation during menopause due to elimination of the negative feedback effect on the pituitary by the failure of ovarian estrogen production, is in agreement with other studies reported in literature (Burger, 1994). LH reference range established in this study for the adult Kenyan male population will detect primary hypogonadism in concentrations less than 7 μU/mL and above 32.7 μU/mL. Low level of LH in male interferes with the process of spermatogenesis whilst high levels affects testosterone functions. Under diagnosis of primary hypogonadism will be made on male adult Kenyan when interpretation is made using either the French or the German adult male reference ranges for LH.

It is evident from the current study that EII concentration levels are low during the follicular phase with a significant surge at ovulation phase and a decline in luteal phase. This pattern corresponds with the physiological activities of EII in a normal female of
productive age. The same pattern is expressed by other studies carried out elsewhere in France (BioMerieux, 2004) and Germany (Heil et al., 2002). This study has established that the EII production decreases in menopause compared with the reproductive period of a female. The mean EII concentration during menopause was very low compared with the concentration in the phases of the menstrual cycle. This EII low concentration could be attributed to significant alterations of hypothalamic-pituitary feedback mechanisms in addition to decreased ovarian function. All the three geographical regions; Kenya, France and Germany are in agreement on the EII concentration patterns during menstrual cycle and low levels during menopause and in men. It is also clear that EII reference ranges differs from one region to another, which therefore dictates the need to use relevant reference range for the interpretation of EII clinical laboratory report.

Current study produced a PRG pattern of low concentrations in the follicular phase followed by a steady rise in the ovulation phase and a high peak in the luteal phase. This pattern corresponds with the physiological pattern of PRG as seen in a healthy adult female of reproductive age (Conneely et al., 2004). Similar studies reported in literature and compared with the findings of the current study have a similar PRG pattern despite the concentration levels of specific menstrual cycle phase. It is evident from the study findings that PRG concentrations in the menopausal female population are very low. The same picture of low concentrations of PRG in menopausal females is portrayed by the findings from other geographical regions in Germany and France.
Adult males have levels similar to those in women during the follicular phase of the menstrual cycle (Dennerstein et al., 2003). Studies by Heil et al 2002 in German population and BioMerieux, 2004 in French population concurred with this similarity between PRG levels in male and in follicular phase in female. Current study differed with this since the PRG levels for males and female follicular phase were different (p<0.001). These differences could be due to the geographical localities of the three populations. Kenya male population PRG reference range; was found to be different from Germany and France male PRG reference ranges. In the investigation of male hypogonadism each region should use reference range of male PRG established from its healthy male population.

5.1.7 Established tumour markers reference ranges for adult Kenyans

This study established reference ranges for CA 125, CA 15-3, CA 19-9, CEA and TPSA. A common reference range for each parameter was established since male and female had no statistical difference (p>0.05). Inclusion of males in the establishment of CA 125 and CA 15-3 reference ranges was necessary since the two parameters are used in the diagnosis and management of lung cancer. In the diagnosis and management of various cancer related pathological disorders, upper reference range limit of specific tumour marker is a major tool for interpretation. Lower reference range limit of any tumour marker is of no clinical significance but a sign of good health. It is worth to mention that clinical chemistry quantitative laboratory results alone can not make a conclusive cancer diagnosis. Other tools such as histological tissue examinations and radiological procedures should be used in conjunction with the clinical chemistry quantitative
laboratory results. This is the first study carried out in Kenya to establish tumour maker reference ranges that will make the diagnosis of cancer independent of literature quoted reference ranges. Consequently, the findings of this study can be used to determine risk, screen for early cancer, estimate prognosis, predict if a specific therapy is effective, or monitor for disease recurrence or progression.

The greatest challenge in dealing with cancer of the ovary like any other type of cancer is failure of diagnosing at an early stage, resulting with majority of patients with advanced stage of ovarian carcinoma dying of the disease. Studies on cancer have been geared into improving diagnostic tools for early cancer detection. Population based reference range is one of these diagnostic tools used for early cancer detection. Since cancer of the ovary is female oriented, current study established reference range of CA125 for adult female Kenyan population. An adult Kenyan female being investigated for ovarian cancer would be diagnosed at CA 125 levels above 42 U/mL. CA 125 upper reference range limit of the Kenyan adult population is higher than that of the French and German populations. Incidentally, French and Germans CA 125 tumour marker reference ranges quoted in diagnostic kits are currently used for diagnosis and management of ovarian cancer in the Kenyan health institutions. Consequently, the female Kenyan population has always been over diagnosed by the use of reference range for CA 125 quoted in the diagnostic kits. The findings of this study will revolutionize the interpretation of CA 125 laboratory report by making it independent from literature based reference range. An adult Kenyan female being investigated for breast cancer would be diagnosed at CA 15-3 levels above 37 U/mL. CA 15-3 upper reference range limit of the Kenyan adult population is higher
than that of the French and German populations. The use of literature based CA 15-3 reference ranges quoted in diagnostic kits from Germany and America over diagnoses the Kenyan female population being investigated for breast cancer. The established CA 153 reference range for the adult female Kenyan will revolutionize the diagnosis and management of breast cancer in Kenya. This Kenyan based CA 153 reference range would be an asset for future research work on breast cancer in Kenya.

In the diagnosis, treatment and management of cancer of the pancreas, CA 19-9 is the best parameter. A lot of information in literature on pancreatic cancer is based on the Americans, Germans and the Britons, since these populations use their own diagnostic and management tools based on their healthy populations. Developing countries, Kenya inclusive have very scarce information on pancreatic cancer. For example, Kenyan health institution use CA 19-9 literature quoted reference ranges for French to diagnose and manage pancreatic cancer in the adult Kenyan population. The upper reference range limit for the French adult population is lower than that established in this study. Considering the fact that CA 19-9 reference range for the French population has always been used to make diagnosis and manage pancreatic cancer in the adult Kenyan population, it can therefore be concluded that there has been over diagnosis of the Kenyan individuals. On the other hand, pancreatic cancer would be under diagnosed whenever German based CA19-9 reference range upper limit is used for interpretation of a laboratory report of a Kenyan individual. It is evident from the findings of this study that reference ranges are affected by the geographical locality of a population as shown by reference ranges of Kenyans, Germans and French populations. With the
establishment of Kenyan based reference range of CA 19-9, proper diagnosis and management of pancreatic cancer would hence forth be achieved and any further dependency of literature based reference range will gradually cease.

Colorectal cancer (CRC) is the third most common cancer worldwide. This cancer is diagnosed in clinical laboratory by the estimation of CEA. The upper CEA reference range limit; 3.4 ng/mL established in the current study is lower than those from Germany and France. A Kenyan individual would be under diagnosed for colorectal cancer in an event the French and German reference range is used for interpretation of laboratory report for a Kenyan individual. These three different reference ranges for Kenyans, Germans and French adult populations is a clear indication of the effect geographical location have on reference ranges. It is therefore appropriate that every individual report should be interpreted using relevant CEA reference range, in order to avoid any misdiagnosis of colorectal cancer.

The current study developed reference ranges of TPSA based on the age group categories of adult male population. It is evident from the current study findings that TPSA concentration increases with age. This finding concurs with the findings of other studies carried out in Frech and German populations. There are two differences observed between the Kenyan population and the other compared populations. First, upper reference range limit which is used to diagnose prostrate cancer, the levels of the Kenyan population are higher than those of the other compared populations. Secondly, the current study developed separate TPSA reference range for age groups 18-28 yrs and
29-39 yrs. The French and Germans populations' each has a common reference range for the two age groups categories. Since the diagnostic TPSA upper reference range limits for the Kenyan population is higher than the French and German populations, it therefore implies that the Kenyan male adult population would be over diagnosed by the use of French and German reference ranges for interpretation of Kenyans TPSA results.

5.1.8 Established reference ranges for fasting profiles and OGTT for adult Kenyans

Current study presents reference ranges for fasting profiles for Kenyan adult population which have not been reported previously in this region of Africa. Studies reported have been on analytes investigated using random specimens. With the large number of study participants of both male and female, the study did not find any gender differences on the studied analytes. Due to the differences between capillary and venous blood glucose, the study established two different types of fasting glucose reference ranges. This was necessary since the two type of glucose analyses are commonly adopted in routine laboratories (David et al., 2002). A study by Roche Diagnostics had fasting capillary blood glucose reference range for adult as 3.3-5.5 mmol/L which was similar with the current study reference range of 3.2-5.4 mmol/L. The current study reference ranges of capillary whole blood differs substantially from that of Ugandan population given as 3.11-7.55 mmol/L, despite the proximate of the two regions.

The fasting plasma glucose reference range established by Roche Diagnostic (3.1-6.4 mmol/L) differs from the current study reference range of 2.1-5.7 mmol/L. The two studies carried out on different populations are in agreement that fasting capillary blood
glucose differs from fasting plasma glucose, with the former being higher (David et al., 2002) as in the case with their corresponding reference ranges. Other documented reference ranges for fasting plasma glucose which differs from that established in this study are those carried out among Kuwaitis (3.75-6.03 mmol/L) (Olusi and Al-Awadhi, 2002), Americans (4.1-5.9 mmol/L) (ADA, 1997) and Israelitis (2.7-5.5 mmol/L).

All along the clinical laboratories in Kenya have been using the WHO recommended reference ranges for OGTT (David et al., 2002). This is the first study on OGTT reference ranges to be carried out in Kenya. The usefulness of OGTT as a gold standard for diagnosing diabetes mellitus (type 1 and 2) has been dropped by the American Diabetes Association (ADA) who only recommends its use for diagnosing gestation diabetes mellitus. Due to these different recommendations by WHO and ADA, it was therefore important to establish Kenyan based OGTT reference ranges. The fact that glucose concentrations during an OGTT in capillary blood are significantly higher than those in venous blood by 20-25% or a mean difference of 1.7 mmol/L (David et al., 2002, Larsson, 1976), is an important factor to consider in comparing the current study findings with what has been published in literature. Despite the fact that the current study did not use venous blood specimen to establish OGTT reference values, it is obvious that the values would be lower than what has been produced using the capillary blood specimens. For example, the 2 hr OGTT capillary blood value of 8 mmol/L translates to 6-6.4 mmol/L venous blood glucose which is lower than WHO recommended value of 7.8 mmol/L. Considering these venous blood glucose values would mean that the impaired
glucose tolerance phase for the Kenyan adult population is greater, therefore avoiding under diagnosis of diabetes and impaired glucose tolerance.

On lipid profile, the current study did not find any gender differences thus the reference ranges established are common in both male and female, which is in consistent with other reports in literature (Owiredu et al., 2009, Bimenya et al.2006). The lipid profile parameters reference ranges upper limits of the current study are higher than manufacturer’s reference values that are currently in use in Kenyan’s clinical laboratories, apart from the triglycerides value. For example the reference range upper limit of total cholesterol in the studied Kenyan population (6.4 mmol/L) is greater than the Manufacturer’s reference range upper limit of 5.2 mmol/L. These differences suggest that Kenyan population have always been over diagnosed on hyperlipidaemia and its related pathological conditions such as cardiovascular diseases. Studies done on fasting lipid profile in other populations in different geographical locations exhibits the same trend of lower parameter values than the Kenyan population.

5.1.9 Glomerular filtration rate assessment using creatinine related parameters for healthy adult Kenyans

Estimation of Glomerular Filtration Rate (GFR) is crucial for the diagnosis, evaluation and management of renal pathological disorders. Reference values for measured creatinine clearance, estimated creatinine clearance, serum creatinine, urine volume and urine creatinine were determined in 265 healthy Kenyan adults ages 18-65 years. These study participants comprised of 106(40%) males and 159(60%) females. Since the
accuracy of measured creatinine clearance depends on the accuracy of the urine collection, intensive sensitization exercise was carried out on the study subjects prior to the commencement of urine specimen collection thus keeping variability in creatinine excretion due to incomplete urine collection to a minimum. In addition, study subjects were not allowed to exercise during or prior to the study thus eliminating potential variability of creatinine due to this factor (Lori et al., 1994). The effect of cooked meat (Jacobsen et al., 1979) on creatinine clearance was controlled in this study as well, with no intake of meat by the subjects during or three days prior to the study period.

Effect of body muscle mass on serum creatinine levels, where males generally have a higher muscle mass than females (Heymsfield et al., 1993) was expressed in this study. Males had higher serum creatinine levels (97.9±15.2) than the female counterparts (90.5±15.7) and the mean difference was significant (p=0.001). It is therefore evident that males have a higher body muscle mass than females and this effect has been expressed in related studies carried out elsewhere (Lori et al., 1994). Sex related serum creatinine levels of the current study population are similar to those obtained in an earlier studied Kenyan adult population (Waithaka et al., 2009).

Determination of glomerular filtration rate for the studied Kenyan population was achieved through two commonly used methods ie measured creatinine clearance and estimated creatinine clearance. The achievement of proper urine collection played a major key role in the establishment of reference values of measured creatinine clearance since urine volume and urine creatinine are integral part of the applied formula. The
current study produced sex-related reference values for both urine volume and urine creatinine whereby the male’s value (1588±364) was higher than the female’s (1447±329) and the mean difference was statistically significant (p=0.001). Significant gender difference (p= 0.001) was found in measured creatinine clearance whereby males (81.3±14.83) value was higher than that of the females (71.6±10.75). Similar gender difference in measured creatinine clearance was found in other population studied elsewhere (Finney et al., 2000).

It is useful and quite convenient to determine creatinine clearance by estimation without the need for urine collections. Estimated creatinine clearance employs serum creatinine levels, age (years) and weight (kg) to predict the creatinine clearance of a study participant. The current study came up with sex related estimated creatinine clearance reference values with the males (85.6±16.4) having a higher value than the females (81.7±12.4). Gender differences in estimated creatinine clearance could be attributed to body muscle mass difference between male and female. Comparatively, generated estimated creatinine clearance values are higher (male: 85.6±16, female: 81.7±12.4) than the measured creatinine clearance values (male: 81.3±14, female: 71.6±10.75) In addition, the difference between these two methods for creatinine clearance analysis was significant in both sex (male: p = 0.021, female: p = 0.001). Current study has shown a relationship between age and creatinine clearance. Creatinine clearance declines as age progress but at different rates depending on the sex of an individual and the method used for analysis. Decline rate is higher in males than in females for measured creatinine clearance whilst the decline rate is almost the same for male and female in estimated
creatinine clearance. A similar study carried out on older women from ages 40 to 95 years was in agreement with the current study in that as age progress creatinine clearance declines (Lori et al., 1994). This fall of creatinine clearance with age across the gender could be attributed to subclinical pathology (Fliser et al., 1997).

5.1.10 Different analytical methods used for analysis of same biochemical parameter

Comparison of analytical methods was performed to evaluate the agreement of results produced by these methods which have been validated for use in clinical chemistry laboratory. Most of the parameters have one method of analysis. Current study identified blood urea nitrogen, calcium, glucose, sodium and potassium as parameters having more than one analytical method. Berthelot reaction and glutamate dehydrogenase methods for analysis of blood urea nitrogen showed no analytical difference (p=0.810). These two methods can therefore be used interchangeably without affecting other tools of analysis such as quality control and reference ranges. O-Cresolphthalein and arsenazo III methods used for calcium analysis showed no analytical differences (p=0.138). This qualified the use of these two methods in calcium analysis without altering any other analytical tool such as quality control and the reference ranges as shown in Table 18. Glucose oxidase and hexokinase methods of analyzing glucose also did not show analytical differences (p=0.632). Therefore, these two methods could be used for glucose analysis interchangeably without affecting the results. Direct and indirect ion selective electrodes for sodium and potassium analysis had no analytical differences (p=0.819 and p= 0.299 respectively) as shown in Table 23. Current study concurred with a similar study by Flegar et al., 2006), on the compatibility
of results by two different methods analyzing same parameter. Another study by Jose et al., 2002, on the permeability of dentin using two different methods concluded that the methods are interchangeable although not identical.

Analytical methods differences were shown by the ion selective electrode methods (direct and indirect) and calorimetric methods for the analysis of potassium and sodium ($p<0.001$). Quality control reports and reference ranges for potassium and sodium should be interpreted differently depending on the method used for analysis whether calorimetric or ion selective electrode. The reason behind the difference between ion selective electrode and calorimetric for the estimation of electrolytes could be due to the sensitivity difference of the two methods. The study suggests that ion selective electrode method is more sensitive than the calorimetric method. The sensitivity superiority of ion selective electrode method was expressed by a similar study by Morimatsu et al., 2003.

5.1.11 Different analytical instruments used for analysis of same biochemical parameter

The study undertook the comparison of automation and manual analytical procedures used for the analysis of biochemical parameters. Olympus 640 AU represented the automated instrument whilst Hitachi spectrophotometer represented the manual instrument. Total protein, albumin and total bilirubin represented the end point reaction analysis whilst alkaline phosphatase represented the kinetic method of analysis. Differences between the results obtained from the two analytical instruments in all the analysed parameters were significant ($p<0.001$). The results produced by the automated
method were higher than those produced by manual methods in all parameters studied. This suggests that automation is more superior to manual methods in terms of accuracy and precision. Despite the superiority nature of automation, both methods produced quality results, since the quality control outcomes were within the acceptable limits.

The decision of whether to use automation or manual methods in this study depended on several factors which included; increase in workload, cost, improvement in quality and reduction of analytical errors. Increase in workload involves number of people and requests patterns prescribed by clinicians. As a result of increase in population, most health institutions have witnessed a surge in the number of people seeking medical assistance. Clinicians on the other hand have resulted in requesting panels of tests to aid in making quick and proper diagnosis. This increase in workload therefore becomes increasingly impossible to handle using manual methods. To cope with the demand, laboratory medicine and in particular clinical chemistry has embraced automated methods.

This study had a population of 6,795 study subjects and generated a total of 94,643 tests through automated methods and 1,116 tests through manual methods. It could have been impossible to achieve the goals of this study using the manual methods. The cost of analysis per test using automated methods is far much cheaper than using manual methods. More reagents are required to analyze a parameter using manual method as opposed to using automated method. It could have been very costly to accomplish this study if the analysis was solely on manual methods. The use of automated methods
greatly improved the quality of results generated and analytical errors completely eradicated. This achievement could not have been possible in manual method since it is prone to many errors thus compromise the quality of results.
5.2 CONCLUSIONS

The current study is the first to establish reference ranges for forty-five biochemical parameters for the adult Kenyan population. Previous studies managed to establish reference ranges of a few parameters whose study subjects were selected from specific locations within the country. Current study has managed to cover all regions of the country thus giving the established reference ranges a national outlook. Reference ranges for special biochemical parameters which includes, fasting profiles, oral glucose tolerance test, creatinine clearance, hormones and tumour maker have now been established for the first time in the Kenyan population.

Gender differences have been expressed in creatinine, creatinine clearance, gamma glutamyl transferase, lactate dehydrogenase, uric acid and all fertility hormones. For proper interpretation of laboratory report in reference to these parameters, it is mandatory to indicate the corresponding reference range. This study has indicated that testosterone levels decline as age progresses in males. It is of paramount importance to indicate the age of the male client whenever testosterone report is being interpreted for diagnostic purposes. Total prostatic specific antigen which is the male oriented tumour maker, showed an increasing trend with advancement in age for males. Interpretation of total prostatic specific antigen report for the diagnosis and management of prostate cancer would require the provision of the individual’s age.

Glucose and uric acid are the only parameters that showed regional differences. The two reference ranges for glucose which are 2.4-6.6 mmol/l for Eastern, Coast region, Nyanza,
Western, North Rift Valley and South Rift Valley and 2.6-7.0 mmol/l for Nairobi, Central and North Eastern have a narrow upper and lower reference range limits. Despite the means differences for the regional groupings being statistically significant, diagnostically this may not have any effect in the indication of hyperglycaemia and hypoglycaemia. The two reference ranges would be appropriate to use in the interpretation of random blood glucose reports across the studied Kenyan regions. South Rift Valley region have higher uric acid levels for both male and female than the rest of the country. It is therefore necessary to establish the area of residence for an individual whose uric acid level is being considered for interpretation.

Determination of GFR for the Kenyan adult population using measured and estimated creatinine clearance methods of analysis has been accomplished for the first time in Kenya. It is evident from this study that male and female have different reference ranges for either the measured or estimated creatinine clearance. Considering the two methods of creatinine clearance analysis, measured creatinine clearance produced lower values than estimated creatinine clearance. This factor is worth considering whenever creatinine clearance reference ranges are used in the interpretation of creatinine clearance laboratory results. There was a progressive decline of creatinine clearance with age in this study; therefore age of an individual should be considered whenever reference ranges from the study are to be made.

The establishment of fasting blood glucose reference ranges using both venous and capillary blood specimens is a major step in the diagnosis of glucose metabolic disorders.
It will be possible for the laboratory personnel to carry out an OGTT using capillary blood specimens which is convenient to the individual under going the investigation and also compatible with the method of analysis using a glucometer. In an event a request is made for fasting blood glucose and fasting lipid profile, it would be appropriate to use the venous blood glucose reference range to interpret the glucose report. Adaptation of lipid profile reference ranges established in this study will ensure that Kenyans are not over diagnosed on hyperlipidaemia and its related pathological conditions such as cardiovascular diseases. Over diagnosis of lipids related pathological disorders has all along been the trend due to the fact that the established reference ranges for lipid profile are higher than what is quoted in literature and widely used as a reference tool for the Kenyan population.

Calcium is the only parameter that did not show any geographical differences among the populations compared with the Kenyan population. Over and under diagnosis of various pathological disorders in the Kenyan population has been brought about by the use of reference ranges quoted in literature. Proper interpretation of clinical laboratory reports will be achieved by the use of established reference ranges of the various biochemical parameters. These Kenyan based reference ranges will revolutionize the diagnostic field since diagnostic decisions will be made using Kenyan based reference ranges independent of what is quoted in literature.

It is evident from this study that different methods used for analysis of the same parameter give similar results especially if the method has a high degree of sensitivity.
This study has identified automated method of analysis as having a high degree of sensitivity thereby making it more superior than the manual method of analysis. The superiority of automated method in terms of precision and accuracy can be attributed to the fact that it is not prone to analytical errors unlike the manual method.

5.3 RECOMMENDATIONS

Clinical chemistry is a wide field having many biochemical parameters used in diagnosis of various pathological disorders. This study has managed to establish reference ranges for forty-five biochemical parameters. There is great need to establish reference ranges for other parameters not included in this study. Once this is achieved, the Kenyan population will be dependent on local reference ranges other than literature-based reference ranges.

It is important to establish reference ranges for children below 18 years and adults above 60 years. These two groups of Kenyan population were not included in the present study.

There is need to establish Kenyan sex and age-specific reference ranges for hematological and immunological parameters.

The age and sex of an individual must be included whenever a health provider makes a laboratory request. This is important since it has been established that some parameters reference ranges are dependent on the age of an individual such as total prostatic specific antigen, testosterone and creatinine clearance. Sex of an individual must always be
considered during analysis since the study has established sex differences in some parameters eg uric acid, creatinine and fertility hormones.

There is need for further studies to determine the cause of high uric acid levels for adult Kenyan population from the South Rift Valley Region.

The type of specimen used should be indicated especially in the interpretation of fasting blood glucose report. The study established two types of fasting blood glucose reference ranges that is whole blood (capillary) and plasma (venous). This would ensure that correct established reference range for fasting blood glucose has been used.

Due to the superiority of automated methods in terms of accuracy and precision which results with quality results, it is important to progressively introduce automated analysers in all clinical laboratories and withdraw manual analysers from all clinical laboratories.

The finding of this study provides baseline data for future applications in the utilization and development of population based clinical chemistry reference ranges.
REFERENCES


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APPENDICES

Appendix 1: CONSENT FORM

STUDY QUESTIONNAIRE / CONSENT FOR RESEARCH PURPOSES.

STUDY TITLE: National Reference Ranges for Routinely Analyzed Biochemical Parameters, Creatinine Clearance, Oral Glucose Tolerance Test, Lipid Profiles, Tumour Makers and Hormones for Adult Kenyans

The principal investigator hereby request your participation in the above mentioned proposed study. Therefore you are requested to grant permission for the use of your blood/urine specimens for the purposes of the proposed study. Please sign the consent statement below as an indication that you have granted permission for your specimens to be used for the proposed study.

QUESTIONNAIRE/CONSENT FORM (Please fill in where appropriate)

(1) Study number ..................Date.............................

(2) Contact: Phone number................P.O.Box....................

(3) Gender (tick) : Male/Female

(4) Age: ...............(Years)

(5) Weight ...............(Kg)

(6) Height..............(metre)

(7) Blood pressure...........(mm/hg)

(8) Occupation..............................

(9) County.................................

(10) Have you fasted (YES/NO)

(11) Health status for the last six months (tick): WELL/UNWELL
If unwell, please state the nature of your illness in the space provided below.

(12) Are you on any medication (tick) : YES/NO

If YES, please specify: ..............................................................

(13) Last monthly periods: Start date................. Stop date .............

I..................................................(study number), have given consent for my
blood/urine to be used ONLY for the proposed study.

Signature........................................
Appendix 2: Ethical approval
Ref: KNH-ERC/ 01/ 379

Mr. Stanley Kinge Waithaka
Clinical Chemistry Laboratory
KNH

Dear Mr. Waithaka

REVISED RESEARCH PROPOSAL: “THE ESTABLISHMENT OF NATIONAL REFERENCE RANGES FOR ROUTINELY ANALYSED BIOCHEMICAL PARAMETERS, CREATININE CLEARANCE, ORAL GLUCOSE TOLERANCE TEST, LIPIDS, TUMOUR MAKERS AND HORMONES FOR THE ADULT KENYAN POPULATION” (P342/11/2007)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and approved your above revised research proposal for the period 6th May, 2008 - 5th May, 2009.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH-ERC

c.c. Prof. K.M. Bhatt, Chairperson, KNH-ERC
The Deputy Director CS, KNH
Co-investigators: Mr. Bernard Chiuri, Dept. of Lab. Medicine, KNH
Mr. Leonard Njagi, Clinical Chemistry Lab., KNH
Mr. Daniel Muturi Mwangi, Emergency Laboratory, KNH
Mr. Stanley Kinge Waithaka  
Clinical Chemistry Laboratory  
KNH

Dear Stanley


This is to grant you annual extension of approval for the research Protocol No.P342/11/2007.

The renewal periods are 6th May 2009 – 5th May 2010.

Yours sincerely

PROF. A N GUANTAI  
SECRETARY, KNH/UON-ERC

c.c. Prof. K.M.Bhatt, Chairperson, KNH-ERC  
The Deputy Director CS, KNH
Appendix 3: TPSA means comparison for the four male age group categories

<table>
<thead>
<tr>
<th>Age groups(I)</th>
<th>Age groups(J)</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-28</td>
<td>29-39</td>
<td>-0.5756(*)</td>
<td>0.1312</td>
<td>0.000</td>
<td>-0.9144</td>
<td>-0.2368</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>-1.564(*)</td>
<td>0.1329</td>
<td>0.000</td>
<td>-1.9047</td>
<td>-1.2182</td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>-2.9471(*)</td>
<td>0.1256</td>
<td>0.000</td>
<td>-3.2712</td>
<td>-2.6229</td>
</tr>
<tr>
<td>29-39</td>
<td>40-50</td>
<td>-0.9858(*)</td>
<td>0.1345</td>
<td>0.000</td>
<td>-1.3331</td>
<td>-0.6385</td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>-2.3715(*)</td>
<td>0.1272</td>
<td>0.000</td>
<td>-2.6999</td>
<td>-2.0431</td>
</tr>
<tr>
<td>40-50</td>
<td>51-61</td>
<td>-1.3857(*)</td>
<td>0.1289</td>
<td>0.000</td>
<td>-1.7187</td>
<td>-1.0527</td>
</tr>
</tbody>
</table>

*= the mean difference significance at .05 level, Sig.= significance, CI= confidence interval

Appendix 4: Testosterone means comparison for the four male age group categories

<table>
<thead>
<tr>
<th>Age groups(I)</th>
<th>Age groups(J)</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-28</td>
<td>29-39</td>
<td>.4580</td>
<td>.2724</td>
<td>0.562</td>
<td>-2.65</td>
<td>1.181</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>3.6960(*)</td>
<td>.2603</td>
<td>0.000</td>
<td>3.005</td>
<td>4.387</td>
</tr>
<tr>
<td></td>
<td>51-61</td>
<td>4.6502(*)</td>
<td>.3220</td>
<td>0.000</td>
<td>3.795</td>
<td>5.505</td>
</tr>
<tr>
<td>29-39</td>
<td>18-28</td>
<td>-0.4580</td>
<td>.2724</td>
<td>0.562</td>
<td>1.181</td>
<td>.265</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>3.2380(*)</td>
<td>.2799</td>
<td>0.000</td>
<td>2.495</td>
<td>3.981</td>
</tr>
<tr>
<td></td>
<td>51-61</td>
<td>4.1921(*)</td>
<td>.3381</td>
<td>0.000</td>
<td>3.294</td>
<td>5.090</td>
</tr>
<tr>
<td>40-50</td>
<td>18-28</td>
<td>-3.6960(*)</td>
<td>.2603</td>
<td>0.000</td>
<td>-4.387</td>
<td>-3.005</td>
</tr>
<tr>
<td></td>
<td>29-39</td>
<td>-3.2380(*)</td>
<td>.2799</td>
<td>0.000</td>
<td>-3.981</td>
<td>-2.495</td>
</tr>
<tr>
<td></td>
<td>51-61</td>
<td>.9541(*)</td>
<td>.3284</td>
<td>0.024</td>
<td>.082</td>
<td>1.826</td>
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<td>51-61</td>
<td>18-28</td>
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<td>.3220</td>
<td>0.000</td>
<td>-5.505</td>
<td>-3.795</td>
</tr>
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<td>-4.1921(*)</td>
<td>.3381</td>
<td>0.000</td>
<td>-5.090</td>
<td>-3.294</td>
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<tr>
<td>40-50</td>
<td>51-61</td>
<td>-0.9541(*)</td>
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<td>0.024</td>
<td>-1.826</td>
<td>-.082</td>
</tr>
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</table>

*= the mean difference significance at .05 level, Sig = significance, CI= confidence interval
### Appendix 5: Female testosterone means comparison for the three menstrual phases and menopause

<table>
<thead>
<tr>
<th>(I) age</th>
<th>(J) age</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>fol</td>
<td>ov</td>
<td>-1.3951(*)</td>
<td>.0865</td>
<td>.000</td>
<td>-1.625 -1.165</td>
</tr>
<tr>
<td></td>
<td>lut</td>
<td>.0343</td>
<td>.0890</td>
<td>1.000</td>
<td>-.203 .271</td>
</tr>
<tr>
<td></td>
<td>meno</td>
<td>-.3519</td>
<td>.1955</td>
<td>.439</td>
<td>-1.872 .169</td>
</tr>
<tr>
<td>ov</td>
<td>fol</td>
<td>1.3951(*)</td>
<td>.0865</td>
<td>.000</td>
<td>1.165 1.625</td>
</tr>
<tr>
<td></td>
<td>lut</td>
<td>1.4295(*)</td>
<td>.0831</td>
<td>.000</td>
<td>1.208 1.651</td>
</tr>
<tr>
<td></td>
<td>meno</td>
<td>1.0433(*)</td>
<td>.1929</td>
<td>.000</td>
<td>.530 1.557</td>
</tr>
<tr>
<td>lut</td>
<td>fol</td>
<td>-.0343</td>
<td>.0890</td>
<td>1.000</td>
<td>-.271 .203</td>
</tr>
<tr>
<td></td>
<td>ov</td>
<td>-1.4295(*)</td>
<td>.0831</td>
<td>.000</td>
<td>-.1651 -1.208</td>
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<tr>
<td></td>
<td>meno</td>
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<td>.1940</td>
<td>.287</td>
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</tr>
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<td>lut</td>
<td>.3862</td>
<td>.1940</td>
<td>.287</td>
<td>-.130 .903</td>
</tr>
</tbody>
</table>

* = mean difference is significant at the .05 level, fol = follicular phase, ov = ovulation phase, lut = luteal phase and meno = menopause.

### Appendix 6: Parameters showing age group category difference among different age groups within the healthy adult Kenyans

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(I) age</th>
<th>(J) age</th>
<th>MD (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creat-Female 18-28</td>
<td>29-39</td>
<td>1.302</td>
<td>0.564</td>
<td>.589</td>
<td>-0.6889 -0.3656</td>
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</tr>
<tr>
<td></td>
<td>40-50</td>
<td>.321</td>
<td>0.032</td>
<td>.042*</td>
<td>-1.7712 1.3542</td>
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</tr>
<tr>
<td></td>
<td>51-61</td>
<td>1.569</td>
<td>-0.412</td>
<td>.021*</td>
<td>-4.3220 2.5734</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>-0.415</td>
<td>.039*</td>
<td>.1987 0.5469</td>
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</tr>
<tr>
<td></td>
<td>40-50</td>
<td>1.159</td>
<td>-0.985</td>
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<td>.9478 1.7982</td>
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</tr>
<tr>
<td></td>
<td>51-61</td>
<td>.547</td>
<td>0.758</td>
<td>.901</td>
<td>-1.7956 -0.9808</td>
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<tr>
<td>Creat-Male 18-28</td>
<td>29-39</td>
<td>1.236</td>
<td>0.258</td>
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<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>BUN 18-28</td>
<td>51-61</td>
<td>.301</td>
<td>0.211</td>
<td>.017*</td>
<td>-0.128 0.317</td>
<td></td>
</tr>
</tbody>
</table>

* = mean difference significance at p<0.05, std = standard, sig = significance