THE ROLE OF SERUM ZINC, COPPER, RETINOL AND ALPHA-TOCOPHEROL IN MODULATING IMMUNITY IN HIV AND AIDS SUBJECTS IN WESTERN KENYA

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DECLARATION

This thesis is my original work and has not been presented elsewhere for the award of any degree.

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DEDICATION

This thesis is dedicated to my parents Andrew Mbakaya, the late Teresina Masinzachi, Benedette Nzambi and Ayuma Mbakaya. Special appreciation goes to my beloved children, Yvonne, Angela, Jasmine, Winnie, Andrew, Gabriel and Daisy.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrophotometer
AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of Variance
ARV	Antiretroviral
AZT	Zidovudine
BLRA	Binary Logistic Regression Analysis
BMI	Body Mass Index
CBS	Central Bureau of Statistics
CDC	Center for Disease Control
CPHR	Center for Public Health Research
CVBCR	Center for Vector Biology Control Research
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonucleic Acid
ESR	Erythrocyte Sedimentation rate/Hr
EDTA	Ethylenediaminetetraacetate
GOK	Government of Kenya
HAART	Highly Active Antiretroviral Therapy
Hb	Hemoglobin
HCT	Haematocrit
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IBR	Infectious Bovine Rhinotracheitis
IQR	Inter-quartile Range (25^{th}- 75^{th})
IL	Interleukin
IU	International Units
KEMRI	Kenya Medical Research Institute
KDHS	Kenya Demographic Health Survey
KAIS	Kenya Aids Indicator Survey
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MFO	Mixed Function Oxidase
MOMS	Ministry of Medical Services
MOPHS	Ministry of Public Health and Sanitation
NACC	National AIDS Control Council
NASCOP	National AIDS Control Program
NC	Nucleocapside
NGOs	Non Governmental Organizations
NK	Natural Killer
PC	Personal Computer
PMCT	Mother to Child Transmission
RBC	Red Blood Cells
RET	Retinol
RDA	Recommended Daily Allowance
ROS	Reactive Oxygen Species
SD	Standard Deviation

SPSS	Statistical Packages for Social Sciences
STD	Sexually Transmitted Diseases
TB	Tuberculosis
Tot Lymp	Total Lymphocytes
USA	United States of America
UNAIDS	United Nations Programme on HIV and AIDS
VCT	Voluntary Counseling and Testing
Vit	Vitamin
Vit E	Vitamin E (α-tocopherol)
WBC	White Blood Cells

ABSTRACT

From the early 1980s, scientists continue to debate on HIV as the cause of AIDS with few arguing that AIDS is caused by chemicals, drugs and malnutrition. A nutrition intervention study in Kenya using VIUSID™, a recommended daily allowance compliant formulation with respect to ascorbic acid, pyridoxal, folic acid and zinc sulphate, was ineffective in restoring serum zinc levels and immunity of HIV and AIDS subjects in 12 weeks. The purpose of this study was to determine serum micro-nutrient levels, assess blood for immune markers and establish safety of an intervention with VIUSID™ administered together with mega doses of zinc, selenium and vitamins A, $B_{12,}$ C and E in HIV and AIDS subjects. Ninety subjects were sequentially recruited from VCT centres in western Kenya and clinically examined at baseline and at the 12th week. For intervention, subjects were administered with VIUSID™ together with citric acid (Arm 1) and VIUSID™ together with citric acid and with mega doses of zinc, selenium and vitamins A, $B_{12,}$ C and E (Arm 2) for 8 weeks. In both Arms however, administration of VIUSID™ alone was continued to the 12th week. Venous blood was obtained for determination of micro-nutrient status, viral load, liver function and immune testing. Serum micro-nutrient levels were determined at monthly intervals, serum zinc and copper being determined using a Flame Atomic Absorption Spectrophotometer (FAAS) while retinol and alpha-tocopherol levels were by a High Performance Liquid Chromatograph (HPLC) as respective test kits were used for immunological, virological and liver function tests. Data entry and analysis was by SPSS/PC+ version 11.5, analysis being mainly by parametric and non-parametric methods between and within Arms 1 and 2. Spearman's Rho correlations were used to establish linkages between measured variables at baseline, during and after intervention. The ninety (90) subjects recruited and randomized into the study were aged 39.0±8.9 years, 70% were female, 47.1% were widows, 22.2% had a history of TB while 54.4 % ate 3 meals a day and 12.2% were confirmed HIV-seronegative despite losing spouses to HIV and AIDS. There were clinical improvements in 74 subjects who completed the study as significant increases in serum zinc levels occurred in both arms by the 4th week, more so in subjects on mega-doses. These declined to near baseline levels at the 12th week, probably suggesting a physiological regulation of the way nutrients were utilized. Although subjects in Arm 2 had significant increase in CD4 cell count ($p = 0.007$) and near significant viral load reduction ($p = 0.104$), those in Arm 1 had insignificant increase in CD4 cell count ($p = 0.722$) but significant decrease in viral load ($p = 0.030$). Low serum zinc correlated with HIV-seropositivity ($p = 0.0001$) and high optical desnsity of HIV antibodies ($p = 0.002$). High serum retinol level at baseline was associated with better management outcomes by the 12th week of supplementation as use of mega-dose supplements had both clinical and immunological benefits and near-significant reduction in viral load. Low doses of micro-nutrients favored viral load reduction, this being in agreement with other studies. Liver function tests of the subjects stayed normal in both study Arms, suggesting the interventions were safe for use. Therefore, use of VIUSID™ with mega-dose micro-nutrients was safe and more effective than VIUSID™ only in management of HIV and AIDS subjects. From this study, nutrition has a role in management of HIV and AIDS and probably supports the view that malnutrition is a key factor in HIV and AIDS. Further work should be undertaken to develop and up-scale this intervention with a view to mainstreaming its use in healthcare delivery in Kenya and beyond, especially considering that HIV and AIDS drugs present several challenges, including adverse health effects, resistance and compliance.

CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Whereas there is on-going debate on the HIV causes AIDS hypothesis (Duesberg, 1988), the care of patients with AIDS includes many biomedical, nutritional, psychological and behavioral interventions (Weidle *et al.,* 2002). Epidemiological observations and clinical findings have strengthened the concept that both nutritional deficiencies and nutritional excesses impair gastro-intestinal responses and alter susceptibility to inflammation and other diseases (Scrimgeour and Condlin, 2009). Human research on the role of foods in modulation of immune functions in intervention studies or randomized controlled trials can be classified into three categories according to the physical state of subjects enrolled for investigation: (i) examination of the effect of foods in healthy individuals; (ii) analysis of the effect of foods on patients with hypersensitivity; and (iii) investigation of the effect of foods on immune-compromised subjects. Research has led to the conclusion that foods can modulate immune functions manifesting as either innate (phagocytic, NK cell activity) or acquired immunity (Kaminogawa and Masanobu, 2004).

A growing body of evidence from cross-sectional epidemiologic studies has shown that HIV-infected persons exhibit abnormal blood levels of key micro-nutrients, arising from interplay of malnutrition, mal-absorption, metabolic alteration and nutrient depletion associated with the risk factors of HIV infection (Jariwalla, 2009). In addition, the studies observe that most of these micro-nutrients are well known in providing protective physiologic functions, serving as vital anti-oxidants in scavenging toxic free radicals and/or immune-modulators in maintaining cell-

mediated/humoral responses. Consequently, micro-nutrient imbalance may contribute to increased oxidative stress/inflammation and abnormalities in immunologic/neurophysiologic functions underlying HIV and AIDS infections. Moreover, improvement of immune functions by foods can normalize the physical state of allergic or cancer patients, and may reduce the risk of diseases in healthy individuals. Consequently, it is valuable to assess the immune-modulating abilities of foods by measuring at least one parameter of either innate or acquired immunity (Kaminogawa and Masanobu, 2004).

In HIV-infected persons, low serum concentrations of vitamins and minerals are associated with an increased risk of AIDS progression and mortality. For instance, a study in South Africa has observed that children infected with HIV and had abnormally low vitamin A levels, had significantly high viral loads and low CD4 cell count than those with normal vitamin A levels (Steenkamp *et al*., 2009). In the same study, children with low serum zinc levels had significantly low median CD4 cell count than those with high levels ($p = 0.002$). Furthermore, it is recognized that micro-nutrient supplements can delay HIV disease progression and reduce mortality in HIV-positive persons not receiving highly active antiretroviral therapy (Drain e*t al.*, 2007). Therefore, the provision of simple, inexpensive micro-nutrient supplements as an adjunct to Highly Active Antiretroviral Therapy (HAART) may have several cellular and clinical benefits. Besides, the spectrum of complications emerging in successfully treated HIV-infected patients has dramatically changed since the advent of HAART (Libre *et al*., 2009). Notably, typical AIDS-defining illnesses have been substituted by new co-morbid conditions that threaten even those patients who maintain virologic suppression. Thus, proper management of cardiovascular risk, and early diagnosis of AIDS-related and, particularly, non-AIDS-related

malignancies (including papilloma-virus-related neoplasms) must be introduced into the routine care. Hot areas of investigation include HIV-associated neuro-cognitive disorders, Hepatitis B and C co-infection, non-alcoholic fatty liver disease, progressive multi-focal leukoencephalopathy and tuberculosis. Furthermore, bone and kidney long-term toxicities and lipoatrophy remain as issues of importance as is the identification and early treatment of immune reconstitution disease; especially in those patients starting antiretroviral treatment with severe CD4 cell depletion (Libre *et al*., 2009).

Oxidative metabolism that is characteristic of HIV and AIDS inevitably leads to generation of reactive oxygen species (ROS) or "free radicals", which have the potential to cause further oxidative reactions, especially to those parts of the cell in a relatively reduced state, such as cell membranes or nucleic acids (Evans and Halliwell, 2001). The potential to cause damage is limited by mechanisms that include direct quenching of oxidant activity by α- tocopherol (vitamin E), or carotenoids (vitamin A), or enzyme systems to dispose of the products of oxidation-superoxide dismutase (zinc/copper or manganese dependent) and glutathione peroxidase (selenium dependent) (Shenkin, 1995).

There have been various nutritional interventions in HIV and AIDS subjects (Catalysis, 1999; Olaniyi and Arinola, 2007; Mbakaya *et al*., 2003; Mbakaya *et al.*, 2004a; 2004b). A study in Nigeria observed that uric acid and zinc were significantly high while vitamin E, Mg, Fe, Mn, Cu, and Se were significantly low in HIV-infected patients compared to healthy controls (Olaniyi and Arinola, 2007). VIUSID^{TM}, a micro-nutrient powder supplement manufactured by a Spanish company, has been administered in intervention studies in both Europe and Kenya (Catalysis,

1999; Mbakaya *et al.*, 2003; Mbakaya *et al.*, 2004a; 2004b). A daily dose of VIUSID™ is comprised of honey (2.5 mg), of malic acid (2.0 g), arginine (2.0 g), glucosamine (2.0 g), glycine (1.0 g), ascorbic acid (0.06 g), pyridoxal (1.0 µg), folic acid (200 µg), glycirrhizinic acid (0.1 g) and hydrated zinc sulphate (15 mg Zn) that is taken three times a day in a glass of juice or water. It was among the first nutritional supplements to be clinically piloted in Kenya in the early 2000s when anti-retroviral (ARV) drugs were not affordable to the majority of HIV and AIDS patients who needed care and presented with challenges of adverse side effects and drug resistance.

In Europe, a study demonstrated beneficial effects of VIUSID™ as a nutritional preparation with enhanced anti-oxidant properties on HIV and AIDS subjects (Catalysis, 1999). In an open label study conducted in Kenya, this preparation was shown to have some beneficial effects after 36 months of observational study on HIV and AIDS subjects (Mbakaya *et al.*, 2004a; 2004b). The proportion of key signs and symptoms associated to HIV and AIDS at baseline and after three months of intervention showed a decrease in night sweats from 57 to 3%; headache from 47 to 7%; diarrhea from 23 to 0%; fever from 40 to 6%; oral thrush from 30 to 3%; and skin lesions from 50 to 6%, respectively. Changes in biochemical parameters after 30 months of administration of VIUSID™ are shown in Table 1.

Furthermore, it was notable that the ratio of IL-2/IL-4 increased from 3.42 to 24.0 after 36 months of supplementation (Mbakaya *et al.*, 2004a). From these results, liver function tests remained within normal values, indicating that VIUSID™ was safe when used over a long period of time. However, the fact that no increase in CD4 cell count was noted possibly alludes to nutritional deficiencies of other key nutrients that were not part of the VIUSID™ formulation

or were in lower dosages. For example, low vitamin B_{12} has been shown to increase the risk of HIV progression to AIDS, while selenium deficiency independently predicts mortality from HIV and AIDS, yet these micro-nutrients were not part of the ingredients in the VIUSID™ formulation (Tang *et al.*, 1997, Baum *et al.*, 1997). Furthermore, despite the fact the HIV-seropositive subjects were supplemented with VIUSID™ formulation for 36 months, the zinc levels fluctuated at very low levels when compared with healthy subjects (Mbakaya *et al.*, 2003).

Table 1: Parameters of HIV and AIDS subjects at baseline and 30th month

Parameter	Month 0 Mean (SD)	Month 30 Mean (SD)	p-Values
Hematology			
WBC ($x10^9$/l)	5.75(1.64)	5.11(1.22)	0.059
RBC ($x10^{12}$/l)	4.38(0.69)	4.59(0.64)	0.047
Hb (g/dl)	12.4(1.75)	12.53(1.75)	0.667
Lymphocytes (%)	41.76(10.29)	39.76(12.18)	0.497
Monocytes (%)	8.69(2.99)	8.24(3.14)	0.596
HCT (%)	38.56(5.69)	35.88(9.13)	0.142
Anthropometry			
BMI (Kg/m^2)	24.01(4.32)	24.52(4.07)	0.138
Biochemistry			
Albumin (g/l)	38.00(4.71)	42.74(6.41)	0.002
AST (U/l)	33.23(12.63)	41.36(13.25)	0.026
Total bilirubin (mg/l)	11.63(2.63)	10.58(2.16)	0.102
Glucose (mmol/l)	4.30(0.89)	5.88(2.59)	0.004
Retinol (µmol/l)	0.47(0.15)	0.75(0.28)	0.0001
S-creatinine (µmol/l)	108.29(8.41)	108.00(10.49)	0.132
Total protein (g/l)	76.93(6.99)	79.21(5.77)	0.163
Serum zinc (µg/dl)	79.28(14.45)	70.99(12.98)	0.016
Immunology			
CD4 cell count ($x10^9$/l)	469(321)	415(377)	0.120
CD8 cell count ($x10^9$/l)	1335(1080)	1171(531)	0.312
CD4/CD8 ratio	0.57(0.60)	0.44(0.50)	0.012
Virology			
Viral load (log_{10}copies/ml)	4.382(0.955)	3.678(0.0001)	0.0001

Source: Mbakaya *et al.*, 2003; n = 29, p-values are 2-tailed, month 0 = baseline

1.2 Problem statement and justification

Various co-factors are known to influence the rate at which HIV progresses to AIDS with nutrition increasingly becoming recognized as one of the most important factor. In a nutritional study conducted in Kenya, use of VIUSID™ to manage HIV and AIDS infection showed some limitations. While remarkable clinical improvements were noted within 3 months, it took 30 months before significant viral load changes could be observed. VIUSID™ did not stimulate the

immune response by increasing the CD4 cell count and the CD4/8 ratio. While it took about 18 months to note some improvements in vitamin A serum levels, use of VIUSID^{TM} did not reverse the decline in the serum zinc status of the patients, yet these nutrients are essential to an efficient immune system. The daily dosage of VIUSID^{TM} contains nutrients marginally compliant with the USA recommended daily allowance (RDA) levels of normal persons; making the preparation inadequate to address the actual demand of such nutrients by HIV and AIDS patients in Kenya. Furthermore, VIUSID^{TM} does not contain vitamins A and E that are essential anti-oxidants critical in human immunity. Lastly, the VIUSID^{TM} formulation was developed largely with the users in affluent societies in mind, yet reports indicate that majority of people in Kenya are not comparable to those populations as nutritional deficiencies are more rampant in the developing countries.

Since preliminary studies on VIUSID^{TM} suggest that its use by HIV and AIDS subjects reduced clinical signs and symptoms associated with this disease, with marginal improvements in biochemical, immunological and virological indicators, it appears that using VIUSID^{TM} with other nutritional formulation is necessary if the results obtained are to be improved and within a short time span. Some synergy could be demonstrated if VIUSID^{TM} were to be used together with other nutrients such as zinc, vitamin-A, B_{12}, C, E and selenium (Baum and Sho-porshner, 1998). This would promote a deeper understanding of the role of nutritional supplementation of HIV and AIDS management in Kenya as well as elucidate how micro-nutrients modulate the immune system. Furthermore, correlations between measured nutritional, virological and immunological variables in the proposed study may elucidate the HIV causes AIDS controversy.

1.3 Null hypothesis

There is no significant difference in the levels of serum zinc, retinol and α–tocopherol and markers of immunity of HIV and AIDS subjects using VIUSID^{TM} with mega-doses of micro-nutrients and VIUSID^{TM} only in management of HIV and AIDS infection in western Kenya.

1.4 General objective

The aim of this study was to determine serum micro-nutrient levels, assess blood for immune markers and establish safety of an intervention with VIUSID^{TM} administered together with mega-doses of zinc, selenium and vitamins A, $B_{12,}$ C and E in HIV and AIDS subjects in western Kenya before, during and after 12 weeks of intervention.

1.4.1 Specific objectives

i) To establish socio-demographic characteristics of the study subjects.

ii) To establish the subjects BMI and clinical signs and symptoms of HIV and AIDS at baseline and after intervention.

iii) To determine blood hemoglobin and markers of immunity, serum zinc, copper, retinol and α-tocopherol levels of the study subjects at baseline, during and after intervention.

iv) To determine the safety of the mega-dose nutrition intervention in the HIV and AIDS subjects.

1.5 Scope and Limitations

Whereas the mega-dose formulation had several micro-nutrients/ingredients including vitamin A, B_{12}, E, selenium zinc, dehydroepiandrosterone (DHEA) and citric acid, only serum retinol, alpha-tocopherol, zinc and copper were determined as specified in the objectives.

Although mega-doses were originally intended for twelve weeks, this was interrupted at 8 weeks when it was noted that serum zinc levels of the subjects had risen substantially by the 4^{th} week of the intervention.

While copper was not part of the supplements provided, serum copper levels were determined since it is known that mega-doses of zinc interfere with its metabolism.

1.6 Conceptual framework

The factors that impact human nutrition and immunity, with emphasis on the HIV disease are summarized in Figure 1.

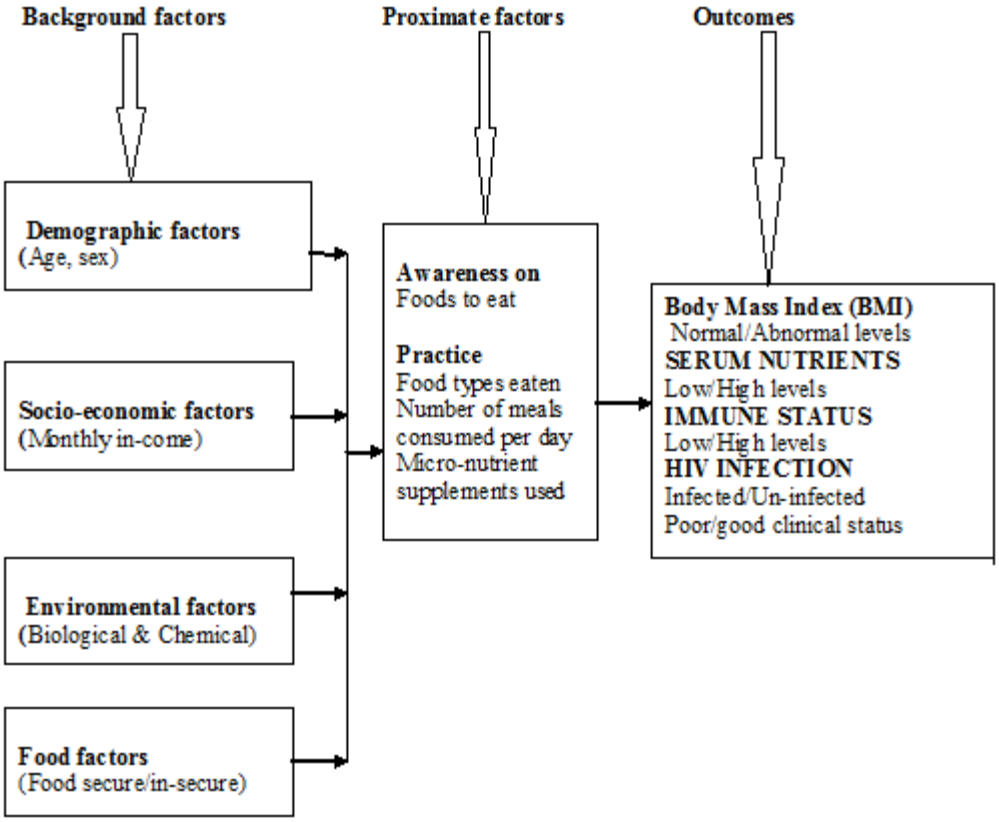

Figure 1: Conceptual framework

There are environmental factors that may cause disease including exposure to biological hazards (viruses, bacteria, fungi, parasites) and chemical hazards (pesticides, aflatoxins, fumonisins). However, other factors do modify effects of such exposures and these include individual chacteristics such as socio-demographic and nutrition status. Consequently, interventions that include awareness-raising and provision of micro-nutrient supplements have a role in determining outcomes of environmental exposures. Outcomes of the interventions may manifest as normal and abnormal levels of BMI, serum micro-nutrient levels, clinical signs and symptoms of disease and immune markers.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 HIV as the cause of AIDS

Acquired Immune Deficiency Syndrome (AIDS) is of major public health importance and concern globally, the assumption proposed that its cause is infection with HIV being founded on the correlation between detection of antibodies to this virus and the onset of AIDS (Duesberg, 1994; Lindermann, 1994). This view became generally accepted, and today it is still the foundation stone of HIV-related measures for the prevention and treatment of AIDS. Although unanimously rejected by AIDS researchers, it has been hypothesized that AIDS is caused by drugs, chemicals and malnutrition and that HIV is only a passenger pathogen that takes advantage of a bad situation (Duesberg, 1994; Lindermann, 1994). While HIV may not be the only cause of AIDS, both nutritional deficiencies and nutritional excesses are among other factors that alter susceptibility to inflammation and other diseases. Further research has led to the view that foods can modulate immune functions manifesting as either innate (phagocytic, NK cell activity) or acquired immunity (Duesberg, 1988; Weidle *et al.*, 2002; Scrimgeour and Condlin, 2009; Kaminogawa and Masanobu, 2004). Studies have shown that HIV-infected persons exhibit abnormal blood levels of key micro-nutrients (Jariwalla, 2009).

The role of consumption of fumonisin-contaminated maize has been linked with increased risk of HIV transmission while consumption of rice contaminated with aflatoxins has been associated with hepatocellular cancer (Williams *et al*., 2010). A review paper has suggested that the search for a cure for HIV and AIDS would remain extremely elusive, and for a long time to come, if the combined role of micro-nutrient zinc and the immune-suppressing dioxins was relegated to the

periphery of biomedical research (Mbakaya and Wakori, 1997). It has been reported that zinc deficiency was responsible for the premature switch from cellular (Th-1) to humoral/antibody (Th-2) immunity (Sprietsma, 1999).

Furthermore, it has been observed that micro-nutrient zinc deficiency was a possible co-factor in the transmission and progression of HIV and AIDS, also supporting earlier observations (Mbakaya *et al.*, 2004a; Duesberg, 1994; Lindermann, 1994). It has been argued that serum zinc levels, and by extension cell-mediated immunity, are physiologically lowered to produce antibodies in adapting to pathogens such as HIV and chemicals such as oral contraceptive agents (Mbakaya *et al*., 2011). It has been observed that the perpetual exposure to malaria parasites in malaria endemic regions in Kenya and the greater Sub-Saharan Africa may be a push factor for the population to enhance humoral immunity, thereby exposing such persons to the risk of HIV, TB, pneumonia and cancer since these diseases require cellular-immunity for effective prevention and control as was recently elucidated (Mbakaya, 2011). This may elucidate the hitherto un-explained co-infection with malaria, HIV and AIDS, TB, pneumonia and cancer since these diseases require cellular-immunity for effective prevention and control (Williams *et al*., 2010, Mbakaya *et al.*, 2011).

An open label study in Kenya showed that by management of HIV and AIDS patients using micro-nutrients with enhanced antioxidant properties, it was possible to reverse the signs and symptoms associated with HIV and AIDS, significantly reduce viral load and favourably increase expression of cellular immunity 8-fold (Mbakaya *et al.*, 2004a). Supplementation with zinc was demonstrated to be effective in preventing immunological failure and diarrhoea in HIV

and AIDS patients receiving Highly Active Antiretroviral Therapy (HAART) in a study in the US (Baum *et al.*, 2010). However, to demonstrate how emotive and deeply entrenched the HIV causes AIDS views are among majority of scientists, the institution of a strict peer-review mechanism at the *Medical Hypotheses Journal*, all because the editor had, without peer-review and within his earlier mandate, published a purportedly controversial paper on the HIV causes AIDS theory is notable (Akst, 2010).

2.2 Prevalence and Management of HIV and AIDS

In July 2008, UNAIDS/WHO estimated that by December 2007, there were 33.0 million people living with HIV infection globally. The report further stated that the number of cases of new HIV infections have decreased from 3.0 million in 2001 to 2.7 million in 2007 while those who died in 2007 increased to 2.0 million compared to 1.7 million who died in 2001. In total, Sub-Saharan Africa is reported to be home to 67% of all people living with HIV and AIDS globally (UNAIDS/WHO, 2008).

However, by the end of 2005, about 40 million people were living with HIV and AIDS, while nearly 5 million persons were newly infected in that year (UNAIDS/WHO, 2005). In 1998, over 33 million people were living with HIV and AIDS, the vast majority in the developing world, where few interventions are available (UNAIDS/WHO, 1998). Although access to HIV medication has been nearly universal to people in developed countries, only 1 in 7 Asians and 1 in 10 Africans who need HIV therapy were receiving the medication (Drain *et al.*, 2007). However, the twin global epidemic of HIV and food scarcity disproportionately affect Sub-Saharan Africa and a significant proportion of patients who require antiretroviral therapy (ART)

are malnourished because of a combination of HIV-associated wasting and inadequate nutrient intake (Koethe and Heimburger, 2010).

In a multi-center cross-sectional study of more than 70 year olds infected with HIV-1 and on HAART, majority had undetectable HIV-1 viremia, CD4 cell count >= 350 and one co-morbid condition, including dyslipidemia (54%), hypertension (36%), hyperglycemia or diabetes (30%), cardiovascular disease (23%), chronic renal failure (18%), history of neoplasmia (17%), lipodystrophy (58%), and cognitive impairment (11%). Rates of hypercholesterolemia, diabetes and cancer were higher than those reported in un-selected local population (Mothe *et al*., 2009).

In Kenya, where the first case of HIV and AIDS was reported in 1984, more than 1.5 million people have died of the disease; leaving an estimated 1.3 million children aged below 15 years as orphans (GOK, 2003). Programs and policies to combat the HIV epidemic began in 1990 but accelerated with the formulation of National Sessional Paper on in 1997 (GOK, 1997). The National HIV and AIDS Plan of 2000-2004 put in place strategies to prevent the spread of HIV and AIDS and mitigate impacts of the disease on the population through promotion of prevention, care and support of those infected and access to anti-retroviral therapy (NACC, 2000). Since the year 2002, national policies have been developed for voluntary testing and counseling (VCT) for HIV infection (NASCOP, 2001). National Guidelines on the prevention of mother to child transmission (PMCT) were introduced in 2002, setting standards for HIV testing and counseling as a routine part of antenatal care (MOH, 2002). The GOK developed the first National HIV and AIDS Strategic Plan (NASP) for 2000-2005. The second NASP for the years 2005/6-2009/10 has been developed and is a basis of the current national response to HIV and

AIDS. Its goals include: reduce the spread of HIV; improve the quality of life of infected and affected people; and mitigate the social and economic effects of the epidemic (NASCOP, 2007).

In the demographic and health survey conducted in 2003 (GOK, 2003) on women aged 15-49 and men aged 15-54 years, HIV prevalences of 8.7 and 4.6%, respectively, were reported (GOK, 2003). However, figures on the prevalence of HIV in pregnant women attending ante-natal clinics at sentinel sites in the same year were as high as 13, 26 and 41% in Kakamega, Kisumu and Suba Districts, respectively (GOK, 2003).

An AIDS indicator survey of 2007 was undertaken with the objective of providing information required for advocacy and planning for appropriate interventions for HIV prevention, treatment and care (NASCOP, 2007). The survey revealed that 7.1% or 1.42 million adults aged 15-64 years, were living with HIV infection in 2007. Furthermore, women were found to be more likely to be infected (8.4%) than men (5.4%); young women aged 15-24 years were four times more likely to be infected (5.6%) than young men of the same age group (1.4%); there was a wide regional variation in prevalence with Nyanza leading (14.9%) and North Eastern Province tailing (0.8%) in HIV prevalence. HIV prevalence among uncircumcised men (13.2%) aged 15-64 years was three times greater than among circumcised (3.9%) men (NASCOP, 2007).

While management of HIV and AIDS has been on activities such as awareness creation, condom distribution and provision of anti-retroviral therapies (NACC, 2000), HIV-patients are under oxidative stress from the infection and loss of CD4 counts (Schwarz, 1996), a number of micro-nutrients are required for fighting infection (Grimble, 1998; Semba, 1998; Nimmagadda *et al*.,

1998). Several HIV-associated clinical conditions including mucosal lesions of the mouth, esophagus, fever and malignancies, decreased appetite while other conditions, such as diarrhoea and mal-absorption increase demand for nutrition and diet control (Carbonnel *et al*., 1997; Timbo and Tollefson, 1994; Macallan, 1993). From the literature, weight loss and wasting are features of HIV disease progression and are predictors of morbidity and mortality (Palenicek *et al.*, 1995). However, basic nutritional and metabolic disturbances that lead to weight loss and wasting in HIV-infected persons may represent an adaptive response to an inflammatory state (Godfried *et al.*, 1993).

The cause of the increasing depletion of CD4 cell count in the transition from healthy carrier to AIDS patients has been investigated by administering cortisol (a hormone produced by the adrenal glands in the response to stress). It was found that CD4 cell count migrated from the blood circulating in the blood vessels into other areas of the body outside the vascular system (Fauci and Dale, 1974; Haynes and Fauci, 1978). After withdrawal of cortisol, the CD4 cell count returned to the circulating blood and CD4/CD8 ratio returned to normal. With regard to where the CD4 cell migrate under the influence of cortisol, it has been shown in animal experiments that they are sequestered mainly into the bone marrow (Fauci and Dale, 1974; Cohen, 1972; Levine and Claman, 1970). It was concluded that the sequestration of CD4 cells to bone marrow may be considered as a general phenomenon in any severe and persistent hypercortisolism (an excess of cortisol in blood) in acute-phase inflammatory reactions in which the whole body responds to an inflammation or injury. In the lymphocytes, dehydroepiandrosterone (DHEA) that is produced in the cortex of the adrenal glands plays an important role as an antagonist to cortisol (Daynes and Hennabold, 1994). DHEA causes

increased production of IL-2 and interpheron gamma (IFN-gamma), but not IL-4, thus playing a central role in the production of Th-1. Therefore, the sequestration of CD4 lymphocytes to the bone marrow is a significant component of a stress-induced Th-2 profile of the CD4 lymphocytes because, in the Th-2 profile in the bone marrow, CD4 cells actively stimulate the B cells, present there in large numbers, to increase the formation of antibodies.

Isolation of highly replicating HIV strains is associated with the weakest type 1 cytokine production, the strongest type 2 cytokine productions and lowest CD4 cell count. It has been noted that antiretroviral therapy, by controlling viral replication alone, invariably fails to achieve the broadest immune reconstitution. This issue has strengthened the rationale to widely explore new adjuvant immunotherapy with most work being performed on IL-2, given its potential to correct HIV-driven immune defects thereby translating to more effective immune competency (Tincati *et al*., 2009).

2. 3 Overview of micro-nutrients and HIV and AIDS

It has been noted that children infected with HIV and had abnormally low vitamin A levels had significantly high viral loads and low CD4 cell count than those with normal vitamin A levels (Steenkamp *et al*., 2009). Micro-nutrient supplements are associated with a delay in HIV disease progression and reduce mortality in HIV-positive persons not receiving highly active antiretroviral therapy (Drain e*t al.,* 2007). There are conflicting outcomes of micro-nutrient supplementations and suggestions that the interventions may not always be beneficial to HIV-seropositive persons (Tang *et al*., 1993; 1996). In South Africa, a study administered 15 mg of zinc daily to HIV-infected children for 6 months, resulting in no gains in viral load reduction

compared to the placebo-group (Bobat *et al*., 2005). Furthermore, failure to increase CD4 cell count in HAART-treated HIV-infected patients with satisfactory virological suppression has been related to low CD4 T-cell product ion, high turnover and death. However, the relative contribution of these factors is still unclear, although recent studies suggest that CD4 T-cell re-population during HAART is determined by CD4 T-cell activation and death (Massanella *et al*., 2010). However, changes in supplementation outcomes have been notecd since the advent of HAART (Libre *et al*., 2009).

Nutritional and metabolic disturbances can lead to altered acute-phase response proteins due to chronic inflammation in persons with advanced HIV infection (Treitinger *et al.,* 2001; Arinola *et al.,* 2004). Changes in acute phase response proteins, mainly decreased albumin and elevated C-reactive protein concentrations, have been associated with low serum concentrations of several micro-nutrients in HIV-seronegative persons (Shenkin, 1995; Thurnham *et al.,* 2003*;* Ghayour-Mobarhan *et al.,* 2005). Other researchers who have shown that repletion of serum zinc during infection with HIV is not a simple task and special supplements are needed (Mburu *et al*., 2010). Cell-mediated immunity is associated with increased serum zinc level (Mbakaya *et al.,* 2011; Cantorna *et al.,* 1994; 1995; Prasad *et al.,* 1997; Bao *et al.,* 2003). It has been shown that zinc supplementation to HIV patients on HAART resulted in prevention of immunological failure and significant repletion of serum zinc levels (Baum *et al*., 2010). While low CD4/CD8 ratio was associated with antibody immunity (Cantorna *et al.,* 1994), vitamin A, zinc and selenium deficiency were found to be common in TB-infected subjects (61, 85 and 87%, respectively) (van Lettow *et al.,* 2004). Disease progression is also associated with low serum zinc (Baum *et al.,* 1995). Furthermore, it has been reported that vitamin B_{12} deficiency impairs neutrophil

function (Benedich and Cohen, 1988) as zinc deficiency decreases lymphocyte concentrations (Fraker *et al.,* 2000). Similarly, selenium deficiency has been associated with impairment of neutrophil and T- lymphocyte responses (Ferencik and Ebringer, 2003).

The human body will increase or lower nutrient concentrations in circulation, the intake doses notwithstanding (Mbakaya *et al.*, 2011). Among these, include considerations such as prevailing infections and what immunity is best required to contain them. Thus, nutrient levels in circulation will be increased or decreased so as to up-regulate cellular or humoral immunity, depending on the form of immunity that may be required to effectively contain the offending agents (Jason *et al.*, 2002; Sprietsma, 1999). Furthermore, iron and zinc may be made available to organs and cells with increased requirements in the HIV disease in what is also believed to be purposeful in making these minerals unavailable to invading micro-organisms (Keusch and Farthing, 1986). Therefore, the need for extra caution when supplementing HIV-seropositive subjects is a reality as confirmed by some of the disappointing findings of such interventions (Filteau, 2010; Prentice, 2010).

There are suggestions that blind supplementation of immune-challenged persons may be counter-productive as one may stimulate one form of immunity, but down-regulate the very one needed to prevent fatal infections (Filteau, 2010; Mbakaya *et al*., 2011). For instance, while elevated zinc levels are needed to up-regulate cellular immunity to prevent HIV, TB, pneumonia and cancer, once infection has occurred, the body must adjust to acquire immunity by lowering zinc so as to produce antibodies against the pathogens (Sprietsma, 1999; Mbakaya *et al*., 2011). This phenomenon, may explain why malaria, HIV and AIDS, TB and cancer are increasingly

occurring as co-infections (Mbakaya *et al*., 2011). Furthermore, vitamin A, zinc and selenium deficiency have been found to be common in the TB-infected subjects (Van- Lettow *et al.,* 2004). Under the circumstances, researchers have worked on some safety limits within which therapeutic levels of micro-nutrients may be administered (Allard *et al.*, 1998; Bruning, 1994; Bryce-Smith, 1989; Dowen and Lancey, 1996). However, while some studies have observed that zinc supplementation is a potential intervention for prevention of acute lower respiratory infection (ALRI) in developing countries, discrepant findings from randomized trials remain unexplained (Roth *et al*., 2010).

There is no scientific unanimity on the benefits of zinc supplementation to HIV-seropositive subjects (Mbakaya *et al.,* 2005). It should not be assumed that administration of micro-nutrients is always beneficial, and therefore proposed micro-nutrient interventions in HIV-infected subjects should be scrutinized by well-designed, completely randomized, placebo controlled trials (Drain *et al,* 2007). Although supplementation may lead to biochemical improvement, showing that it leads to improved clinical outcome remains controversial (Irlam *et al*., 2005). However, one study does suggest that supplementation will delay progression of the severity of HIV (Fawzi *et al.,* 2004). Low vitamin B_{12} has been shown to increase the risk of HIV progression to AIDS, while selenium deficiency independently predicts mortality from HIV and AIDS (Tang *et al*., 1997a, Baum *et al.*, 1997). Other studies have shown serum zinc levels of HIV and AIDS patients fluctuated, and at low levels (Mbakaya *et al*., 2003).

It is reported that recognizing the presence of inflammation can help interpret nutrition data (Thurnham, 2010). However, the disproportionate burden of disease in Sub-Saharan Africa

might partly be attributable to the evolutionary dynamics of adaptation, which lowers serum zinc levels, and by extension, cellular immunity in favour of antibody production against pathogens and chemical agents that abound in the environment (Mbakaya *et al*., 2011). Large-scale intervention trials in Africa and Asia have shown that iron and zinc do not have the benefits for survival rates that were anticipated and revealed that iron may elevate mortality in malarious areas and that vitamin A supplementation of mothers yielded some adverse effects in terms of mother-to-child transmission of HIV (Prentice, 2010). Furthermore, epidemiological studies in affluent settings have frequently suggested associations between micro-nutrient status and outcomes such as cancers, heart disease and pre-eclampsia, but subsequent intervention trials have almost universally failed. In a study in India on residential school children with biochemical evidence of poor status for several micro-nutrients, a multi-micro-nutrient supplement did not significantly reduce the incidence of common childhood infections. However, the duration of such common childhood illnesses were significantly reduced (Sarma *et al*., 2006).

Although the mechanism by which inflammation may block zinc absorption is reportedly not known, there are reports that correlation between low serum zinc, HIV-seropositivity, enhanced optical density of HIV antibodies and significant viral load reduction in HIV and AIDS subjects on mega-doses of micro-nutrients in western Kenya suggested an evolutionary interplay and a way forward in management of HIV and AIDS (Mburu *et al.,* 2010, Mbakaya *et al.*, 2011). However, nutritional and metabolic disturbances can lead to altered acute-phase response proteins due to chronic inflammation in persons with advanced HIV infection (Arinola *et al.,* 2004).

A study found that blood from HIV-seropositive persons lacked the non-typhoidal *Salmonella*-specific antibodies needed to kill the pathogen that is a major opportunistic infection in people infected with HIV (MacLennan *et al*., 2010). In addition, the blood samples unable to eliminate the infection had higher levels of immunoglobulin G (IgG) that specifically binds to the bacteria than blood samples that could kill *Salmonella*. On further examination, it was found that an antibody specific to lipopolysaccharide (LPS), a membrane protein common to most gram negative bacteria, including *Salmonella,* was the inhibitory factor. Paradoxically, whereas blood samples from HIV patients were less effective at killing *Salmonella*, it also had lots of antibodies. Furthermore, it was observed that some antibodies were protective while others were on the contrary inhibitory/facilitating infection, a rather unusual finding. Both healthy and HIV-infected individuals had both inhibitory and protective antibodies, the difference stemming from the ratio of the two; with HIV patients having much higher levels of the inhibitory kind relative to the protective ones. Although the mechanism for these observations is unclear, it may be associated with the Th-1/Th-2 immunological dynamics where zinc sufficiency supports the protective while deficiency up-regulates inhibitory/facilitatory Th-2 immune systems, respectively (Sprietsma, 1999). This is further supported by observations that there was a significant correlation between low serum zinc, HIV-seropositivity, high HIV antibody production and significant viral load reduction in HIV seropositive subjects using mega doses of micro-nutrients that included zinc (Mbakaya *et al*., 2011).

While a study has shown that high pre-infection plasma vitamin E levels are, for poorly understood reasons, associated with high mortality in HIV-1-infected Kenyan women, another has shown that high serum zinc correlates with low serum α-tocopherol levels and vice versa

(Graham *et al.*, 2007). Furthermore, oxidative metabolism that is characteristic of HIV and AIDS inevitably leads to generation of reactive oxygen species (ROS) or "free radicals", which have the potential to cause further oxidative reactions, especially to those parts of the cell in a relatively reduced state, such as cell membranes or nucleic acids (Evans and Halliwell, 2001). In another study, vitamin A supplements in populations with poor vitamin A status have been shown to reduce mortality from diarrhoea in community studies, and deaths from pneumonia in measles studies (Glasziou and Mackerras, 1993).

Although anemia is more common with advanced HIV disease progression, studies disagree on whether this is principally due to iron-deficiency or chronic disease (Belperio and Rhew, 2004; van de Broek and Letsky, 2000; Totin *et al*., 2002; Semba and Gray, 2001). Whereas micro-nutrient minerals like Zn, Cu and Se are essential for maintaining proper immunologic function; their deficiency decreases lymphocyte concentration, reduces cytokine response and impairs neutrophil and T-lymphocyte responses respectively (Fraker *et al.*, 2000; Percival, 1998; Ferencik and Ebringer, 2003).

In a randomized control trial in Uganda, children aged one to five years and attending HIV clinics were administered with twice the recommended dietary allowance of 14 micro-nutrients as the intervention arm (vitamins A, B_1, B_2, niacin, B_6, B_{12}, C, D and E, folate, zinc, copper, iodine and selenium) or the standard recommended dietary allowances of 6 mult-ivitamins (vitamins A, D_2, B_1, B_2,, C and niacin) as a comparative standard of care arm for six months (Ndeezi *et al*., 2010). From this study, it was concluded that there was no significant difference in how the two micro-nutrient formulations were tolerated, effects on mortality, growth or CD4

cell count, leading to suggestions that future studies should carefully consider the composition and dosing of the supplements and the power needed to detect a difference between arms.

A study in India has established serum micro-nutrient zinc, copper and selenium levels in subjects with and without HIV and AIDS (Malviya *et al.,* 2009). Comparably, the mean micro-nutrient concentrations in HIV-seronegative and seropositive subjects were 136.55±9.30 vs 92.60±18.55 µg/dl ($p = 0.001$) for zinc; 104.74±5.06 vs 119.95±7.94 µg/dl ($p = 0.001$) for copper and 113.34±4.27 vs 72.87±22.00 µg/L ($p = 0.001$) for selenium, respectively. For serum retinol level in adults, it is reported to range from 1.6-2.3 µmol/L (Abbott-Johnson *et al.,* 2011). As for serum alpha-tocopherol concentrations, not much difference between HIV-seronegative and seropositive subjects has been reported in the US, the median values being 18.4 (15.2-22.0 and 18.0 (15.2-21.8) µg/L, respectively (Stephensen *et al*., 2006). In Nigeria, a micro-nutrient study of HIV-seropositive and seronegative subjects have shown that CD4 cell count positively correlated with serum alpha-tocopherol, zinc, and copper levels (Bilbis *et al.,* 2010). Furthermore, lower levels of alpha-tocopherol in HIV-seropositive subjects compared with controls were a result of its increased utilization in quenching free radicals. Also, serum zinc, iron, copper and CD4 cell count in HIV-seronegative subjects were significantly lower compared with seropositive ones ($p < 0.05$). To ensure normal serum values of micro-nutrients in the population, Dietary Reference Intakes (DRI) have been recommended as follows: Vitamins A, 3000 IU; C, 90 mg; E, 33 IU and B_{12}, 4.4 µg while for minerals it is zinc, 15 mg; and selenium, 55µg (USDA, 2010).

Anemia is more common with advanced HIV disease progression and studies disagree on whether this is principally due to iron-deficiency or chronic disease (Belperio and Rhew, 2004; van de Broek and Letsky, 2000; Totin *et al*., 2002; Clark and Semba, 2001; Semba and Gray, 2001). There are reports that zinc and iron compete during intestinal absorption but the post-absorptive interactions between these ions are not well understood (Donangelo *et al.,* 2002). However, infections may reduce intake and absorption, as well as increase utilization and excretion of micro-nutrients, thus impairing host micro-nutrient status (Friis and Michaelsen, 1998). Acute and even chronic generalized infections give rise to an acute-phase response, which is a generalized, stereotypic host reaction caused by a cascade of cytokines released by activated phagocytic cells (Dinarello, 1984). While the acute-phase response serves to enable the host to withstand invading pathogens and repair tissue damage, it also compromises host nutritional status (Beisel, 1992). Anorexia and fever are prominent constitutional signs of acute-phase response, leading to reduced intake of food and, hence, micro-nutrients. Fever is accompanied by catabolism of muscle tissues with mobilization of amino acids required for proliferation of neutrophils, lymphocytes, and fibroblasts and for synthesis of immunoglobulin and hepatic acute phase proteins. Consequently, the serum zinc and iron concentrations fall drastically due to redistribution within the body, with accumulation in the liver (Friis and Michaelsen, 1998). This serves to make the iron and zinc available to organs and cells with increased requirements, but is also believed to be purposeful in making these minerals unavailable to invading micro-organisms (Keusch and Farthing, 1986). There are reports that high serum iron levels favor micro-organisms such as viral and parasitic infections (Beisel, 1992).

There are reports that supplementation of HIV-seropositive subjects with multi-micro-nutrients is beneficial and has a favourable effect on reconstituting the nutritional status, cytokine production and immune status (Grimble, 1998). Furthermore, use of highly active anti-viral therapy (HAART) restores immunologic function but does not eliminate weight loss and wasting (Autran *et al.,* 1997; Wanke *et al.,* 2000; Tang *et al*., 2005). Consequently, some researchers have called for micro-nutrient supplements as adjunct therapy to HAART (Singhal and Austin, 2002; Lanzillotti and Tang, 2005; Baum *et al.*, 2010).

In South Africa, administration of 15 mg of zinc daily to HIV-infected children for 6 months resulted in no gains in viral load reduction compared to the placebo-group (Bobat *et al*., 2005). Furthermore, the failure to increase CD4 cell count in HAART-treated HIV-infected patients with satisfactory virological suppression has been related to low CD4 T-cell production, high turnover and death. However, the relative contribution of these factors is still unclear, although there are suggestions that CD4 T-cell re-population during HAART is determined by CD4 T-cell activation and death (Massanella *et al*., 2010). It has been noted that development of deficiency of vitamin A or vitamin B_{12} is associated with a decline in CD4 cell count ($p = 0.0255$ and $p = 0.0377$, respectively) , while normalization of vitamin A, vitamin B_{12} and zinc was associated with higher CD4 cell count ($p = 0.0492$, 0.0061 and 0.0112; respectively) (Baum *et al.*, 1995). It has also been observed that a deficiency in zinc leads to a premature transition from the efficient T-helper type 1 (Th-1) dependent cellular anti-viral immune function to the less efficient T-helper type 2 (Th-2) dependent humoral immunity (Sprietsma *et al.,* 1999). Furthermore, it is known that zinc determines progress and outcomes of many diseases through the Th-1/Th-2 balance.

An average serum zinc level of about 120 µg/100 ml has been observed in normal control subjects (Surendra and Elmer, 1970). There are views that the effective management of HIV and AIDS could remain extremely elusive and for a very long time if the dual health effects of dioxins and zinc as well other pertinent micro-nutrients are to continue being relegated to the periphery of biomedical research (Mbakaya *et al.,* 1997). However, better management could be obtained by providing patients with micro-nutrients such as zinc, vitamin A, vitamin B_{12}, vitamin E and selenium that work in concert with it since toxicity occurs when serum micro-nutrient levels exceed 150 µg/100 ml zinc, 20.6 µg/100 ml selenium and 2.44 µmol/l retinol (Surendra and Elmer, 1970; Allaway *et al*., 1968; Gofman *et al*., 1964; Pillch, 1985).

Kenya is classified as a country with clinical vitamin A deficiency of public health significance (WHO, 1996). Several studies conducted in Kenya confirm the classification and show a prevalence of vitamin A deficiency that is of public health concern in several segments of the Kenyan population (Etyang *et al.*, 2003; Mwaniki *et al.,* 2002; MacDonald *et al*., 2001). A cross-sectional study conducted in Kenya has shown that vitamin A deficiency is associated with up to 13 fold increased risk of HIV-1 DNA vaginal shedding, after controlling for CD4 cell count (Mostad *et al*., 1997). In a randomized controlled study involving 49 HIV-infected Canadian adult patients of whom 24 had AIDS, the average viral load was approximately 1.0 $\pm$ 0.4 log copies/ml lower in the vitamin taking group (800 IU vitamin-E and 1000 mg vitamin C daily for 3 months) than in the placebo group, a figure comparable to the reduction brought about by daily single therapy of AZT for 12 weeks (Allard *et al*., 1998).

2.3.1 Zinc and HIV and AIDS

Zinc is an essential element that is naturally present in some foods, added to others, and available as a dietary supplement. It is involved in many aspects of cellular metabolism, required for the catalytic activity of about 100 enzymes, plays a role in the immune function, protein synthesis, wound healing, DNA synthesis and cell division and possesses anti-viral, anti-bacterial and anti-cancer properties (Bryce-Smith, 1989; Cantorna *et al.,* 1994; Prasad *et al.,* 1997; Bao *et al.,* 2003). Furthermore, high serum zinc concentration is associated with up-regulation of cell-mediated immunity (Cantorna *et al.,* 1994, 1995; Prasad *et al.,* 1997; Bao *et al.*, 2003).

Serum and plasma zinc concentrations in adults range from 80 to 150 mg/dl, although circadian diurnal fluctuations occur in concentration. About 2 g of zinc is distributed throughout the body (average 10 to 200 mg/g) of an adult human being. Absorption of dietary zinc occurs over the duodenal region of the gastrointestinal tract. Active transport of zinc into portal blood is mediated by metallothionein. Zinc-albumin complexes account for about 5 percent of the zinc, and the metal is readily exchangeable throughout the peripheral circulation. About 7 to 8 percent is loosely bound to amino acid constituents in plasma and the remaining percentage of plasma zinc is largely bound to macroglobulins and unavailable for nutritional purposes (Cotton and Williamson, 1988; Mbakaya and Wakori, 1997).

Plasma or serum zinc levels are the most commonly used indices for evaluating zinc deficiency, but these levels do not necessarily reflect cellular zinc status due to tight homeostatic control mechanisms (Bryce-Smith, 1989). Furthermore, zinc deficiency is characterized by growth retardation, loss of appetite, and impaired immune function. In more severe cases, zinc

deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions. Weight loss, delayed healing of wounds, taste abnormalities, and mental lethargy can also occur. Many of these symptoms are non-specific and often associated with other health conditions; therefore, a medical examination is necessary to ascertain whether a zinc deficiency is present. Zinc nutritional status is difficult to measure adequately using laboratory tests due to its distribution throughout the body as a component of various proteins and nucleic acids (Bryce-Smith, 1989).

Common measurements of zinc levels in the blood don't necessarily make it possible to predict low body levels (Bogden *et al.*, 1990). In a double blind controlled clinical trial to investigate the effect of zinc supplementation in HIV-infected women in Tanzania, no viral load reduction or prevention of mother to child transmission was observed (Villamor *et al.,* 2006*).* In a study on 800 children (aged 12–35 months) in Bangladesh, a two-week supplement of zinc, vitamin A, both, or placebo was given to children who were followed up for six months. Combined zinc and vitamin A synergistically reduced the prevalence of persistent diarrhoea and dysentery. Zinc was associated with a significant increase in acute lower respiratory infection, but this adverse effect was reduced by interaction between zinc and vitamin A, suggesting the importance of both immune responses in the fight against diseases (Sprietsma, 1999; Jason *et al.*, 2002).

While children in South Africa had high viral load before and after supplementation with micro-nutrient zinc for six months, high antibody densities are required for effective clearance of viral load and correlate with low serum zinc levels (Bobat *et al.*, 2005; Mbakaya *et al.*, 2011). Low serum zinc status has been associated severally with humoral immunity (Bao *et al.*, 2003). Since

humoral immunity was associated with significant reduction in viral load, however much HIV-seropositive subjects are supplemented with zinc, the serum levels often returned to near baseline levels (Mbakaya *et al.*, 2011). Low serum zinc level is associated to an up-regulated antibody immunity that has been noted to be effective in clearance of viral load in infected persons (Cantorna *et al.*, 1995; Prasad *et al.*, 1997; Mbakaya *et al.*, 2011). Furthermore, it has been reported that vitamin B_{12} deficiency impairs neutrophil function (Benedich and Cohen, 1988) as zinc deficiency decreases lymphocyte concentrations (Fraker *et al.*, 2000). Zinc ions reportedly have anti-viral, anti-bacterial and anti-cancer properties (Bryce-Smith, 1989; Sprietsma, 1999)

TB is an opportunistic infection in HIV and AIDS probably because up-regulation of humoral immunity against the virus lowers the serum zinc level and by extension the cellular immunity which is required to prevent TB infection. In a study of tuberculosis in Indonesia, supplementation with zinc and vitamin A led to much earlier resolution of radiological changes and time to sputum negativity (Karyadi *et al*., 2002). However, copper deficiency due to overuse of zinc supplementation has been reported (Rowin and Lewis, 2005; Igic *et al.*, 2002).

In asymptomatic HIV-positive men, higher zinc intake in foods and supplements has been associated with faster disease progression and mortality in a dose-response relationship (Tang *et al*., 1993; 1996). A study examined the efficacy of daily supplementation with nutritional doses of zinc (12 and 15 mg of elemental zinc for women and men, respectively) for 18 months in 231 HIV-infected patients who had plasma zinc levels < 75 µg/dl and found that it delayed HIV disease progression and prevented associated morbidity and mortality (Baum *et al*., 2010). Furthermore, the intervention reduced 4-fold the risk of immunological failure, defined as CD4

cell count < 200 cell/mm^3 but had no effect on viral load or mortality but decreased the rate of diarrhea by more than half compared to placebo. There are several biologically plausible mechanisms that can explain these findings that include; zinc deficiency is associated with suppression of cell-mediated immunity, decreased levels of thymulin that is known to induce T cell differentiation, decreased CD4/CD8 ratio, IL-2 levels and natural killer (NK) cell count (Sprietsma, 1999; Mehta and Fawzi, 2010).

Zinc deficiency has also been associated with a higher susceptibility to infectious diseases such as pneumonia, malaria and diarrhea (Black, 2003). Zinc deficiency in people living with HIV may account for an improper maturation of CD4 cell count mediated through low levels of the zinc-dependent hormone, thymulin; thereby leading to a less effective immune response and a higher susceptibility to opportunistic infections (Mocchegiani and Muzzioli., 2000). While one study in the USA has reported high zinc intake to be significantly associated with faster HIV progression (Tang *et al*., 1996), another has shown high serum zinc levels were inversely associated with mortality (Lai *et al*., 2001). A study with RDA doses of zinc in children with severe pneumonia in Nepal did not achieve any difference comparable to placebo (Valemtiner-Branth *et al*., 2010).

Compared to healthy controls, a study in Iran found that serum zinc in human immuno-deficiency virus infected subjects were significantly low ($p = 0.01$) (Khalili *et al.*, 2008). HIV infected individuals are vulnerable to malnutrition due to several factors including inadequate nutrient intake, nutrient loss, metabolic alteration and drug nutrient interactions (Dudgeon *et al.*, 2006; Colecraft, 2008). In India, HIV patients with various sexually transmitted diseases have

been found to be deficient in zinc (Pradeep *et al*., 2010). In a study of pregnant women in Ethiopia, low plasma zinc concentration was observed in 72% of the subjects while 99% were at risk for inadequate zinc intake given their low dietary intake of zinc (Abebe *et al*., 2007).

Zinc deficiency is prevalent in children in developing countries where diarrhoea is also a big problem. In six of nine trials, zinc supplementation significantly reduced the incidence of diarrhoea, and in five of these there was a lower incidence of pneumonia (Black, 2003). Moreover, in acute diarrhoea trials, zinc supplemented children had a 15% lower probability of continuing diarrhoea on a given day. In persistent diarrhoea trials, there was a 24% lower probability of continuing diarrhea in zinc supplemented children. A study in Nigeria showed that uric acid and zinc were significantly high while vitamin E and all other trace elements (except zinc) were significantly low in HIV-seropositive subjects compared to healthy controls, suggesting the need for routine assessment and appropriate supplementation of micro-nutrients in such vulnerable groups (Olaniyi and Arinola, 2007).

It has been reported that HIV infected adults receiving micro-nutrient zinc supplements for 3 years in Nairobi, Kenya, did not show much repletion in the serum zinc levels (Mbakaya *et al.,* 2004b). Furthermore, the levels were found to be consistent with the extent of disease progression (subjects in the later stages of disease progression exhibited much lower levels than asymptomatic counterparts). Serum zinc levels in all subjects declined even when provided with 1 x RDA doses, suggesting the need for use of higher dosages. Populations in Sub-Saharan Africa and South East Asia are at greater risk of zinc deficiency due to the inadequate zinc intakes in one-third of the population (Shrimpton *et al.,* 2005). As a micro-nutrient, zinc has a

unique property of forming mercaptides with thiols, inhibits cyclic reduction-autoxidation of thiols and superoxide generation (Reid, 2000). Furthermore, when the mixed function oxidase (MFO) system acts as an activator and not as a detoxifier, zinc inhibits the oxidation.

Common measurements of zinc levels in the blood don't necessarily make it possible to predict low body levels (Bogden *et al.*, 1990). In a double blind controlled clinical trial to investigate the effect of zinc supplementation in HIV-infected women in Tanzania, no viral load reduction or prevention of mother to child transmission was observed (Villamor *et al.*, 2006). A possible interaction between zinc and vitamin A status has been explored (Raham *et al*., 2002). In a study on 800 children (12–35 months) in Bangladesh, a two-week supplement of zinc, vitamin A, both, or placebo was given to children who were followed up for six months. Combined zinc and vitamin A synergistically reduced the prevalence of persistent diarrhoea and dysentery. However, zinc alone was associated with a significant increase in acute lower respiratory infection, but this adverse effect was reduced by interaction between zinc and vitamin A, suggesting the importance of both immune responses in the fight against diseases (Sprietsma, 1999; Jason *et al.*, 2002).

In Iran, HIV-infected subjects had significantly lower serum zinc and selenium than healthy subjects ($p = 0.01$, $p = 0.02$ respectively) (Khalili *et al.*, 2008). Furthermore, patients who may have been infected by used syringes had significantly lower serum zinc (32.4 ± 10.6 vs. 67.2 ± 14.3 μg/dl) and selenium (55.8 ± 14.6 vs. 84.1 ± 9.9 μg/l) concentrations compared to those who were probably infected via sexual contact ($p = 0.001$ for both comparisons).

2.3.2 Alpha-tocopherol (Vitamin E), vitamin C and HIV and AIDS

The term vitamin E describes a family of eight antioxidants: four tocopherols (alpha-, beta-, gamma-, and delta-) and four tocotrienols (alpha-, beta-, gamma-, and delta-). Alpha- tocopherol is the only form of vitamin E that is actively maintained in the human body. Therefore, it is the form of vitamin E found in the largest quantities in blood and tissues and appears to have the greatest nutritional significance. In longitudinal studies, dietary intake of vitamin E and high serum vitamin E levels were associated with high CD4 cell count and reduced risk of HIV progression (Baum *et al.*, 1994; Tang *et al*., 1997b). In one study, drug use was not independently associated with micro-nutrient alterations. Furthermore, elevated triglycerides were associated with higher serum retinol and α-tocopherol as hepatitis C was an independent determinant of low micro-nutrient status (Forrester *et al.*, 2009). Previous studies demonstrated that vitamin A deficiency reduces lymphocyte response (Semba, 1999) while vitamin C and selenium deficiency depresses cell-mediated immune response (Benedich, 1988) as impairment of neutrophil plus T-lymphocyte responses, respectively (Ferencik and Ebringer, 2003).

A study in Kenya has shown that high pre-infection levels of α-tocopherol were associated with increased mortality, suggesting the need for more research to elucidate the role of vitamin E in HIV-1 pathogenesis (Graham *et al.*, 2007). Furthermore, deficiency of vitamins A, C, E and B_{12} reduces lymphocyte response, depresses cell-mediated immune response, impairs T cell-mediated function, and impairs neutrophil function, respectively (Benedich, 1988; Benedich and Cohen, 1988). In a placebo-controlled trial where 29 HIV-seropositive patients received either 6 months of vitamin E supplements or placebo while simultaneously initiating HAART, no significant differences were observed in CD4 cell count, CD4/CD8 and viral load between the

groups but a greater increase in lymphocyte viability in the vitamin E-supplemented group (De Souza *et al.,* 2005). Consumption of balanced diets provides the necessary nutritional resources for producing assorted immune proteins that are efficient in preventing disease progression (Tang *et al*., 1993; 1996). In several observational studies, vitamins C and E were found to be low in people living with HIV (Macallan *et al*., 1995; Beach *et al*., 1992). Low serum levels of vitamins C and E were related to significantly higher levels of oxidative stress and increased viral load replication (Macallan *et al*., 1995; Kanter *et al*., 1999). High pre-infection plasma vitamin E levels were, for poorly understood reasons, associated with high mortality in HIV-1-infected Kenyan women (Graham *et al.,* 2007). Furthermore, oxidative metabolism that is characteristic of HIV and AIDS inevitably leads to generation of reactive oxygen species (ROS) or "free radicals", which have the potential to cause further oxidative reactions, especially to those parts of the cell in a relatively reduced state, such as cell membranes or nucleic acids (Evans and Halliwell, 2001). However, it has been observed that use of micro-nutrients may be beneficial in reduction of the inflammatory response (Shenkin, 1995; Thurnham *et al.,* 2003*;* Ghayour-Mobarhan *et al.,* 2005).

2.3.3 Retinol and HIV and AIDS

Vitamin A is a generic term for a large number of related compounds. Retinol (an alcohol) and retinal (an aldehyde) are often referred to as preformed vitamin A. Retinal can be converted by the body to retinoic acid, the form of vitamin A known to affect gene transcription. Retinol, retinal, retinoic acid, and related compounds are known as retinoids. Beta-carotene and other carotenoids that can be converted by the body into retinol are referred to as pro-vitamin A carotenoids. Hundreds of different carotenoids are synthesized by plants, but only about 10% of

them are pro-vitamin A carotenoids. Vitamin A is a fat-soluble vitamin that is derived from two sources: preformed retinoids and pro-vitamin carotenoids (Underwood and Barbara, 2004).

Vitamin A deficiency usually results from malnutrition, but can also be due to abnormalities in intestinal absorption of retinol or carotenoids. Deficiency is prevalent in humans, especially children, in certain underdeveloped countries. There are reports that low and high serum retinol levels are associated with cellular and humoral immunity, respectively (Jason *et al.*, 2002). During infections, plasma retinol levels may decrease due to poor food intake, an increased utilization of retinol by target tissues or increased urinary losses of vitamin A (Campos *et al*., 1987; Stephenson *et al.*, 1994). In addition, serum concentrations of several nutrients decline during the acute-phase response, either because they are re-distributed in the body or because they are bound to acute-phase proteins (Visser *et al*., 2003). Some of the manifestations of vitamin A deficiency include: blindness due to inability to synthesize adequate quantities of rhodopsin. Moderate deficiency leads to deficits in vision under conditions of low light ("night blindness"), while severe deficiency can result in severe dryness and opacity of the cornea (xeropthalmia). Supplementation with vitamin A has been shown to substantially reduce mortality from diseases such as measles and gastrointestinal infections. Abnormal function of many epithelial cells manifests by such diverse conditions as dry, scaly skin, inadequate secretion from mucosal surfaces, infertility, decreased synthesis of thyroid hormones and elevated cerebrospinal fluid pressure due to inadequate absorption in meninges. Vitamin A also acts in the body as an antioxidant, a protective chemical that may reduce the risk of certain cancers. In the intestine, vitamin A is protected from being chemically changed by vitamin E. The Dietary Reference Intake (DRI) or Recommended Daily Amount (RDA) for vitamin A for a

25-year old male is 900 µg/day, or 3000 IU. The Food Standards Agency states that an average adult should not consume more than 1500 µg/day or (5000 IU) per day, because this increases the chance of osteoporosis (WHO, 1996).

Deficiency of vitamin A has consistently been associated with an increase in mother-to-child transmission of HIV (Semba *et al.,* 1994). However, there is conflicting evidence coming from observational studies with one reporting that increased mortality and low CD4 cell count was associated with low serum vitamin A (Semba *et al.*, 1993) while another did not find a relationship between low serum retinol status and HIV disease progression (Tang *et al*., 1997b) as another in Tanzania showed that vitamin A supplementation increased mother-to-child transmission of HIV by an additional 38% compared to placebo (Fawzi *et al*., 2002). Furthermore, association of high HIV load, rapid progression, and low serum retinol late but not early in disease progression has been reported (Camp *et al.,* 1998). While hyporetinolemia is an independent prognosis factor in AIDS patients, acute-phase inflammatory markers (C-reactive protein and tumor necrosis factor-alpha) are associated with low serum retinol levels in these patients (Neves *et al.,* 2010).

The HIV disease progression has been associated with low serum retinol level at baseline and low serum zinc at baseline and after intervention (Baum *et al.,* 1995), implying that for disease to progress, cellular and humoral immunity that are up-regulated by zinc and retinol respectively, will be low due to lowered nutritional status of the micro-nutrients. A study has shown that a single large dose of vitamin A to neonates improved survival by the 6^{th} week in those who were HIV-seropositive by polymerase chain reaction (Humphrey *et al.,* 2006). This immunological

reality may explain observations that use of HAART restores immunologic function but does not eliminate weight loss and wasting (Autran *et al.,* 1997; Wanke *et al.,* 2000). Consequently, some researchers have called for micro-nutrient supplements as adjunct therapy to HAART (Singhal and Austin, 2002). Vitamin A, iron and zinc have been associated with adverse effects and caution is warranted for their use in management of HIV and AIDS (Hummelen *et al*., 2010).

In South Africa, independent predictors of low serum retinol levels in adults attending an HIV clinic were high WHO clinical staging of disease and low body weight while low zinc levels were predicted by low body weight only (Visser *et al*., 2003). High serum retinol concentration is associated with an up-regulated humoral immunity, nutritional soundness and high BMIs (Palenicek et al., 1995; van Lettow *et al.,* 2004). A study has shown that a single large dose of vitamin A to neonates improved survival by the 6^{th} week in subjects who were HIV-seropositive by polymerase chain reaction (Humphrey *et al.,* 2006). In addition, elevated serum retinol levels have been shown to be beneficial during HIV infection (Nimmagadda *et al.,* 1998; Semba and Gray, 2001). Furthermore, low serum retinol is associated with up-regulation of cellular immunity (Jiamton *et al*., 2003).

2.3.4 Copper and HIV and AIDS

Copper is a trace mineral which plays a role in health as it is a required component for many redox enzymes including; cytochrome C oxidase (Lippard and Berg, 1994). Serum copper levels have been reported to be significantly higher in infection and inflammatory states (Sinha and Gabrieli, 1970). According to some researchers, zinc and copper inadequacy are defined to be < 75 and 85 µg/dl respectively (Lai *et al.,* 2001). Many investigators have utilized plasma Cu/Zn

ratio for clinical assessment of zinc deficiency in several diseases (Bogden *et al*., 1990). In a study involving 121 HIV positive homosexual men, it was found that a Cu/Zn ratio > 1 was associated with increased mortality (Lai *et al.,* 1998). The copper/zinc ratio has been shown to be a valuable clinical marker for many diseases, such as neoplasms of lung, breast, head, and neck and with pancreatic cancer, asthma, skin and cardiovascular diseases (Kadrabova *et al.*, 1996; Tasaki *et al*., 1993; Suciu *et al*., 1992). However, there are reports that suggest that zinc interferes with copper nutriture (Rowin and Lewis, 2005). Furthermore, copper deficiency due to over-use of zinc supplementation has been reported (Igic *et al.,* 2002).

2.3.5 Iron and HIV and AIDS

In people living with HIV, anemia (defined as low haemoglobin) is highly prevalent and is associated with increased mortality and enhanced disease progression (Sullivan *et al*., 1998; O'Brien *et al.,* 2005). However, there are concerns that iron supplementation may adversely affect HIV progression and increase mortality (Gordeuk *et al.,* 2001). In a retrospective study in thalassemia major patients, the rate of progression of HIV was significantly faster amongst those receiving low-doses of iron chelating agent, desferrioxamine, and who had higher serum ferritin concentrations (Costagliola *et al.,* 1994; Salhi *et al*., 1998). Furthermore, administration of low doses of iron with dapsone for prophylaxis of pneumocystis carinii pneumonia in HIV-positive patients was associated with an increased risk of mortality (Salmon-Ceron *et al*., 1995).

A retrospective study of iron load in bone marrow macrophages in HIV patients suggested that high iron stores were associated with shorter survival (Monye *et al*., 1999). Given the potential adverse role of iron in HIV progression, caution is warranted for iron supplementation and this

should not be routinely undertaken as it is contra-indicated (Hummelen *et al.*, 2010). In Tanzania, zinc supplementation to HIV positive women was inversely associated with haemoglobin levels and a three-fold increase in the probability of wasting (Villamor *et al.*, 2006).

2.4 Tuberculosis and HIV and AIDS

HIV and AIDS continue to fuel the tuberculosis epidemic, a key opportunistic infection, especially in Africa (Lonnroth *et al.*, 2010). Poor nutritional status impairs the immune system and may increase the risk of active tuberculosis (Cegielski and McMurray, 2004). Since TB is an opportunistic infection in HIV and AIDS and considering that patients with active TB usually lose considerable weight and suffer from multiple micro-nutrient deficiencies, nutritional support was a cornerstone of TB treatment before the antibiotics era (Daniel, 2006). However, since the development of anti-TB drugs in the 1940s, nutritional support has ceased to play a prominent role (Ramakrishnan *et al.*, 1961). According to some researchers, use of zinc in supplements for TB patients is not advisable because observational studies have suggested that high dietary intake of zinc is associated with decreased survival in HIV-infected individuals (Villamor *et al*., 2008). It has been suggested that the research community should agree on a package of micro-nutrients with doses that meet the demands of patients with TB during treatment but that still can be considered safe (Benn *et al.*, 2008). From another study, there is evidence that multiple micro-nutrients improve CD4 cell count and HIV-related morbidity and mortality in adults compared with supplementation with two or few micro-nutrients (Allen *et al.*, 2009). In another study, it was demonstrated that among a group of HIV-infected patients who were co-infected

with TB, patients who exhibited with weight loss had significantly low vitamin A levels (Rwangabwoba *et al.*, 1998).

In a study of tuberculosis in Indonesia, supplementation with zinc and vitamin A led to much earlier resolution of radiological changes and time to sputum negativity (Karyadi *et al*., 2002). In a large study in India on residential school children with biochemical evidence of poor status for several micro-nutrients, a multi-micro-nutrient supplement did not reduce the incidence of common childhood infections, but reduced the duration of such illnesses (Sarma *et al*., 2006).

2.5 Methods of Analysis

2.5.1 Principle of Atomic Absorption Spectrometry (AAS)

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample (Skoog and Leary, 1992). It therefore relies heavily on the Beer-Lambert law. The electrons of the atoms in the atomizer can be promoted to higher orbitals for a short amount of time by absorbing a set quantity of energy (i.e. light of a given wavelength). This amount of energy (or wavelength) is specific to a particular electron transition in a particular element, and in general, each wavelength corresponds to only one element. This gives the technique its elemental selectivity. As the quantity of energy (the power) put into the flame is known, and the quantity remaining at the detector can be measured, it is possible, from Beer-Lambert law, to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured.

2.5.2 Principle of High-performance liquid chromatography (HPLC)

This is a separation technique that can be used to identify, quantify and purify the components of a mixture (Skoog and Leary, 1992). The sample to be analyzed is introduced (small volumes), into the stream of mobile phase (liquid) and moved through the column with rates depending on specific chemical or physical interactions with the stationary phase (partition chromatography) based on the nature of the sample and on the compositions of the stationary phase. The retention time for a specific solute under particular conditions (type of stationary phase, ratio/composition of solvent(s) used, and the flow rate of the mobile phase) is used to identify it. This liquid chromatographic technique utilizes smaller column size, smaller media inside the column, and higher mobile phase pressures.

2.5.3 Principle of Eliza and Amplicor

HIV tests are used to detect the presence of the HIV, in serum, saliva or urine to detect antibodies or antigen using a biochemical technique called Enzyme-linked immunosorbent assay (ELISA) used as a diagnostic tool in medicine. In ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal, most commonly a colour change in a chemical substrate (Leng *et al*., 2008; Adler *et al*., 2009).

Viral load is a measure of the severity of a viral infection and is calculated by estimating the amount of virus in an involved body fluid such as plasma and is given in copies per milliliter.

Tracking viral load is used to monitor therapy during chronic viral infections, and in immuno-compromised patients (Puren *et al*., 2010).

2.5.4 Principle of Facscalibur

This is a simple, rapid, and affordable method for counting CD4, CD8 and NK lymphocytes (Rodriguez *et al*., 2005). Microliter volumes of blood without further sample preparation are stained with fluorescent antibodies, captured on a membrane within a miniaturized flow cell and imaged through microscope optics with the type of charge-coupled device developed for digital camera technology. An associated computer algorithm converts the raw digital image into absolute CD4, CD 8 and NK cell count percentages in real time.

2.5.5 Principle of Coulter Counter

The Coulter Principle is most commonly employed in a COULTER COUNTER, which is an analytical instrument designed for counting cells in hematology used to obtain information about blood cells (Stephen *et al*., 2007). The Coulter Principle relies on the fact that particles moving in an electric field cause measurable disturbances in that field. The magnitudes of these disturbances are proportional to the size of the particles in the field. The particles are suspended in a conducting liquid and the electrical field physically constricted so that the movement of particles in the field causes detectable changes in the current. The particles are then diluted enough so that only one at a time passes through the physical constriction, preventing an artifact known as coincidence. In addition to clinical blood cells (~6-10 µm, typically), the Coulter principle has established itself as the most reliable laboratory method for counting a wide variety of cells.

The technique has been used to diagnose a variety of diseases, and is the standard method for obtaining red blood cell count (RBC) and white blood cell count (WBC) as well as several other common parameters. When combined with other technologies such as fluorescence tagging and light scattering, the Coulter Principle can help produce a detailed profile of patients' blood cells.

2.5.6 Principle of Westergren's Method

This is used to determine the Erythrocyte sedimentation rate (ESR) of the blood sample. ESR is measured as the length in millimeters of the clear column of plasma that collects at the top of a vertical column of anti-coagulated blood kept undisturbed for one hour. ESR is the rate at which erythrocytes sediment in anti-coagulated blood kept undisturbed. RBCs settle down due to their greater specific gravity than that of plasma. The normal values of ESR for Males are 1-4 mm/ Hr while those of females are 3-10 mm/ Hr.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study design

This was a randomized controlled intervention study using VIUSID^{TM} with citric acid together with micro-nutrient doses of zinc, selenium and vitamins A, $B_{12,}$ C and E. HIV and AIDS subjects were sequentially recruited and randomly allocated to the following interventions Arms:

Arm 1: VIUSID^{TM} with citric acid alone

Arm 2: VIUSID^{TM} and mega multi-micro-nutrient doses of vitamins-A, B_{12}, C and E, zinc, selenium, dehydroepiandrosterone (DHEA) and citric acid.

The interventions above were blinded (to the clinicians, subjects and laboratory analysts). Unmasking was done during data analysis.

3.2 Sample size

Hypothesis testing for continuous data is given by the equation below (Lemeshow *et al*., 1991**)**:

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \, 2\sigma^2}{d^2}$$

Where $Z_{1-\alpha/2} = 1.96$ and $Z_{1-\beta} = 1.28$ for $\beta = 10\%$ (i.e. for 90% power of study)

The equation reduces to $n = 21\sigma^2 / d^2$

Where σ^2 is variance; and d the difference to be detected at $\mu_1 - \mu_2 = 10$ µg/dl serum zinc and $\sigma^2 = 14^2$, with respect to zinc measured in an earlier study (Mbakaya *et al*., 2003).

The sample size is therefore,

$n = 21(14)^2/10^2$ $\quad$ $n = 41.16$

Allowing for a loss to follow-up of 10%, 45 subjects were recruited per Arm, giving a total sample size of 90 patients in Arms 1 and 2. The sample size was adequate and compares with that of other peer-reviewed published studies (Mburu *et al.*, 2010; Pradeep *et al.*, 2010; Steenkam *et al*., 2009; Graham *et al*., 2007).

3.3 Study population and site

This study was undertaken in western Kenya, where the prevalence of HIV infection is 5.4% and 14.9% in Western and Nyanza Provinces, respectively (NASCOP, 2007; NACC, 2000). The western Kenya region was selected for considerations given that national statistics indicated that this was one of the regions reporting high prevalence of HIV and AIDS in the country (NACC, 2000). Consenting subjects from sugar cane processing companies, clinics, Teachers' Unions and Societies of people living with HIV and AIDS in the study areas were recruited. In these sites, the study team worked in close consultations with human resource departmental staff and healthcare providers at the company staff clinics, Provincial Administration and the Ministry of Health staff as well as local NGOs.

3.4 Inclusion and exclusion criteria

HIV-1 infected men and women aged 18-60 years and working and or living in the selected sites were included in the study. The main study inclusion criterion was that a subject had a positive HIV test result from a voluntary testing and counseling centre (VCT).

Subjects using anti-retroviral drugs, those already using other nutritional supplements and those who refused to give a written informed consent were excluded from the study.

3.5 Recruitment and randomization of subjects

The Provincial Administration and Medical Officers of Health in the selected study sites were informed of the objectives of the study and their support sought. The Teachers and Plantation Workers' Union representatives in the districts were also informed of the study and asked to sensitize their members with a view to mobilizing potential study clients to participate in the study. Priority was given to proper counseling before patients were recruited. Kenya National Teachers Union and the Kenya Plantation Workers Union in the Sugar Industries assisted with identification of infected workers who upon counseling and consenting to be included in the study were screened with a view to selecting those who met the study inclusion criteria.

Written informed consent was obtained from the study subjects by completion of a consent form (Appendix I). Consenting HIV positive subjects who met the inclusion criteria were recruited. Baseline demographic and socio-economic data were obtained using a quality of life assessment questionnaire (Appendix II). In this tool, the food consumption data obtained was used to indicate how many times food was consumed in a day. This information was correlated with clinical, micro-nutrient and immunological status of the subjects.

At baseline the patients were randomized into two blocks, Arm and Arm 2. Arm 1 received VIUSID^{TM} (comprising of honey (2.5 mg), of malic acid (2.0 g), arginine (2.0 g), glucosamine (2.0 g), glycine (1.0 g), ascorbic acid (0.06 g), pyridoxal (1.0 µg), folic acid (200 µg) ,

glycirrhizinic acid (0.1 g) and hydrated zinc sulphate (15 mg Zn) with citric acid (for matching the acidic taste of vitamin C in the mega-dose syrup). Arm 2 received VIUSID^{TM} and mega-multi-micro-nutrient supplement comprising zinc (100 mg daily), vitamin A (200,000 IU single monthly), vitamin B_{12} (500 µg daily), vitamin C (1,000 mg daily), citric acid, vitamin E (1,000 IU daily), selenium (200 µg daily). The mega-dose formulation was well within safety limits as separately observed by other researchers (Allard *et al.*, 1998; Bruning, 1994; Bryce-Smith, 1989; Dowen and Lancey, 1996).

To promote compliance, subjects were educated on the importance of un-interrupted use of the supplements provided and also informed to stick to their regimen that had been selected on the basis of individual nutritional needs. Sharing of products within or without the subjects in the study was discouraged. To additionally tighten on compliance, subjects were asked to return used containers of the supplements at their next visit for replenishment.

3.6 Procedures for cleaning containers

The containers were soaked in hot water containing detergent for two hours then cleaned thoroughly with a brush and rinsed with tap water then with de-ionized water before being soaked in 50% nitric acid for the glassware and 2 % for the plastic bottles for 18 hours. They were then rinsed twice with cold de-ionized water, filled with the hot de-ionized water and let stand for one hour before being rinsed with hot de-ionized water and dried in an oven.

3.7 Clinical examinations and management of subjects

The project clinicians, assisted by qualified district healthcare personnel and local worksite medical personnel screened and enrolled subjects who met the inclusion criteria and recorded results on a specific form (Appendix II). The subjects were examined at baseline and at 12th week post intervention and their bio-data and clinical signs and symptoms data obtained using questionnaires based on the Center for Disease Control Criteria (CDC, 1993) criteria of HIV and AIDS staging. The study subjects' vital signs which include temperature, pulse rate and respiratory rate were determined by the project clinician at baseline and at the 12th week.

Use of the mega-dose supplements in Arm 2 and a marched placebo in Arm 1 was discontinued at the 8th week to ensure safety of the subjects, having realized that the serum zinc levels had raised substantially by the 4th week. However, VIUSID^{TM} supplements were provided to both arms for the entire study period of 12 weeks since it was compliant with the RDA. Blood was obtained at four time points to enable toxicity checks to be undertaken monthly over the 12 weeks follow-up period.

Study subjects were clinically examined at baseline and at the 12th week and details of HIV-associated opportunistic infections recorded. Diagnosis was done according to the CDC criteria for staging HIV and AIDS patients at baseline and post intervention (CDC, 1993). Treatment of any conditions requiring management was undertaken accordingly at the cost of the project or where specialized treatment was needed, study subjects were referred to medical facilities commensurate with their financial capabilities.

3.8 Reagents, Chemicals and Kits

Specifications of various reagents, chemicals and kits used in the study are provided. AAS standards of zinc and copper (Analar grade, Sigma, Steinheim, Germany); HPLC grade alpha-tocopherol acetate, alpha-tocopherol standard, *all trans*-retinol, retinal acetate standard, methanol, ethanol, HPLC grade hexane, acetonitrile and sodium chloride (Analar grade, Sigma, Steinheim, Germany); viral load Roche Amplicor kits (Cobas Amplicor V1.5, Roche, Germany); immunology Beckton Dickson CD4, CD8 kits (BD, London, UK); haematology cell clean and eluent (Sysmex, Born, Germany) and clinical diagnostic products for liver function tests (Pointe Scientific Inc, Michigan, USA).

3.9 Instruments

3.9.1 Analytical instruments

The instrumental settings for the AAS (Spectra AA-10, Varian, Oxford, UK) were:-

wavelength of 213.9 and 324.8 nm for zinc and copper respectively; slit width of 1.0 nm; Flame of Air/Acetylene.

The instrumental settings for HPLC (Hitachi Ltd, L-600, Tokyo, Japan) were:- Column (Bondapak TMC 18; C-18), guard column, mobile phase of methanol: distilled water (95:5 v/v), Flow rate of 2 ml/min, Detector (Perkin Elmer UV variable detector) with molecular extinction of E1cm % 1560 at 325 and 292 nm for retinol and α-tocopherol respectively, Integrator (D 520 GPC, Hitachi), Pump (L6000 Hitachi), Rheodyne valve with 20 µl loop, Chart recorder speed of 10 cm/min.

3.9.2 Instruments for medical tests

Automated Haematology Analyzer (Sysmex KX-2IN, Kobe, Japan); IncAxmax Intelligent Clinical Chemistry Analyzer (Diconex, Buenos Aires, Argentina); FacsCount Machine (BD, London, UK); Cobas Amplicor V1.5 (Roche, Born, Germany).

3.10 Sample analysis

3.10.1 Sampling and sample pre-treatment

Blood (20 ml) was obtained from the subjects using a 20 ml syringe and a 21 gauge needle. For serum zinc, retinol, alpha-tocopherol and copper determination, blood was obtained at baseline (week 0), 4^{th}, 8^{th} and 12^{th} weeks while for CD4, CD8 and NK cell count blood was obtained at baseline and 12^{th} week. The blood sample was apportioned into well labeled plain 4 ml (one tube) vacutainer wrapped in aluminum foil and 4 ml vacutainers (4 tubes) containing ethylenediaminetetraacetate (EDTA). For haemogram, blood was obtained at baseline, 8^{th} and 12^{th} week.

The blood samples in the plain vacutainers were allowed to settle, spanned in a centrifuge (3000 rpm) for 2 minutes to obtain serum that was pipetted into a 3 ml cryovial wrapped in aluminum foil and the vial stored at -20 °C in the field prior to transportation to KEMRI Nairobi laboratories in cool boxes for storage at -80 °C until analysis for retinol, zinc, copper and alpha-tocopherol. One of the vacutainers containing EDTA was used to determine full haemogram and ESR on whole blood at KEMRI Kisumu laboratories.

The other blood samples in two EDTA containing vacutainers were transported to KEMRI laboratories in Nairobi within 12 hours. One of these vacutainers was used to for cytometry to determine CD4, CD8, NK cell count on whole blood. The remaining EDTA containing vacutainer was centrifuged (3000 rpm) for 2 minutes to obtain plasma. Plasma was pippetted into a 3 ml cryovial and stored at -80C° until viral load determination.

3.10.2 HIV status and HIV-1 viral load tests

Both rapid and ELISA tests were used for confirmation of the HIV status of the subjects and the viral load determined using Roche kits (Amplicor version 1.5).

3.10.3 CD4/8 count and CD4/8 ratios

These were analyzed using a Cytometer (Facscalibur, Becton & Dickson International, UK).

3.10.4 Full haemogram

White blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), haematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and erythrocyte sedimentation rate (ESR) were determined using a Coulter Counter.

3.10.5 Serum zinc, copper, α-tocopherol and retinol

Serum retinol and α-tocopherol was determined using a High Performance Liquid Chromatograph (HPLC). The serum zinc and copper levels were determined using Flame Atomic Absorption Spectrometer (FAAS).

3.10.5.1 Extraction for serum retinol and alpha-tocopherol analysis

This was performed at room temperature in dim light where 250 µl of homogenized serum was pipetted into aluminum covered centrifuge tube (with a Teflon-sealed screw cap). An internal standard (250 µl of 0.5 µg/ml retinal acetate) was added and vortexed at intermittent intervals of 15 seconds for 1 minute before addition of 1.5 ml of HPLC grade hexane and further vortexing. Centrifuging was done at 3000 rpm for 2 minutes and the upper layer removed with a pasture pipette and placed into second aluminum covered tube. The lower phase was re-extracted and the two extracts pooled together. The extracts were evaporated under a gentle stream of pure nitrogen gas in a water bath at 37^0C and reconstituted (the residue) into 250 µl of the mobile phase before injection of 30 µl into the HPLC reverse phase column. Analysis was done in triplicates and averages obtained.

3.10.5.2 Preparations of serum for zinc and copper analysis

Exactly 0.5 ml of serum was pipetted into a well labeled plastic tube containing 4.5 ml of de-ionized water, vortexed for 30 seconds and let to settle for two minutes before being aspirated into the AAS for zinc or copper determination. Analysis was performed in triplicates and averages obtained.

3.11 Standard curve and quantification of retinol and α-tocopherol

Preparation of all standards was performed in a darkened room. For calibration of retinol, a few grains of retinol, *all trans*-retinol were dissolved in a small amount of absolute ethanol to make a stock solution of 1 liter of 2µg/µl retinol. A calibration curve was prepared by pippeting 0, 25,

50, 100 and 150 µl of the retinol standard (2µg/µl) onto 250 µl vitamin A deficient serum sample and 62.5 µl of 2 µg/ µl retinal acetate as an internal standard in a 500 µl volumetric flask. This was made to mark with methanol of varying volumes as presented in table 2 giving a serial retinol concentration of 0, 0.1, 0.2, 0.6 µg/µl.

The same procedure was repeated for calibration of α-tocopherol where the retinol standard and retinal acetate internal standard were replaced by α-tocopherol and α-tocopherol acetate internal standards respectively.

Table 2: Parameters for preparation of standard curve for serum retinol

Retinol concentration (µg/ µl)	0	0.1	0.2	0.4	0.6
Vitamin A deficient serum (µl)	250	250	250	250	250
Retinol (2µg/ µl) standard (µl)	0	25	50	100	150
Retinal acetate(2 µg/ µl) std (µl)	62.5	62.5	62.5	62.5	62.5
Methanol (µl)	187.5	162.5	137.5	87.5	37.5

Retinol concentrations in unknown samples were determined from a standard curve of the peak area ratios of retinol internal standard (retinyl acetate) versus the concentration added to the plasma pool used to prepare the standard curve. The peak area ratios for retinol to its internal standard (retinyl acetate) were plotted against the final retinol concentration for the spike pool standards. Regression analysis of the data was performed for the retinol and the Y (vertical) axis intercept value subtracted from each peak area value for retinol to correct for the contribution of endogenous retinol in the pool. Regression analysis of the corrected peak area ratio versus the concentrations of the spiked retinol yielded a regression formula that was used by the coputer to calculate concentrations in the samples.

For α-tocopherol, concentrations in unknown samples are determined from a standard curve prepared by the standard addition (α-tocopheryl acetate) versus the concentration added to the serum pool used to prepare the standard curve. The concentrations of the unknown samples are determined through extrapolation from the standard addition curve which had known concentrations and values for the α-tocopherol.

3.12 Standard curve and quantification of zinc and copper

1 liter of sodium stock solution was prepared by dissolving 8.2 g sodium chloride (zinc free) in de-ionized water. Into each of the five 100 ml volumetric flasks 2.5, 5, 7.5, 10 and 15 ml of a 100 µg/ml of zinc stock solution was added followed by 10 ml of the sodium stock solution. This was made to the mark with de-ionized water to make standards of 0.25, 5.0, 7.5, 10.0 and 15.0 µg/100 ml which were used in obtaining a standard calibration curve for serum zinc quantification, taking note of the dilution factors. The same procedure was repeated using the same concentration of a copper stock solution to make the same serial dilution of copper standards.

3.13 Validation of Chemical Method

This work was undertaken at the KEMRI nutrition laboratory which participates in a series of inter-laboratory calibration exercises to validate its analytical results on serum retinol, alpha-tocopherol, zinc and copper. As for serum retinol and alpha-tocopherol, internal standards were also used for preparation of calibration curves. In addition, the analytical parameters and calibration curves were optimized to obtain analyte values of reference samples within the

expected ranges. This was done before samples were run while every after reading 10 samples, instruments were re-calibrated.

3.14 Data management

Data entry and analysis was conducted using the SPSS/PC+ Vers. 11.5 program. Continuous data was analyzed by parametric tests using t-tests and ANOVA. Unpaired tests on data that was not normally distributed were done using non-parametric tests. For continuous data measured several times, two-way repeated measures ANOVA were determined. Pearson's χ^2 and McNemar's χ^2 were used in the analysis and Mann-Whitney U-tests also undertake on categorical data. Paired T-tests on continuous data that is not normally distributed ($n < 30$) was undertaken using non-parametric tests of the median using Wilcoxon Signed Ranks Test. To better elucidate predictors of supplementation outcomes, chemo-informatic techniques of data mining including Spearman's Rho tests of correlations between biochemical and clinical variables, at baseline, during and after supplementation were undertaken. This way, computer aided innovative interpretation of the link between chemical data and immune parameters was made possible both at baseline, during and after intervention; significance being tested at the $p < 0.05$ level.

3.15 Ethical considerations

The study was subjected to scientific and ethical review by KEMRI and a letter granting approval obtained (Appendix III). Upon consenting in writing to participate, subjects were assured of confidentiality and informed that no adverse effects had been demonstrated with use of VIUSID^{TM} or the additional micro-nutrients that would be provided in the study. The subjects

benefited from free management of opportunistic infections and at the end of the study, received dietary counseling to maximize their intake of foods rich in anti-oxidants and other essential nutrients in a bid to ensure sustainability in the improvement of their nutritional and immunological status. Subjects were informed that their participation in the study had no collateral effects and that VIUSID^{TM} or the other micro-nutrients used had no demonstrated incompatibility with other types of treatments.

CHAPTER 4

4.0 RESULTS AND DISCUSSIONS

4.1 Introduction

The results of this study are presented and discussed in five sections namely: i) characteristics of the subjects; ii) baseline and after intervention clinical data from evaluation by a clinician, iii) nutritional parameters of subjects at baseline, during and after intervention; iv) immune profiles at baseline and after intervention; and v) outcomes of liver function tests that were used to assess safety of the interventions.

Whereas the study was intended for 90 persons diagnosed with HIV at VCT centres, eleven subjects were later confirmed HIV-seronegative on further tests. This raises concerns on the accuracy of some test results from VCT centres, given the implication of the results to the tested subjects. Since all the 90 subjects recruited and randomized into the two study Arms, 7 of the HIV-seronegative subjects being in Arm 1 while the other 4 were in Arm 2, a brief account is presented comparing management outcomes of HIV-seropositive and seropositive subjects. Though un-intended, the HIV-seronegative subjects provided additional insights to the study, beyond its original objectives.

4.2 Characteristics of subjects

At baseline, 90 subjects (distributed across the age bracket of 18-60 years with a mean age of 39.0$\pm$8.9 years) were sequentially recruited from societies of people living with HIV and AIDS in western Kenya. The socio-demographic and other characteristics of the subjects at baseline are provided in table 3.

Table 3: Baseline socio-demographic and other characteristics of study subjects

Parameter	Frequency (%)
Sex	
Female	63 (70.0)
Male	27 (30.0)
Marital status	
Single	5 (5.9)
Married	35 (41.2)
Widow	40 (47.1)
Widower	3 (3.5)
Divorced	2 (2.4)
Dietary habits	
Eats 3 meals/day	51 (56.7)
Eats< 3 meals/day	39 (43.3)
Occupation	
Company workers and small scale farmers	25 (27.8)
Informal sector (Jua Kali)	57 (63.3)
Teachers	8 (8.9)
HIV Seropositive	**79 (87.8)**

Women were a majority (70%) of the study population probably because they are more willing to get help for issues affecting their health. Most subjects were either married (41.2%) or widowed (47.1%). From the dietary practices reported, 56.7% of the subjects ate 3 meals a day, this possibly being an underlying problem for their compromised immunity. Majority had attained primary education, worked in the informal sector, had large families and earned less than a dollar a day.

4.3 Clinical signs and symptoms

Eleven subjects were confirmed HIV-seronegative, seven (7) of whom were randomized into Arm 1 (VIUSID^{TM} only) and 4 into Arm 2 (VIUSID^{TM} with mega-dose). These HIV-

seronegative subjects were retained and completed the study alongside the HIV-seropositive subjects. By the 12th week, 9 subjects had dropped out citing various reasons that included stigma, 7 had died largely of respiratory complications (6 in Arm 1 and 1 in Arm 2), leaving 74 subjects at the end of the study. In total, the HIV-seropositive subjects who completed the study were 33 in Arm 1 and 30 in Arm 2. Eighty eight percent (88%) of the subjects reported that the interventions were beneficial to them in improving their energy levels, capacity to work and appetite. Analysis of the pooled clinical data of the HIV-seronegative and HIV-seropositive subjects who completed participation by the 12th week of study is presented in table 4.

Table 4: Signs and symptoms of HIV and AIDS at baseline and after intervention

Sign/symptom	Arm 1 (n = 40)			Arm 2 (n = 34)		
	0-weeks number (%)	12-weeks number(%)	P-Value	0-weeks number(%)	12-weeks number(%)	p-Value
Headache	20(54.1)	8(32.0)	0.146	26(61.9)	12(34.3)	0.049
Skin rash	18(48.6)	3(12.0)	0.012	19(45.2)	7(20.0)	0.012
Diarrhoea	9(24.3)	2(8.0)	0.688	11(26.2)	6(17.1)	0.508
Cough	16(43.2)	11(44.0)	0.688	14(33.3)	9(25.1)	1.000
Fever	15(40.5)	4(16.0)	0.267	16(38.1)	5(14.3)	0.267
Oral thrush	10(27.0)	5(20.0)	1.00	12(28.6)	8(22.9)	1.000
Loss of appetite	15(40.5)	8(32.0)	0.754	17(38.1)	8(22.9)	0.549
Fatigue	18(48.6)	8(32.0)	1.000	24(57.1)	11(31.4)	0.092
Pneumonia	5(13.5)	1(4.0)	0.375	14(33.3)	1(2.9)	0.004
Boils	7(18.9)	3(12.0)	1.000	9(21.4)	1(2.9)	0.039
Itchy genitals	13(35.1)	4(16.0)	0.109	14(33.3)	4(11.4)	0.180
Pallor	2(5.7)	5(20.8)	0.125	14(10.5)	9(25.7)	0.016
Loss of weight	20(54.1)	6(24.0)	0.344	23(54.8)	5(14.3)	0.002
Treated for malaria	4(12.1)	7(28.0)	0.219	15(40.5)	12(34.3)	0.454

There was a significant reduction in prevalence of signs and symptoms associated with HIV and AIDS in subjects on the mega-dose supplements (Arm 2), including headache ($p = 0.049$), skin rash ($p = 0.012$), boils ($p = 0.039$), pneumonia ($p = 0.004$) and loss of weight ($p = 0.002$). This suggests that the mega-dose supplementation was more effective in management of HIV and AIDS. The significant results obtained in reduction of the prevalence of pneumonia are notable, especially because a study with lower zinc doses in children with severe pneumonia in Nepal did not achieve any difference comparable to placebo (Valemtiner-Branth *et al*., 2010). This may suggest that mega-doses of zinc, as administered in this study, are more appropriate and should be explored further considering that pneumonia is a leading cause of illness and death not only in HIV and AIDS patients but also among children in developing countries.

4.4 Trends in serum micro-nutrient levels in all subjects

Trends in medians and the 25^{th}-75^{th} interquartile ranges (IQR) of nutritional parameters of the subjects in both Arms are presented in table 5.

Table 5: Nutritional parameters of all subjects by Arms of study

Parameter	Time (Weeks)	Arm 1 Median (IQR), (n = 40)	Arm 2 Median (IQR), (n = 34)	p-Value
BMI	0	19.63 (18.04 – 21.95)	20.31 (18.50 – 22.66)	0.400
BMI	2	19.72 (18.75 – 21.56)	19.96 (18.18 – 22.54)	0.843
BMI	6	19.80 (18.64 – 22.34)	20.70 (18.95 - 22.66)	0.345
BMI	12	20.03 (18.29 – 23.34)	19.96 (18.18 – 22.54)	0.843
Zn	0	119.00 (86.00 – 183.25)	128.0 (69.0 – 171.5)	0.625
Zn	4	210.00 (135.00 – 262.00)	220.0 (160.0 – 28.0)	0.564
Zn	8	118.00 (102.25 – 130.50)	115.0 (96.8 – 128.0)	0.282
Zn	12	119.00 (93.50 – 159.00)	106.0 (90.0 – 129.3)	0.202
Retinol	0	0.95 (0.72 – 1.24)	1.03 90.76 – 1.24)	0.716
Retinol	4	1.00 (0.81 – 1.35)	0.99(0.81 – 1.12)	0.870
Retinol	8	0.88 90.58 – 1.16)	0.79 (0.60 – 1.16)	0.933
Retinol	12	0.99 (0.61 – 1.14)	0.87 (0.70 – 1.07)	0.703
Vit E	0	154.24 (85.52 – 242.75)	97.19 (40.91 – 177.00)	0.570
Vit E	4	109.16 (72.78 – 144.81)	48.80 (20.10 – 68.96)	0.731
Vit E	8	130.25 (49.07 – 170.35)	133.85 (77.84 – 251.86)	0.012
Vit E	12	58.88 (27.16 – 122.34)	60.68 (33.49 – 175.91)	0.391
Cu	0	260.00 (182.25 - 327.50)	220.00(190.00 – 250.00)	0.431
Cu	4	206.50 (165.00 – 258.75)	210.00 (205.00- 240.00)	0.565
Cu	8	208.00 (160.00 – 252.50)	170.00 (160.00 – 190.00)	0.099
Cu	12	240.00 (171.25 – 270.00)	210.00 (160.00 – 235.00)	0.390
Hb	0	11.85 (10.08 – 13.40)	11.50 (10.20 – 12.90)	0.716
Hb	8	9.00 (7.80 – 10.10)	9.20 (7.45 – 10.30)	0.685
Hb	12	10.75(9.38 – 12.20)	10.20(9.20 – 11.90)	0.254

BMI (Kg/M^2); retinol (µmol/l); α-Tocopherol (µmol/l); zinc (µg/dl); copper (µg/dl); Hb (g/dl)

Nutritionally, the interventions did not result in significant changes between the Arms at respective time points. However, there was some slight increase in BMI between baseline and the 12^{th} week in Arm 1. Studies have shown that weight loss and wasting are features of HIV disease progression and are predictors of morbidity and mortality (Palenicek *et al.*, 1995). However, basic nutritional and metabolic disturbances that lead to weight loss and wasting in

HIV-infected persons may represent an adaptive response to an inflammatory state (Godfried *et al.*, 1993), hence the un-predictable response to supplementation in this clinical marker.

In both Arms, there was an initial increase in serum zinc levels, attaining a maximum at the 4th week. However, at the 8th week when mega-dose supplementation was stopped, serum zinc levels that had attained a downward trend returned to baseline levels in both Arms. As serum zinc levels appeared to be controlled by other physiological processes that did not dependent on the dose of micro-nutrients administered, this may explain why common measurements of zinc levels in the blood don't necessarily make it possible to predict low body levels (Bogden *et al.*, 1990). In a double blind controlled clinical trial to investigate the effect of zinc supplementation in HIV-infected women in Tanzania, no viral load reduction or prevention of mother to child transmission was observed (Villamor *et al.*, 2006*).* Since high and low serum zinc levels are associated with cellular and humoral immunity, respectively, supplementation with mega-doses of zinc in this study initially favored reconstitution of cellular and thereafter humoral immunity and has implications on how diseases can be managed (Sprietsma, 1999; Jason *et al.*, 2002). Furthermore, significant repletion of serum zinc levels that occurred initially in both Arms was a key factor predicting stimulation of cell-mediated immunity (Sprietsma, 1999).

There was an initial decline and subsequent increase in serum retinol levels by the 8th week in both Arms despite only Arm 2 subjects receiving mega-doses of vitamin A. The observation supports earlier reports that low and high serum retinol levels are associated with cellular and humoral immunity, respectively suggesting that the interventions initially stimulated reconstitution of cellular immunity that was later followed with an enhancement of humoral

immunity (Jason *et al.*, 2002). The trends in serum zinc levels appear to support this observation (Sprietsma, 1999). A possible interaction between zinc and vitamin A status has been explored (Raham *et al*., 2002). In a study on 800 children (12–35 months) in Bangladesh, a two-week supplement of zinc, vitamin A, both, or placebo was given to children who were then followed up for six months. Combined zinc and vitamin A synergistically reduced the prevalence of persistent diarrhoea and dysentery. However, zinc alone was associated with a significant increase in acute lower respiratory infection, but this adverse effect was reduced by interaction between zinc and vitamin A, suggesting the importance of both immune responses in the fight against diseases (Sprietsma, 1999; Jason *et al.*, 2002).

There was an initial significant decline of serum α-tocopherol levels by the 4^{th} week, followed by a rise by the 8^{th} week and a decline by the 12^{th} week. Apparently, stimulation of cellular immunity as suggested by the increase in serum zinc concentrations (Sprietsma, 199) by the 4^{th} week was associated with reduced levels of vitamin E in both Arms despite only Arm 2 receiving mega- doses of vitamin E. Another study has shown that high pre-infection plasma vitamin E levels are, for poorly understood reasons, associated with high mortality in HIV-1-infected Kenyan women (Graham *et al.*, 2007). Furthermore, oxidative metabolism that is characteristic of HIV and AIDS inevitably leads to generation of reactive oxygen species (ROS) or "free radicals", which have the potential to cause further oxidative reactions, especially to those parts of the cell in a relatively reduced state, such as cell membranes or nucleic acids (Evans and Halliwell, 2001).

Serum copper levels initially declined to a minimum at the 8th week, returning to near baseline levels by the 12th week in both Arms. Also, serum copper levels by the 8th week were lower in Arm 2. Since copper was not one of the nutrients in the micro-nutrient formulation, the changes seen in serum levels may be attributed to physiological reconstitution as other micro-nutrients with which it interacts were provided (Igic *et al*., 2002).

Hemoglobin levels in both Arms declined to a minimum by the 8th week and rose to baseline values after intervention. Anemia is more common with advanced HIV disease progression and studies disagree on whether this is principally due to iron-deficiency or chronic disease (Belperio and Rhew, 2004; van de Broek and Letsky, 2000; Totin *et al*., 2002; Clark and Semba, 2001; Semba and Gray, 2001). However, as haemoglobin levels declined and rose to baseline values, serum zinc levels rose from baseline levels to a maximum at the 8th week and returned to baseline levels by the 12th week, suggesting that the fall in hemoglobin levels was not due to zinc replacing iron in the subjects but due to iron relocating from circulation to reserves. Studies have shown that zinc and iron compete during intestinal absorption but the post absorptive interactions between these ions are not well understood (Donangelo *et al.*, 2002).

Infections such as HIV and AIDS may reduce intake and absorption, as well as increase utilization and excretion of micro-nutrients, thus impairing host micro-nutrient status (Friis and Michaelsen, 1998). Acute and even chronic generalized infections give rise to an acute-phase response, which is a generalized, stereotypic host reaction caused by a cascade of cytokines released by activated phagocytic cells (Dinarello, 1984). While the acute-phase response serves to enable the host to withstand invading pathogens and repair tissue damage, it also compromises

host nutritional status (Beisel, 1992). Anorexia and fever are prominent constitutional signs of acute-phase response, leading to reduced intake of food and, hence, micro-nutrients. Fever is accompanied by catabolism of muscle tissues with mobilization of amino acids required for proliferation of neutrophils, lymphocytes, and fibroblasts and for synthesis of immunoglobulin and hepatic acute phase proteins. Consequently, serum zinc and iron concentrations fall drastically due to redistribution within the body and accumulation in the liver (Friis and Michaelsen, 1998). This serves to make the iron and zinc available to organs and cells with increased requirements, but is also believed to be purposeful in making these minerals unavailable to invading micro-organisms (Keusch and Farthing, 1986). Therefore, the trends in serum iron levels observed may be beneficial to the host defense mechanisms since high serum iron levels favor micro-organisms such as viral and parasitic infections (Beisel, 1992). The micro-nutrient supplements may have assisted the body to physiologically boost its anti-oxidant defenses against HIV and related opportunistic infections.

4.4.1 Trends in nutritional status by HIV-sero-status

Comparison of the subjects' nutritional data by HIV status is presented in table 6 and illustrated in figures 2-5.

Table 6: Nutritional status ofn subjects by sero-status

Parameter	Time (Weeks)	(HIV+ves) Median (IQR), (n = 63)	(HIV-ves) Median (IQR), (n =11)	p-Value
Zn	0	117.5 (78.0 – 173.0)	165.0 (119.0 – 183.0)	0.051
Zn	4	229.0 (141.0 – 278.0)	207.0 (63.5 – 26.5)	0.315
Zn	8	113.0 (100.0 – 127.5)	117.0 (111.0 – 137.0)	0.085
Zn	12	103.5 (89.0 – 130.0)	191.0 (127.0 – 227.0)	0.0001
Retinol	0	0.98 (0.73 – 1.22)	1.10 (0.85 – 1.54)	0.276
Retinol	4	0.97 (0.81 – 1.09)	1.38 (1.14 – 1.73)	0.008
Retinol	8	0.77 (0.59 – 1.07)	1.16 (0.64 - 1.55)	0.046
Retinol	12	0.92 (0.71 – 1.13)	1.06 (0.54 – 1.49)	0.700
Vit E	0	99.46 (78.64 – 176.95)	135.9(40.9 – 231.2)	0.391
Vit E	4	64.22 (50.28 – 79.38)	69.0 (20.1 – 137.1)	0.705
Vit E	8	108.65(74.73 – 87.34)	141.6(0.0 – 185.9)	0.886
Vit E	12	160.78(28.74 – 196.62)	58.9 (33.5 – 83.9)	0.291
Cu	0	230 (146.25 – 257.50)	240.0 (210.0 – 350.0)	0.283
Cu	4	211.50 (168.75 – 243.75)	210.0 (200.0 – 270.0)	0.943
Cu	8	185.00 (137.5 – 212.5)	190.0 (170.0 – 220.0)	0.720
Cu	12	172.50 (115.75 – 242.50)	235.0 (210.0 – 330.0)	0.099
Hb	0	11.45 (10.15 – 12.95)	11.80 (11.30 – 13.70)	0.298
Hb	8	8.60 (7.50 – 9.95)	10.10 (9.70 – 11.40)	0.010
Hb	12	10.20 (9.20 – 11.75)	11.70 (10.70 – 12.80)	0.033

BMI (Kg/M^2); retinol (µmol/l), α-tocopherol (µmol/l); zinc (µg/dl); copper (µg/dl), Hb (g/dl)

HIV-seronegative subjects had higher serum zinc values at all the other time points except the 4^{th} week when HIV-seropositive subjects had higher levels. The trend in serum zinc for subjects based on their sero-status is illustrated in figure 2. The HIV-seronegative subjects had significantly higher serum zinc levels at baseline ($p = 0.051$) and at the 12^{th} week ($p = 0.0001$). In Iran, HIV-infected subjects were also found to have significantly lower serum zinc than healthy subjects ($p = 0.01$) (Khalili *et al.*, 2008). This maybe attributed to the differences in immunological challenges in the HIV-seronegative and sero-positive subjects. It would appear that while HIV sero-negative subjects have higher serum zinc levels and a more efficient cellular immunity (Sprietsma, 1999), the sero-positives have lower serum zinc to up-regulate humoral immunity that is crucial in viral load reduction (Mbakaya *et al*., 2011). These findings are

supported by observations that zinc deficiency results in a transition from cellular to humoral immunity (Sprietsma, 1999).

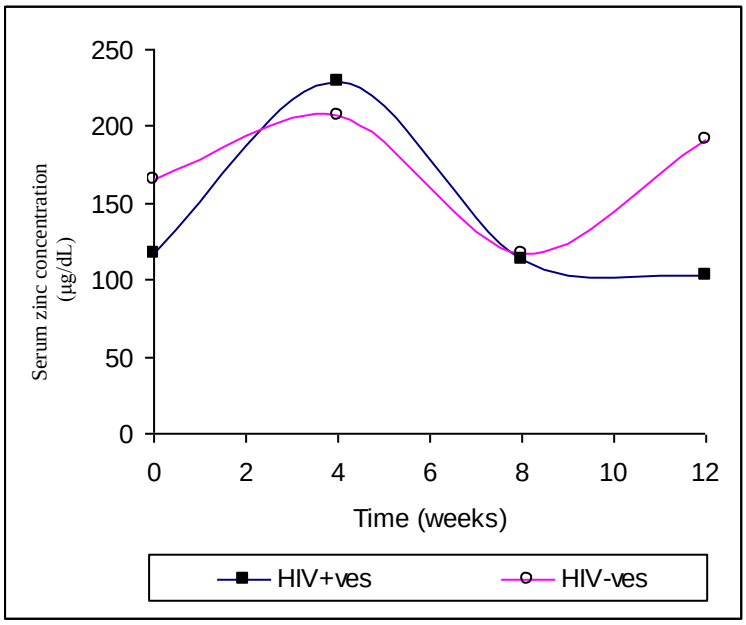

Figure 2: Trend in median serum zinc levels by HIV-serostatus

As a micro-nutrient, zinc has a unique property of forming mercaptides with thiols, inhibits cyclic reduction-autoxidation of thiols and superoxide generation (Reid, 2000). Furthermore, when the mixed function oxidase (MFO) system acts as an activator and not a detoxifier, zinc inhibits the oxidation. An example of increased toxicity with increased MFO activity is the toxin 3-methylindole (3-M), a toxic product of intestinal bacterial putrifaction which reactivates the infectious bovine rhinotracheitis virus (IBR). Therefore, while zinc reduces MFO activity and in this regard it functions synergistically with antioxidants in protecting cell membranes, it has been hypothesized that stable zinc complexes inhibit activity of proteases in the virus nucleocapside (NC) proteins in the virus coat because zinc also inactivates some toxins that are thiol depleters or virus reactivators.

The serum retinol levels observed in both HIV-seropositive and sero-negative subjects (table 6) were below the adult range of 1.6-2.3 µmol/L (Abbott-Johnson *et al.*, 2011) and have immunological implications in the subjects and the region. Serum retinol levels of HIV-seropositive subjects declined from baseline to the 8th week and rose by the 12th week while the levels in sero-negative subjects increased to the 4th week and declined to the 12th week. These trends in serum retinol levels are illustrated in figure 3.

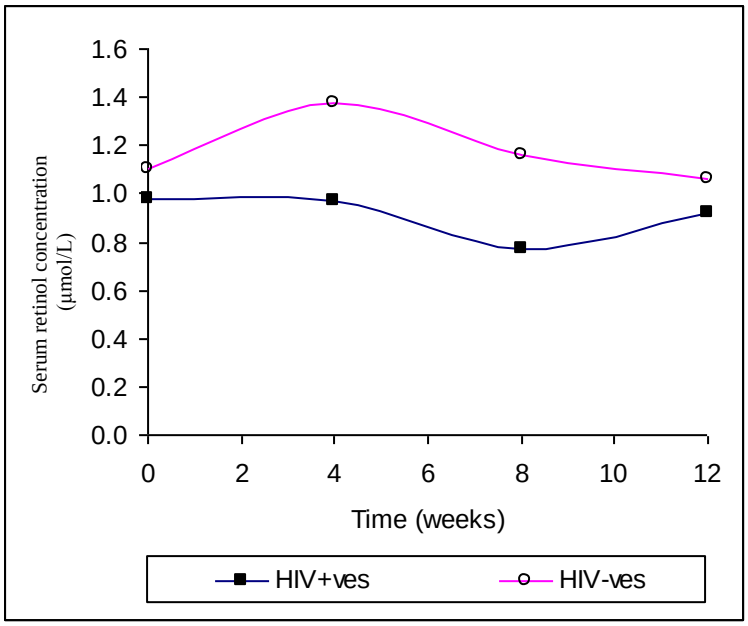

Figure 3: Trend in median serum retinol levels by HIV-serostatus

Although the serum retinol levels were statistically similar at baseline, HIV-seronegative subjects had statistically higher levels at the 4th and 8th weeks ($p = 0.008$, $p = 0.046$, respectively). As a study has associated low serum retinol level to cellular immunity (Jason *et al.*, 2002) and high serum zinc to celluar immunity (Sprietsma, 1999), the data suggests that the HIV-seronegative subjects had both their humoral and celluar immunity up-regulated and this

probably explains their immunity to HIV, considerring that spouses with whom they had unprotected sex had succumbed to HIV and AIDS.

The median α-tocopherol levels declined to a minimum at the 4th week in both HIV-seropositive and seronegative subjects, rose to the 8th and 12th week in the seropositives but declined by the 12th week in the seronegative subjects as presented in table 6 and illustrated in figure 4.

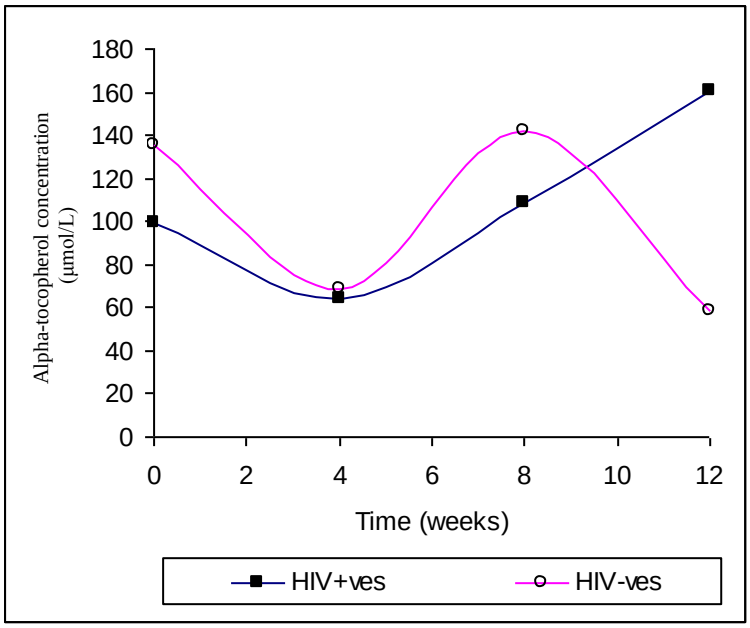

Figure 4: Trend in median α-tocopherol levels by HIV-serostatus

The median serum α-tocopherol levels declined at the 4th week when median serum zinc levels were at the highest (figure 1), then rose again to the 8th week when serum zinc levels were low. When serum zinc was highest by the 12th week in HIV-seronegative subjects, α-tocopherol levels were lower than in the HIV-seropositive subjects. This might suggest high serum α-tocopherol levels were needed to enhance humoral immunity (Jason, 2002). These trends in nutrient levels

are supported by the unpredictable nature of nutritional intervention outcomes (Filteau, 2010; Prentice, 2010).

The α-tocopherol levels in this study are higher than reported in the US in HIV-seronegative and seropositive subjects whose median values were 18.4 (15.2-22.0 and 18.0 (15.2-21.8) µg/L, respectively (Stephensen *et al*., 2006). As low serum alpha-tocopherol levels corresponded with high serum zinc levels in this study, the low levels of alpha-tocopherol in subjects in the US may suggest their serum zinc levels were higher as has been reported (Surendra and Elmer, 1970). The immunological import of these observations are that low alpha-tocopherol levels may suggest enhanced free radical quenching in cells and tissues (Bilbis *et al*., 2010). Another study in Kenya has observed that high pre-infection levels of vitamin E (alpha-tocopherol) were associated with increased mortality and recommended that more research be undertaken to elucidate the role of vitamin E in HIV-1 pathogenesis (Graham *et al.*, 2007). While low serum alpha-tocopherol is associated with high serum zinc levels, supplementation with zinc has been shown to prevent immunological failure (Baum *et al.*, 2010), probably due to increased quenching of free radicals in the cells (Bilbis *et al*., 2010). The increased fatality in subjects with high pre-infection vitamin E in Kenya (Graham *et al.*, 2007) may suggest they were low in zinc and prone to immunological failure (Baum *et al.*, 2010). The data further supports the hypothesis that zinc deficiency results in premature transition from cellular to humoral immunity, yet it is the former immune system that has elite anti-viral properties (Sprietsma, 1999; Bryce-Smith, 1989).

There was a general decline in serum copper levels in HIV sero-positive subjects to the 12th week (table 6). On the contary, the levels in HIV- seronegative ones declined to the 8th week and rose to the 12th week. This trend is illustrated in figure 5 and indicates that the differences in serum copper levels between the sero-positive and sero-negative subjects were insignificant at all the time points.

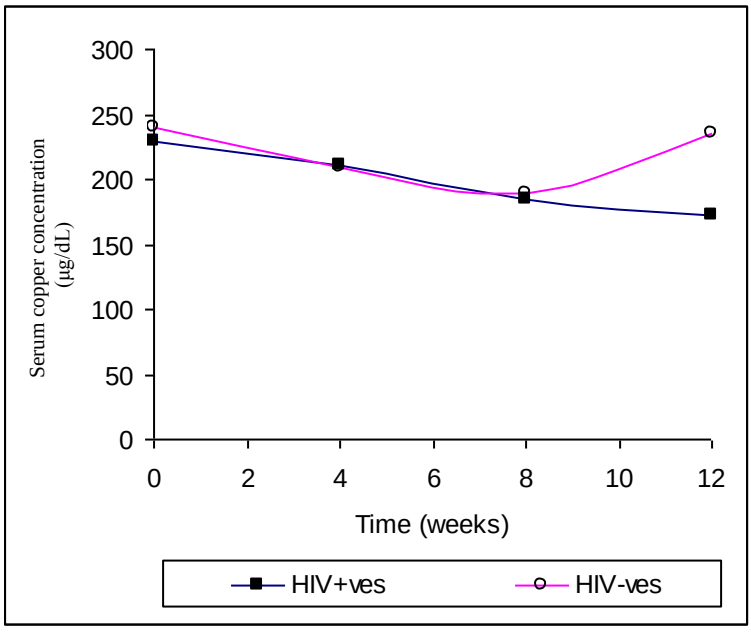

Figure 5: Trend in serum copper levels by HIV-serostatus

4.5 Nutritional status of HIV-seropositive by study Arms

Data on nutritional trends for HIV-seropositive subjects by Arm of study is given in table 7 and illustrated in figures 6-9.

Table 7: Nutritional status for HIV–seropositive subjects by Arms

Parameter	Time (Weeks)	Arm 2 Median (IQR), (n = 33)	Arm 1 Median (IQR), (n = 30)	p- value
BMI	0	20.38 (18.50 – 22.66)	19.96(18.38 – 22.22)	0.555
BMI	2	19.98 (18.18 – 22.54)	19.69 (18.75 – 22.06)	0.853
BMI	6	20.57 (18.84 – 22.56)	19.78 (18.66 – 22.75)	0.621
BMI	12	20.15 (18.80 – 23.03)	20.07 (19.33 – 23.72)	0.711
Zn	0	125.00 (63.50 – 167.50)	115.00 (82.00 – 184.00)	0.617
Zn	4	231.00 (160.00 – 296.00)	225.00 (132.00 – 264.50)	0.660
Zn	8	106.50(96.00 – 127.00)	119.00(101.50 – 128.00)	0.386
Zn	12	100.50 (89.25 – 124.75)	104.00(89.00 – 135.50)	0.679
Retinol	0	(0.74 – 1.21)	0.92 (0.72 – 1.29)	0.937
Retinol	4	0.91 (0.81 – 1.07)	1.00 (0.81 – 1.18)	0.696
Retinol	8	0.75 (0.59 – 0.96)	0.84 (0.59 – 1.19)	0.501
Retinol	12	0.87 (0.70 – 1.06)	1.10 (0.71 – 1.14)	0.483
Vit E	0	97.19 (92.55 – 185.05)	101.72 (36.92 – 174.25)	0.827
Vit E	2	54.71 (48.80 – 73.73)	81.26 (81.26 – 81.26)	0.180
Vit E	8	98.40 (77.84 – 253.83)	118.90 (65.43 – 165.18)	0.827
Vit E	12	175.91 (39.84 – 217.32)	89.21 (17.64 – 160.78)	0.248
Cu	0	220.00 (175.00 – 250.00)	240.00 (60.00 – 280.00)	0.827
Cu	4	210.00 (205.00 – 240.00)	213.00 (60.00 – 255.00)	0.827
Cu	8	180.00 (160.00 – 205.00)	190.00 (70.00 – 235.00)	0.827
Cu	12	160.00 (126.00 – 185.00)	240.00 (85.00 – 250.00)	0.513
Hb	0	11.60 (10.20 0 12.90)	11.20 (9.70 – 13.10)	0.460
Hb	8	8.70 (7.35 – 9.70)	8.45 (7.80 – 10.78)	0.510
Hb	12	10.00 (9.00 – 11.50)	10.40 (9.30 – 12.80)	0.116

BMI (Kg/M^2); retinol (µmol/l), α-tocopherol (µmol/l); zinc (µg/dl); copper (µg/dl), Hb (g/dl)

There were no significant differences in nutritional parameters between the Arms at all time points in the HIV-seropositive subjects. However, trends in median serum zinc levels showed significant increase in Arm 2 ($p = 0.0001$) and Arm 1 ($p = 0.001$) between baseline and 4^{th} week levels as illustrated (figure 6). This was followed by a decline in both Arms by the 8^{th} week,

even as supplementation was in progress. A further decline was noted in median serum zinc levels, reaching a minimum in both Arms by the 12th week.

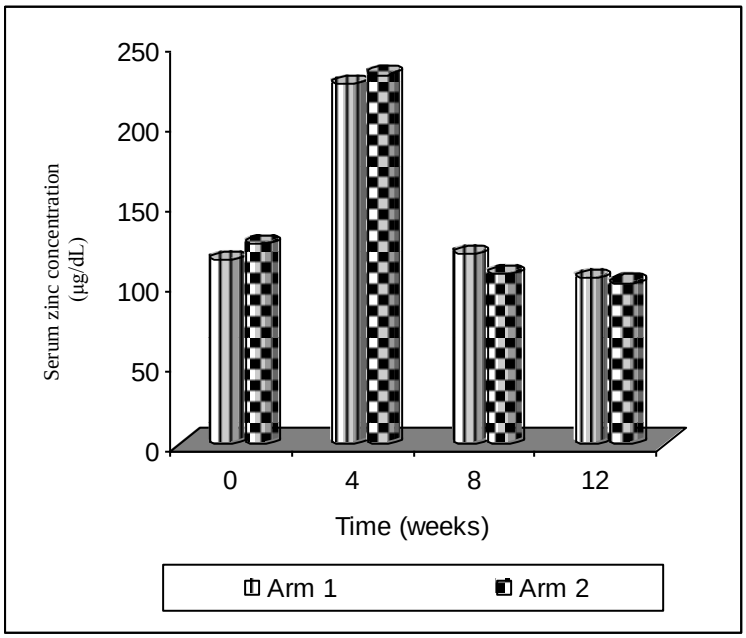

Figure 6: Trend in median serum zinc in HIV-seropositive subjects

Use of citric acid in both arms resulted in enhanced bio-availability of zinc from the supplements administered. Similarly, diet or body stores may have contributed to the rise in serum zinc concentrations in the presence of citric acid, although the observations are preliminary and require further investigations. Since zinc repletion is associated with Th-1 immunity, the initial rise in serum zinc levels suggested that the interventions initially stimulated cellular immunity in both Arms and later humoral immunity in the later stages of the intervention when serum zinc levels dropped in both arms even as mega-doses were administered (Mbakaya *et al.*, 2011). In Nigeria, a micro-nutrient study of HIV-seropositive and seronegative subjects has shown that CD4 cell count positively correlated with serum alpha-tocopherol, zinc, and copper levels (Bilbis *et al.*, 2010) and that serum zinc, iron, copper and CD4 cell count in HIV-seronegative subjects

were significantly lower compared with seropositive ones ($p < 0.05$). It has been shown that only marginal zinc increments occur when using an RDA compliant micro-nutrient supplement for three months ($p = 0.023$) in HIV-seropositive subjects because inflammation blocked increases in plasma zinc levels (Mburu *et al.*, 2010). Therefore, the high increase in serum zinc levels in this study suggests that the supplements administered were more effective in overriding the blockage that arises from inflammation.

Analysis of selected nutritional parameters revealed significant differences within the Arms between baseline and the 12^{th} week as shown as shown in table 8.

Table 8: Medians (IQR) of selected parameters at baseline and 12th week

Parameter by study arm	Baseline	12 weeks	p-value
Arm 2			
Serum retinol	1.01 (0.74-1.21)	0.87 (0.70-1.06)	0.062
Serum zinc	125.0 (63.5-167.5)	100.5 (89.3-124.8)	0.147
Hb	11.6 (10.2-12.9)	10.0 (9.0-11.5)	0.0001
Blood glucose	4.16 (2.98-5.12)	3.94 (3.28-5.29)	0.686
Arm 1			
Serum retinol	0.92 (0.72-1.29)	0.97 (0.71-1.14)	0.042
Serum zinc	115.0 (82.0-184.0)	104.0 (89.0-135.5)	0.081
Hb	11.2 (9.7-13.1)	10.4 (9.3-12.8)	0.046
Blood glucose	4.45 (4.00-5.64)	3.64 (2.91-4.52)	0.138

Arm 1, n =30; Arm 2, n =32; Hb (g/dl); blood glucose (mmol/l); serum retinol (µmol/l); serum zinc (µg/dl)

A decrease in Hb (p = 0.0001) was noted in Arm 2, probably because iron was being displaced from circulation by mega-dose zinc. An increase in serum retinol concentration (p = 0.042) and a decrease in Hb level (p = 0.046) were noted in the Arm 1.

Serum retinol trends in HIV-seropositive subjects by Arm of study are presented in figure 7.

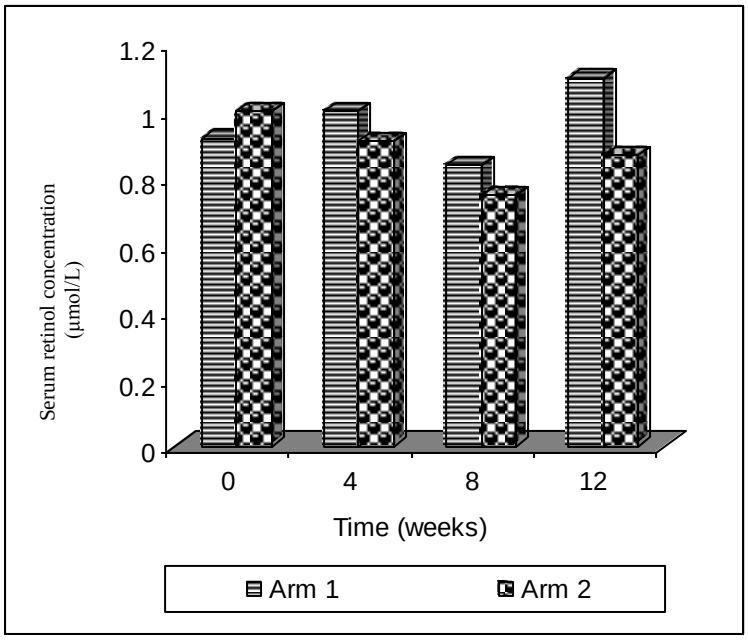

Figure 7: Trend in median serum retinol levels in HIV-seropositive subjects

Arm 2 subjects experienced a decline in serum retinol levels to the 8th week despite receiving mega-dose vitamin A at baseline and monthly thereafter. A slight increase was noted in serum retinol levels by the 12th week, when supplementation dosages had reverted to RDA values. On the contrary, the median serum retinol levels in Arm 1, whose subjects received lower micro-nutrient dosages, had an increase at the 4th week, decreasing at the 8th week and increasing again at the 12th week. Studies have shown that low serum retinol levels favor cellular immunity while high concentrations favor humoral immunity (Jason *et al.,* 2002). It appears that while cellular immunity was enhanced in Arm 2, humoral immunity was enhanced in Arm 1. Considering that humoral immunity was associated with significant decrease in viral load following supplementation of HIV and AIDS patients (Mbakaya *et al*., 2011).

In South Africa, children infected with HIV and had abnormally low vitamin A levels had significantly high viral loads and low CD4 cell count than those with normal vitamin A levels

(Steenkamp *et al*., 2009). It has been shown that micro-nutrient supplements are associated with a delay in HIV disease progression and reduce mortality in HIV-positive persons not receiving highly active antiretroviral therapy (Drain e*t al.,* 2007).

The trend in serum alpha-tocopherol levels in HIV-seropositive subjects by Arms of study is illustrated in figure 8.

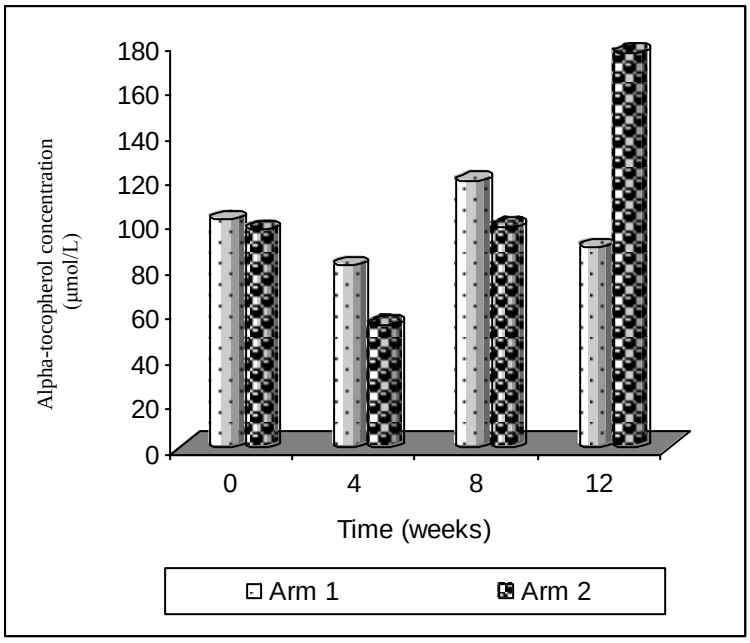

Figure 8: Trend in serum α-tocopherol levels in HIV-seropositive subjects

The trend in serum alpha-tocopherol was similar to that of serum retinol, probably because these vitamins work in concert in modulating humoral immunity. Serum alpha-tocopherol levels in Arm 2 subjects declined by the 4th followed by significant increases at the 8th and 12th weeks. On the contrary, the trend in Arm 1 showed an initial decline at the 4th week followed by an increase by the 8th, then a decline at the 12th week.

However, the differences in alpha-tocopherol were not significant between the Arms, notwithstanding that Arm 2 subjects were administered with mega-doses of this micro-nutrient. Since oxidative metabolism that is characteristic of HIV and AIDS inevitably leads to generation of reactive oxygen species (ROS) or "free radicals", which have the potential to cause further oxidative reactions (Evans and Halliwell, 2001), damage is limited by mechanisms that include direct quenching of oxidant activity by α- tocopherol (Shenkin, 1995). This may be one of the reasons serum alpha-tocopherol levels fluctuated during mega-dose intervention, but requires more in-depth investigations. However, low alpha-tocopherol levels have been noted in HIV-infected patients compared to healthy controls (Olaniyi and Arinola, 2007).

The trend in serum copper levels is shown in figure 9.

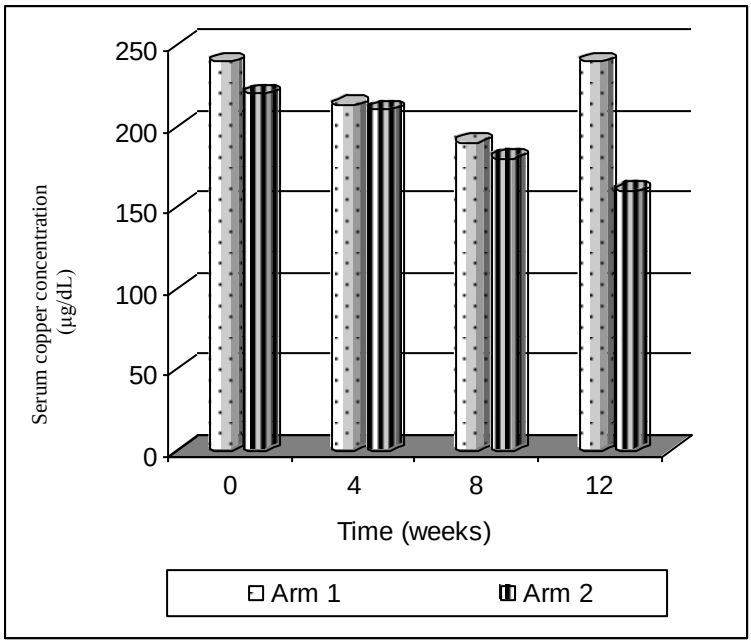

Figure 9: Trend in serum copper levels in HIV-seropositive subjects

For median serum copper levels, subjects in Arm 2 experienced a decline from baseline to the 12th week. In Arm 1, there was an initial decline up to the 8th week followed by an increase in median serum copper levels to baseline levels by the 12th week. However, the differences between the Arms were not statistically significant. The observations are consistent with earlier reports suggesting that zinc interferes with copper nutriture (Rowin and Lewis, 2005). A greater effect of this phenomenon was noted in Arm 2, where mega-doses of zinc were administered. However, when supplementation with mega-doses was stopped at the 8th week to ensure safety of study subjects, serum copper levels reverted to baseline values. While the copper levels in both intervention groups were different, though not significantly, this allayed the fear that the mega-dose zinc administered may have been un-safe in interfering with copper nutriture (Igic *et al*., 2002).

4.6 Immune parameters of the subjects by HIV-sero-status

The data on immune parameters of the subjects by their HIV-sero status is provided in table 9. While median total lymphocyte cell count of HIV-seropositive and seronegative subjects were not significantly different at baseline, HIV-seronegative subjects had significantly higher values after intervention than the seropositive ones ($p = 0.005$). The differences in the CD4 cell count were statistical different at baseline ($p = 0.0001$) and after intervention ($p = 0.0001$).

Table 9: Immune parameters of subjects by HIV-sero-status

Parameter	Time (Weeks)	HIV – seronegative Median (IQR) (n = 11)	HIV – seropositive Median (IQR) (n = 63)	*p-value*
Total lymphocytes	0	1826 (1404 – 2346)	1810 (1249 – 2305)	0.573
Total lymphocytes	12	2806 (2050 – 3132)	1920 (1400 – 2530)	0.005
CD4	0	737 (564 – 1042)	290 (154 – 465)	0.0001
CD4	12	1003 (973 – 1311)	335(193 – 557)	0.0001
CD8	0	368 (309 – 512)	880 (599 – 1318)	0.0001
CD 8	12	620 (434 – 690)	977 (650 – 1513)	0.008
NK	0	330 (184 – 491)	305 (176 – 466)	0.591
NK	12	402 (316 – 699)	247 (162 – 374)	0.003
CD4/CD8	0	1.97(1.67 – 2.35)	0.31(0.15 – 0.47)	0.0001
CD4/CD8	12	1.81 (1.46 – 2.18)	0.31 (0.17 – 0.53)	0.0001
CD3	0	1222 (929 – 1884)	1447 (805 – 1805)	0.948
CD3	12	1727 (1522 – 2333)	1404 (1004 – 2024)	0.100
ESR	0	33.0 (6.0 – 46.0)	47.0 (29.0 – 60.0)	0.010
ESR	12	21.0 (16.0 – 34.0)	46.0 (17.5 – 55.0)	0.081
Eosinophils	0	-	-	-
Eosinophils	12	2.0 (2.0 – 7.0)	3.0 (2.0 – 6.0)	0.552
WBC	0	4.2 (2.9 – 6.9)	4.3 (3.5 – 5.0)	0.498
WBC	12	5.2 (4.6 – 6.1)	4.6 (3.5 – 5.1)	0.032

WBC ($x10^9$/l); total lymphocytes($x10^9$ /l); CD3 cell count ($x10^9$ /l); CD4 cell count ($x10^9$ /l); CD8 cell; Count ($x10^9$/l); CD4/CD8 (ratio); NK cell count ($x10^9$ /l); ESR (mm/hr); neutrophils (%); eosinophils (%)

There was an increase in CD8 cell count in both groups following nutritional supplementation. However, the CD8 cell count for HIV-seropositive subjects were higher than those of HIV-seronegative subjects at baseline (p = 0.0001) and after intervention (p = 0.008). There were no significant differences between the NK cell count of the groups at baseline. However, whereas the NK cell count increased in HIV-seronegative subjects, there was a drastic decline in seropositive ones after intervention (p = 0.003), suggesting that whereas HIV-seronegative subjects enhanced cellular immunity after nutritional support, the seropositive ones up-regulated humoral immunity. While total lymphocyte cell count was not different between HIV-

seronegative and seropositive subjects at baseline, the difference attained significance with the former having higher values after nutritional intervention ($p = 0.005$).

Both at baseline and after intervention, CD4 cell count were significantly different between the groups ($p = 0.0001$). Significant increases in this immunity marker were observed in both groups between baseline and after intervention, a higher increase being evident in the HIV-seronegative subjects. Similarly, substantial increases were noted in CD8 count of the subjects in both groups. However, the CD8 cell count of HIV- seropositive subjects were higher at baseline ($p = 0.0001$) and after intervention ($p = 0.008$) than with the HIV-seronegative subjects.

With respect to NK cell count, HIV-seronegative subjects had a higher count than the seropositive ones at baseline, though there was no significant difference between the groups. Whereas the NK cell count increased among HIV-seronegative subjects, there was a significant decline ($p = 0.003$) among HIV-serpositives. In both groups, there was a decrease in neutrophil count between baseline and after intervention. Furthermore, there was an increase in WBC counts in both groups though it was much higher in HIV-seronegative subjects after intervention ($p = 0.032$).

4.6.1 Immune status of HIV-seropositive subjects by Arm

The immune parameters of HIV-seropositive subjects by Arms are presented in table 10.

Table 10: Immune profile of HIV-seropositive subjects by Arms

Parameter	Time (Weeks)	Arms 2 Median (IQR), (n = 42)	Arm 1 Median (IQR), (n = 37)	p-value
Tot lymphocytes	0	1848 (1200 – 2183)	1773 (1334 – 2310)	0.478
Tot lymphocytes	12	2083 (1600 – 2714)	1220 (1220 – 1716)	0.053
CD4	0	290 (159 – 483)	290 (147 – 456)	0.442
CD4	12	380(226 – 628)	271 (167 – 402)	0.056
CD8	0	838 (509 – 1325)	892 (669 – 1279)	0.439
CD8	12	1097(689 – 1659	780 (600 – 1256)	0.816
NK	0	289 (166 – 398)	321(176 – 498)	0.327
NK	12	253 (156 – 401)	245(170 – 338)	0.816
CD4/CD8	0	0.33(0.16 – 0.49)	0.30 (0.10 – 0.46)	0.404
CD4/CD8	12	0.34 (0.18 – 0.55)	0.29 (0.15 – 0.52)	0.551
CD3	0	1436 (752 – 1817)	1450 (972 – 1736)	0.721
CD3	12	1697 (1083 – 2160)	1220 (827 – 1716)	0.072
ESR	0	50.50 (26.75 – 61.00)	44.00 (33.50 – 57.50)	0.452
ESR	12	48.00 (18.00 – 55.00)	38.00(16.00 – 55.00)	0.579
Log_{10} viral load	0	5.11 (3.82 – 55.2)	4.79 (3.85 – 5.56)	0.9878
Log_{10} viral load	12	4.43 (3.48 – 5.13)	4.09 (3.78 – 4.84)	0.475
Neutrophils	0	55 (40 – 66)	51(44 – 64)	0.867
Neutrophils	12	40 (37 – 58)	52 (46 – 58)	0.145
Eosinophisl	0	-	-	-
Eosinophils	12	3.0 (2.0 – 6.0)	3.0 (2.0 – 6.0)	0.886
WBC	0	4.3 (3.5 – 4.9)	4.4 (3.4 – 5.1)	0.575
WBC	12	4.7 (3.4 – 5.2)	4.2 (3.5 – 4.8)	0.177

WBC ($x10^9$/l); total lymphocytes($x10^9$ /l); CD3 cell count ($x10^9$ /l); CD4 cell count ($x10^9$ /l); CD8 cell count ($x10^9$/l); CD4/CD8 (ratio); NK cell count ($x10^9$ /l); ESR (mm/hr); neutrophils (%); eosinophils (%)

The median total lymphocyte count was not significantly different between the Arms at baseline (p = 0.478). However, the count was much higher in Arm 2 subjects following intervention (p = 0.053). Whereas the CD4 cell count of the subjects were not different in both Arms at baseline (p = 0.442), the values were different after intervention (p = 0.056). However, other immune parameters were not significantly different in both Arms at baseline and after intervention. Nonetheless, substantial increase in CD8 cell count was noted in Arm 2 whereas the count reduced in Arm 1.

While the total lymphocyte cell count was not different between the groups at baseline (table 11), after intervention median cell count was much higher in the group receiving mega-dose supplements ($p = 0.053$). Although the median CD4 cell count in both groups was not different at baseline, it was much higher after intervention in the group on mega dose supplements ($p = 0.056$). This observation indicates that supplementation of HIV-seropositive subjects with mega dose multi-micro-nutrients maybe beneficial and has a favourable effect on reconstituting the nutritional status, cytokine production and immune status (Grimble, 1998).

4.6.2 Changes in key immune parameters by Arms

Medians of key immune parameters were compared for subjects in Arm 1 and 2 were compared within the Arms at baseline and after intervention and are as presented table 11. For subjects in Arm 2, significant changes in immunity were noted in increased CD4 cell count ($p = 0.007$) and increased CD8 cell count ($p = 0.007$) after intervention. For subjects in Arm 1, significant changes were noted in the reduction of viral load ($p = 0.030$). Consequently, Arm 2 demonstrated greater immunological benefits to the subjects than arm 1, indicating that use of mega-dose micro-nutrients had superior outcomes in immune reconstitution, the short intervention period of 12 weeks for which the intervention was undertaken notwithstanding.

Table 11: Medians of selected immune parameters by Arms

Parameter by study arms	Baseline	12 weeks	p-value
Arm 2			
Log_{10} viral load	5.11 (3.82-5.52)	4.43 (3.48-5.13)	0.104
CD4	290 (159-483)	380 (226-628)	0.007
CD8	838 (509-1325)	1097 (689-1659)	0.007
Arm 1			
Log_{10} viral load	4.79 (3.85-5.56)	4.09 (3.38-4.84)	0.030
CD4	290 (147-456)	271 (167-402)	0.722
CD8	892 (669-1279)	780 (600-1256)	0.751

Arm 1, n = 33; arm 2, n = 30; CD4 cell count ($x10^9$ /l); CD8 cell count ($x10^9$l); viral load (log_{10}copies/ml)

A nutrition intervention in Uganda, where the doses used on children were much lower, produced no significant changes, leading to a suggestion that future interventions should consider the micro-nutrient dosing level as well as the power needed to detect differences (Ndeezi *et al*., 2010).

Arm 1 subjects experienced more significant viral load reductions than those in Arm 2, suggesting that use of mega-doses of zinc may have been ineffective. The insignificant viral load reductions in Arm 2 subjects may have partly been because they started with baseline viral load levels that were higher than those in Arm 1. However, these findings are in agreement with others obtained from studies that have evaluated the role of RDA compliant micro-nutrients in management of HIV and AIDS subjects in Thailand, South Africa, Kenya and Uganda, respectively (Bobat *et al*., 2005; Mburu *et al*., 2010, Ndeezi *et al*., 2010).

4.7 Correlation of measured variables

4.7.0 Introduction

To better elucidate predictors of intervention outcomes, chemo-informatic techniques of data mining, including Spearman's Rho tests of correlations between chemical and clinical variables, at baseline, during and after intervention were undertaken using computer aided packages that produced innovative results and interpretations linking clinical, nutritional and immunological parameters; significance being tested at the $p < 0.05$ level. While it was not envisaged that intervention outcomes by the 4^{th}, 8^{th} and 12^{th} week would correlate with variable outcomes at baseline, during and after intervention, it turned out that there were many significant interactions in measured parameters throughout the time of study. This was probably due to an intrinsic physiological mechanism by humans that controls biochemical and biological markers, the composition and dosage of the nutritional interventions notwithstanding. Variables that predicted critical clinical, nutritional and immunological intervention outcomes are presented and discussed.

4.7.1 Predictors of socio-demorgraphic characteristics

4.7.1.1 Factors associated with gender

Factors associated with gender were several and are summarized in table 12.

Table 12: Factors associated with gender

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
Headache	12	0.321	0.007
No herpes zoster	12	0.225	0.061
Fatigue	12	0.254	0.034
No diarrhea	0	0.246	0.020
No boils	0	0.254	0.017
Low WHO clinical staging of disease	0	0.238	0.025
Biochemical			
High CD4/CD8	12	0.208	0.089
High CD4/CD8	0	0.205	0.062
High ESR	0	0.182	0.088
Low serum zinc	8	0.249	0.036
High serum copper	8	0.648	0.017
Low Hb	12	0.327	0.005
Low Hb	8	0.284	0.016
Low Hb	0	0.314	0.005
Low RBC	12	0.204	0.086
Low RBC	0	0.223	0.037

Comparable to males, females had high prevalence of headache after intervention ($p = 0.007$), less diarrhoea at baseline ($p = 0.020$), less prevalence of boils at baseline ($p = 0.017$), high CD4/CD8 ratio at baseline ($p = 0.062$), low serum zinc concentration by the 8^{th} week ($p = 0.036$) and low Hb by the 12^{th} week ($p = 0.005$). Since zinc ions reportedly have anti-viral, anti-bacterial and anti-cancer properties (Bryce-Smith, 1989; Sprietsma, 1999), low prevalence of boils and diarrhoea in women at baseline suggested presence of high serum zinc levels. Similarly, women with high baseline serum zinc levels achieved much higher serum zinc levels

during intervention, resulting in some interference with copper nutriture and lowering of Hb concentrations leading to headache after intervention. However, the headaches may be associated with the monthly menstrual challenges and cannot be associated entirely to the micro-nutrient interventions.

4.7.2 Correlations with clinical data

4.7.2.1 Factors associated with HIV– seropositivity

Many factors were associated with HIV-seropositivity as presented in table 13.

Table 13: Factors associated with HIV-seropositivity at baseline

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
Skin rashes	0	0.250	0.017
Weight loss	0	0.237	0.024
Fever	0	0.206	0.051
Oral thrush	0	0.212	0.045
No malaria parasitaemia	0	0.328	0.002
High WHO clinical staging of disease	0	0.436	0.0001
Biochemical			
Low CD4	12	0.580	0.0001
Low total lymphocytes	12	0.333	0.004
High CD8	12	0.315	0.007
Low NK	12	0.349	0.003
Low CD4	0	0.473	0.0001
High CD8	0	0.398	0.0001
Low CD4/CD8	0	0.371	0.0001
High ESR	0	0.273	0.009
High ESR	12	0.206	0.081
Low serum retinol	8	0.273	0.045
Low serum retinol	4	0.303	0.007
Low serum retinol	0	0.116	0.278
Low serum zinc	12	0.449	0.0001
Low serum zinc	0	0.211	0.050
Low Hb	12	0.252	0.032
Low Hb	8	0.307	0.009
Low WBC	12	0.252	0.031
High total protein	0	0.635	0.006

HIV-seropositivity was associated with several factors including, absence of malaria parasitaemia ($p = 0.002$), low serum retinol levels by the 4^{th} week ($p = 0.007$), low serum zinc concentration at baseline ($p = 0.050$) and low serum zinc level after intervention ($p = 0.0001$). Nutritional and metabolic disturbances can lead to altered acute-phase response proteins due to chronic inflammation in persons with advanced HIV infection (Treitinger *et al.,* 2001; Arinola *et al.,* 2004). Changes in acute phase response proteins, mainly decreased albumin and elevated C-reactive protein concentrations, have been associated with low serum concentrations of several

micro-nutrients in HIV-seronegative persons (Shenkin, 1995; Thurnham *et al.*, 2003*;* Ghayour-Mobarhan *et al.,* 2005). The significant link between HIV-seropositivity and low prevalence of malaria parasitaemia suggested that HIV-seropositive subjects mounted a more aggressive humoral immunity that is also effective in clearing malaria parasites. The HIV-seronegative subjects had a hyperactive cellular immunity that ensured malaria parasites were destroyed as they attacked cells.

Since low serum retinol concentration is associated with cellular immunity, it appears that stimulation of the collapsed cellular immunity by the 4th week of supplementation was priority in HIV-seropositive subjects. However, humoral immunity was stimulated after intervention as manifested in the low serum zinc level in HIV-seropositive subjects at baseline and after intervention, mega-doses of zinc administered during supplementation notwithstanding. This observation is supported by the work of other researchers who have shown that repletion of serum zinc during infection with HIV is not a simple task and special supplements are needed (Mburu *et al*., 2010). Consequently, the serum zinc status in this study returned to baseline level after going through a peak value by the 4th week of supplementation, implying that once infected with HIV, the human host up-regulates humoral immunity until the virus is cleared from the system before it can switch back to a predominantly cell-mediated immunity that is associated with increased serum zinc level (Mbakaya *et al.*, 2011; Cantorna *et al.,* 1994; 1995; Prasad *et al.,* 1997; Bao *et al.*, 2003). Furthermore, it is probably because of these inflammatory/evolutionary processes that serum zinc levels in HIV-seropositive subjects who used VIUSID^{TM} for 30 months did not change appreciably (Mbakaya *et al.*, 2004b). Indeed, according to that study, it was observed that subjects who experienced significant viral load reduction were those whose

serum zinc levels reduced significantly. Although the findings led to speculation that zinc is consumed during the fight against HIV, this study has elucidated the phenomenon by observing correlation between low serum zinc, HIV-seropositivity, enhanced HIV antibody production and significant viral load reduction in adult subjects supplemented with mega-doses of micro-nutrients for 12 weeks (Mbakaya *et al.*, 2011). However, a recent study has also shown that zinc supplementation to HIV patients on HAART resulted in prevention of immunological failure and significant repletion of serum zinc levels (Baum *et al*., 2010). Correlation of HIV-seropositivity with high total proteins at baseline and not after intervention, low micro-nutrient zinc and retinol, significant correlation with high ESR at baseline ($p = 0.009$) but insignificantly high ESR after intervention ($p = 0.081$) suggested that the interventions reduced the inflammatory response as observed by other researchers (Shenkin, 1995; Thurnham *et al.*, 2003*;* Ghayour-Mobarhan *et al.*, 2005).

However, there is no unanimity in the scientific literature on the hypothesis that HIV is the cause of AIDS (Duesberg, 1988). The assumption proposed in 1984 that the cause of AIDS is infection with HIV was founded on the correlation between detection of antibodies to this virus and the onset of AIDS. Although unanimously rejected by AIDS researchers to date, Duesberg opposed these views and suggested that AIDS is caused by drugs and malnutrition and that HIV is only a passenger pathogen (Duesberg, 1994; Lindermann, 1994). The emerging evidence probably suggests the need to redefine the cause of AIDS (Mbakaya and Wakori, 1997; Mbakaya *et al.*, 2004a). Accordingly, it has been preliminarily hypothesized that AIDS is caused not only by HIV but by multiple factors, including primarily zinc deficiency as a result of malnutrition and/or evolutionary dynamics that shift human immunological responses from predominantly

Th-1 to Th-2 to adapt to offending pathogens (e.g. viruses, fungi, parasites, semen in the rectum and bacteria) and chemicals (e.g. pesticides, drugs, contraceptives, dioxins, fumonisins, aflatoxins and dibenzofurans) (Mbakaya *et al.*, 2011).

4.7.2.2 Predictors of high HIV antibodies at baseline

Several factors were associated with high HIV antibodies at baseline in both HIV-seronegative and HIV-seropositive subjects as summarized in table 14.

Table 14: Factors associated with high antibodies at baseline

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
High BMI	0	0.291	0.012
High BMI	12	0.223	0.070
Skin rashes	0	0.334	0.004
Pneumonia	0	0.244	0.037
High WHO classification of disease	0	0.247	0.035
HIV–seropositivity	0	0.641	0.0001
Balanced diets	0	0.207	0.081
Biochemical			
Significant viral load reduction	12	0.282	0.016
High HIV antibody optical density	12	0.301	0.010
High $\log_{10}$ viral load	0	0.446	0.0001
Low CD4	12	0.414	0.0001
High CD8	12	0.200	0.094
Low CD4/CD8	12	0.448	0.0001
Low CD4	0	0.338	0.004
High CD8	0	0.247	0.041
Low CD4/CD8	0	0.344	0.004
High ESR	0	0.212	0.072
High serum retinol	8	0.213	0.087
Low serum zinc	12	0.223	0.062
High serum copper	12	0.613	0.034
Low serum copper	0	0.367	0.240
Low Hb	8	0.258	0.036
Low WBC	12	0.222	0.061
High total blood protein	0	0.671	0.004
Low serum zinc	0	0.157	0.195
Low serum retinol	0	0.040	0.739
High serum retinol	12	0.021	0.864
Low NK	0	0.053	0.665
Low NK	12	0.102	0.399

HIV-seropositivity (p = 0.0001), skin rashes at baseline (p = 0.004), high WHO classification of disease at baseline (p = 0.035), high total blood protein at baseline (p = 0.004), low serum copper levels by the 12th week (p = 0.034), low serum zinc concentration by the 12th week (p = 0.062) and low CD4/CD8 ratio at baseline (p = 0.004) were identified as key factors corresponding to high HIV antibodies in the subjects.

Overall, skin rashes are associated with humoral (antibody) immunity. Biochemically, low serum zinc concentration is also associated with antibody immunity that is required to fight HIV. Low CD4/CD8 ratio was associated with antibody immunity (Cantorna *et al.,* 1994). Furthermore, high antibodies at baseline correlated significantly with viral load reduction after intervention, suggesting that provision of micro-nutrients helped enhance effective antibody immunity against the virus.

4.5.2.3 Predictors of high optical density of HIV antibodies by the 12th week

Many factors correlated to high HIV antibody production after intervention as summarized in Table 15. High WHO clinical staging of disease at baseline (p = 0.0001), cough at baseline (p = 0.008), fever at baseline (p = 0.005), low CD4/CD8 ratio at baseline (p = 0.016), low serum zinc concentration at baseline (p = 0.002), significant viral load reduction post intervention (p = 0.013), high optical density of HIV antibodies at baseline (p = 0.002) and low serum zinc after intervention (p = 0.007) were found to correspond to high optical density of HIV antibodies in the subjects.

Table 15: Factors associated with high HIV antibodies after intervention

Factor	Time (weeks)	Correlation coefficient	p- value
Clinical		0.313	0.007
HIV-seropositivity	0	0.215	0.068
No headache	0	0.296	0.011
Skin rashes	0	0.307	0.008
Cough	0	0.322	0.005
Fever	0	0.451	0.0001
High WHO clinical staging of disease	0		
Biochemical			
Significant log_{10} viral load reduction	0	0.290	0.013
High HIV antibody optical density	0	0.358	0.002
High log_{10} viral load	0	0.209	0.090
High CD8	12	0.236	0.043
Low CD4/CD8	12	0.235	0.044
High CD8	0	0.211	0.082
Low CD4/CD8	0	0.290	0.016
Low serum zinc	12	0.313	0.007
Low serum zinc	0	0.367	0.002
High RBC	0	0.239	0.042
High blood glucose	0	0.506	0.046
High serum albumin	0	0.465	0.069
High blood glucose	12	0150	0.578
Randomized into low dose micro-nutrients	0	0.108	0.361
Low serum retinol	0	0.107	0.366
High serum retinol	12	0.014	0.906

The data associate low serum zinc level to an up-regulated antibody immunity that has been noted to be effective in clearance of viral load in infected persons (Cantorna *et al.,* 1995; Prasad *et al.,* 1997; Mbakaya *et al.,* 2011). Furthermore, high antibody optical density after intervention was associated with high CD8 cell count and significant viral load reduction; possibly suggesting that the reason CD8 cell count is raised is because it is effective in reduction of viral load.

4.7.2.4 Predictors of malaria parasitaemia at baseline

Few factors were associated with malaria parasitaemia in cohort at baseline as summarized in Table 16.

Table 16: Factors associated with malaria parasitaemia at baseline

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
No cough	0	0.183	0.084
No oral thrush	0	0.190	0.073
Low WHO clinical staging of disease	0	0.179	0.091
HIV-seronegativity	0	0.328	0.002
Biochemical			
Low CD8	12	0.280	0.017
High CD4/CD8	12	0.228	0.054
High total lymphocytes	0	0.197	0.071
High CD4	0	0.255	0.019
High CD4/CD8	0	0.244	0.024
Low serum retinol	12	0.206	0.080
High serum zinc	12	0.226	0.051
High serum copper	4	0.540	0.057
High serum vitamin E	0	0.488	0.091
High Hb	0	0.238	0.033
High WBC	0	0.208	0.050

Most significantly, HIV-seronegativity at baseline was associated with malaria parasitaemia ($p = 0.002$). Others factors associated with malaria included low CD8 cell count by the 12^{th} week ($p = 0.017$), high CD4 cell count at baseline ($p = 0.019$), high CD4/CD8 ratio at baseline ($p = 0.024$), low serum retinol levels by the 12^{th} week ($p = 0.080$) and high serum zinc concentration after intervention ($p = 0.054$). Whereas humoral immunity was effective in clearing parasitaemia in HIV-seropositive subjects, cellular immunity prevented development of clinical malaria. Under the circumstances, malaria infections may only be serious in HIV and AIDS subjects when they are at the last stage of disease progression, when even humoral immunity has severely

been compromised. Therefore, perpetual exposure to malaria parasites in malaria endemic regions in Kenya and the greater Sub-Saharan Africa may be a push factor for the population to enhance humoral immunity, thereby exposing such persons to the risk of HIV, TB, pneumonia and cancer since these diseases require cellular-immunity for effective prevention and control as was recently elucidated (Mbakaya, 2011).

4.7.2.5 Factors associated with history of TB at baseline

Factors correlating with a history of having TB at baseline in the cohorts are presented in table 17. Several factors, including high WHO clinical staging of the disease at baseline ($p = 0.0001$), low serum zinc concentration after intervention ($p = 0.003$), low serum zinc level at baseline ($p = 0.028$), low CD4/CD8 ratio at baseline ($p = 0.005$) and high blood glucose level at baseline ($p = 0.049$) correlated with history of TB at baseline.

Table 17: Factors associated with a history of TB at baseline

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
Use of TB drugs	12	0.638	0.0001
Weight loss	0	0.267	0.011
Cough	0	0.245	0.020
Oral thrush	0	0.258	0.015
Pneumonia	0	0.313	0.003
Consumption of balanced diets	0	0.185	0.082
High WHO clinical staging of disease	0	0.419	0.0001
HIV-seropositivity	0	0.199	0.059
Young	0	0.184	0.086
Biochemical			
Low CD4	12	0.225	0.057
High CD4/CD8	12	0.262	0.026
Low CD4	0	0.196	0.072
Insignificant change in CD4	12	0.251	0.017
Low CD4/CD8	0	0.299	0.005
High ESR	12	0.336	0.004
Low serum retinol	4	0.282	0.013
Low serum zinc	12	0.342	0.003
Low serum zinc	8	0.302	0.010
High serum zinc	4	0.243	0.046
Low serum zinc	0	0.236	0.028
Low Hb	12	0.196	0.096
Low Hb	8	0.232	0.050
Low WBC	0	0.182	0.086
High blood glucose	0	0.484	0.049
Low serum retinol	0	0.135	0.204
Low serum retinol	12	0.126	0.290

Since subjects with low CD4/CD8 ratio at baseline also had history of TB at baseline, it may suggest that the subjects had lowered immunity and preferred up-regulation of humoral immunity to effectively fight HIV which is achievable by lowering serum zinc level at baseline and even after substantial zinc repletion during the supplementation (Mbakaya *et al.*, 2011). However, since HIV is an intra-cellular pathogen requiring cellular immunity for effective

containment, infection with the same favoured up-regulation of humoral immunity; making TB a dangerous opportunistic infection in the HIV disease. Furthermore, high blood glucose level in subjects with history of TB suggested that the pathophysiology of the disease involved the endocrine system where stress levels were up-regulated for the purpose of increasing the sugar levels to enable those infected with TB to fight. A cross-sectional study investigating the interaction between tuberculosis, micro-nutrient malnutrition and HIV viral load in Malawi revealed that plasma viral load is inversely associated with BMI, plasma retinol, carotenoid and selenium concentration. Similarly, vitamin A, zinc and selenium deficiency were found to be common in the TB-infected subjects (61, 85 and 87%, respectively) (van Lettow *et al.*, 2004).

4.7.2.6 Factors associated with pneumonia at the 12th Week

Predictors of infection with pneumonia at the 12th week of intervention are as shown in table 18. Loss of weight, malaria parasitaemia at baseline, high monthly income as well as high serum zinc at baseline were associated with pneumonia at the 12th week.

Table 18: Factors associated with a history of pneumonia at 12th week

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
Headache	12	0.263	0.043
Loss of weight	12	0.392	0.002
Fever	12	0.442	0.0001
Fatigue	12	0.273	0.035
Boils	12	0.323	0.012
Cough	0	0.273	0.035
Malaria parasitaemia	0	0.280	0.030
High monthly income	0	0.258	0.047
Biochemical			
High CD8 cell count	12	0.294	0.025
High serum zinc	0	0.284	0.032

Whereas this may suggest that supplementation of HIV seropositive subjects who had high baseline serum zinc levels resulted in faster attainment of peak zinc levels, the subsequent decline that was noted all the way to the 12th week may have resulted in up-regulation of humoral immunity at the expense of cellular immunity, thereby causing an immune suppression of cellular immunity, leading to increased susceptibility to pneumonia at the 12th week. This may suggest the need for extra caution when supplementing HIV-seropositive subjects as is confirmed by some of the disappointing findings of such interventions (Filteau, 2010; Prentice, 2010). Furthermore, high monthly income appears to be associated with development of humoral immunity, probably because of improved access to adequate food. However, more research is needed to shed more light on these preliminary observations.

4.7.2.7 Factors associated with diarrhoea at 12th week

Several factors predicted presence of diarrhea by the 12th week of intervention as shown in table 19.

Table 19: Factors associated with diarrhoea at 12th week

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
Headache	12	0.347	0.007
Weight loss	12	0.448	0.0001
Fever	12	0.384	0.002
Oral thrush	12	0.389	0.002
Fatigue	12	0.365	0.004
Low BMI	12	0.294	0.025
Biochemical			
Low CD4/CD8 ratio	12	0.290	0.027
High ESR	0	0.426	0.001
High ESR	12	0.460	0.0001
Low serum retinol	12	0.450	0.0001
Low serum retinol	8	0.381	0.004
Low serum retinol	4	0.322	0.017
Low serum zinc	8	0.320	0.019
Low Hb	12	0.410	0.001
Low Hb	8	0.407	0.002
Low Rbc	12	0.430	0.001

Low serum retinol, zinc, Hb, and BMI as well as high ESR and weight loss were significantly associated with diarrhea. These data shows how important micro-nutrients, especially zinc are important in managment of diarrhea in the HIV disease. While high ESR is a measure of acute inflammation that is also associated with blockage of zinc up-take (Mburu et al., 2010), these factors are also associated with diarrhea in the HIV and AIDS disease. Other studies have shown zinc supplementation to be beneficial in controlling diarrhea in HIV/AIDS subjects (Bobat *et al*., 2005; Baum *et al*., 2010).

4.7.2.8 Predictors of advanced clinical stage of disease at baseline

Several factors were associated with disease progression in the subjects (table 20).

Table 20: Factors associated with advanced clinical disease at baseline

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
No increase in CD4 after intervention	12	0.262	0.013
Low BMI	0	0.358	0.001
High HIV antibody optical density	0	0.316	0.002
High HIV antibody optical density	12	0.451	0.0001
High $\log_{10}$ viral load	0	0.425	0.0001
Using TB drugs	12	0.325	0.006
Diarrhoea	0	0.356	0.001
Weight loss	0	0.346	0.001
Cough	0	0.286	0.006
Fever	0	0.325	0.002
Oral thrush	0	0.345	0.001
Fatigue	0	0.310	0.003
Pneumonia	0	0.340	0.001
Boils	0	0.248	0.018
TB	0	0.419	0.0001
Biochemical			
Low CD4	12	0.487	0.0001
Low total lymphocytes	12	0.306	0.009
Low CD4/CD8	12	0.548	0.0001
Low NK	12	0.388	0.001
Low CD4	0	0.409	0.0001
High CD8	0	0.245	0.024
Low CD4/CD8	0	0.546	0.0001
High ESR	12	0.233	0.047
Low serum retinol	8	0.234	0.048
Low serum retinol	0	0.217	0.040
Low serum zinc	12	0.346	0.003
Low serum zinc	0	0.298	0.005
Low Hb	12	0.281	0.016
Low Hb	8	0.372	0.001
Low RBC	12	0.341	0.003
High neutrophils	12	0.252	0.032
High total blood protein	0	0.491	0.045

Factors associated with disease progression at baseline included: low BMI at baseline ($p = 0.001$), high optical densities of HIV antibodies after intervention ($p = 0.0001$), high baseline viral load ($p = 0.0001$), low CD4/CD8 ratio at baseline ($p = 0.0001$), low serum retinol

concentration at baseline ($p = 0.040$), low serum zinc level by 12^{th} week ($p = 0.003$), low serum zinc concentration at baseline ($p = 0.005$), and low neutrophil count by 12^{th} week ($p = 0.032$).

Some studies have associated disease progression with low serum retinol and zinc (Baum *et al.*, 1995), implying that for disease to progress, cellular and humoral immunity that are up-regulated by zinc and retinol respectively, will be low due to lowered nutritional status of the micro-nutrients. In a study in India on residential school children with biochemical evidence of poor status for several micro-nutrients, a multi-micro-nutrient supplement did not significantly reduce the incidence of common childhood infections. However, the duration of such common childhood illnesses were significantly reduced (Sarma *et al*., 2006). The differences in the observations probably suggest that the potency of the supplements administered were not similar.

4.7.3 Correlation with nutritional data

4.7.3.1. Factors correlated with eating 3 meals per day

Few factors that were associated with eating 3 meals per day are presented in table 21.

Table 21: Factors associated with consumption of 3 meals per day

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
History of pneumonia	0	0.218	0.040
High BMI	0	0.695	0.068
History of TB	0	0.185	0.082
High number of daily meals	0	0.364	0.0001
Biochemical			
Low total lymphocytes	0	0.214	0.050
Low CD4	0	0.215	0.049
Low eosinophils	12	0.336	0.004
Low serum copper	0	0.592	0.033
Low serum copper	4	0.723	0.005
Low WBC	0	0.218	0.040
Significant viral load reduction	12	0.186	0.083
High optical density of HIV antibodies	0	0.186	0.083

The factors associated with eating 3 meals per day include: high number of daily meal intake ($p = 0.0001$), high BMI at baseline ($p = 0.068$), history of pneumonia at baseline ($p = 0.040$), low CD4 cell count at baseline ($p = 0.049$), low eosinophils by the 12^{th} week ($p = 0.004$), and low serum copper levels at baseline ($p = 0.033$) and 4^{th} week ($p = 0.005$). The link between eating 3 meals per day and a history of pneumonia and low CD4 cell count in HIV-seropositive subjects may suggest that with such nutritional resources, the subjects preferentially up-regulated humoral immunity to fight the virus at the expense of cellular immunity that is required to prevent pneumonia, cancer and TB. This is in tandem with evolutionary principles of survival for the fittest. Consequently, as consumption of 3 meals a day favoured up-regulation of acquired

immunity against HIV, it lowered cellular (innate) immunity that is required to effectively fight pneumonia! These results may be supported by a recent study on the influence of inflammation on plasma zinc concentration in apparently healthy HIV-seropositive Kenyan adults that showed that those supplemented with maize and beans ended up with low serum zinc levels after intervention (Mburu *et al*., 2010), yet it is known that zinc is needed to up-regulate cellular immunity against pneumonia (Jason *et al*., 2002). Furthermore, the unexpected result of where improved food intake has been counter-productive in management of HIV subjects has been observed and frustrated some researchers (Filteau, 2010; Prentice, 2010). However, it has been suggested that, when given adequate food, the human body will judiciously utilize it to up-regulate humoral immunity that serves its immediate survival needs, notwithstanding that in so doing it paves way for attack from opportunistic infections such as TB, cancer and pneumonia that require the down-regulated cellular immunity for prevention and effective control (Mbakaya, 2011). What this means is that nutritional interventions require to be programmed and tailor-made in a manner that best suits all the immunological needs of HIV-seropositive subjects. Otherwise, intervention outcomes will produce results that lack unanimity as has been observed (Prentice, 2010).

4.7.3.2 Factors associated with the Arm of intervention

Few factors were associated with using mega or low-dose micro-nutrients. A significant increase in CD4 cell count was associated with administration of mega-dose micro-nutrients ($p = 0.022$) while subjects receiving mega-doses of supplements had high total lymphocyte cell count after intervention compared to those on low dose ($p = 0.052$). However, for all subjects combined, only serum retinol by the 4^{th} week was high in subjects on low-dose micro-nutrients (Arm 1)

than those on mega-doses (p = 0.005), despite omission of vitamin A supplements in that Arm (Arm 1).

These observations suggest that the mega-dose micro-nutrients administered were beneficial in stimulating immunity in Arm 2 subjects. The significantly high serum retinol status by the 4th week suggests that subjects on low-dose (Arm 1) had high serum zinc and retinol levels, an indication that both the cellular and humoral immune responses had been up-regulated (Jason, 2002). Furthermore, use of citric acid in the formulations appears to have made zinc significantly bio-available in both study Arms to the extent that it narrowed the observed differences in the two intervention groups. This may suggest that zinc is a very critical micro-nutrient in human immunity, especially because deficiency of this micro-nutrient results in a premature transission from cellular (Th-1) to humoral (Th-2) immunity (Sprietsma, 1999).

4.7.3.3 Predictors of high BMI at baseline

High baseline BMI, absence of fatigue, low WHO clinical staging of disease and high serum retinol and Hb levels at the 12th week of supplementation were associated with high BMI at baseline as shown in table 22.

Table 22: Predictors of high BMI at baseline in all subjects

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
Eating 3 meals per day	0	0.195	0.068
High HIV antibodies	0	0.232	0.029
Skin rashes	12	0.224	0.060
Fever	12	0.224	0.061
Absence of fatigue	12	0.350	0.003
Absence of cough	0	0.253	0.017
Few symptoms of disease	0	0.278	0.008
High BMI	12	0.868	0.0001
Biochemical			
Low ESR	0	0.230	0.030
Low ESR	12	0.279	0.017
High retinol	12	0.312	0.007
High retinol	0	0.260	0.014
High zinc	12	0.195	0.098
High Hb	12	0.346	0.003
High Hb	8	0.257	0.029
High Rbc	12	0.364	0.002

High BMI was associated with consumption of 3 meals per day ($p = 0.068$) and low staging of disease ($p = 0.008$). Furthermore, high BMI at baseline was associated with high serum retinol ($p = 0.014$), low inflammation as measured by low ESR ($p = 0.030$) and high HIV antibodies. The data suggests that BMI is a good indicator of stage of disease progression and since it is easy to measure; it can be used as one of the routine markers for monitoring disease progression and retinol status.

4.7.3.4 Factors associated with high BMI after intervention

Clinically, significant viral load reduction ($p = 0.023$), absence of diarrhea ($p = 0.028$) and absence of fatigue ($p = 0.001$) at the 12^{th} week were associated with high BMI after supplementation as was high BMI at baseline ($p = 0.0001$) as presented in table 23.

Table 23: Factors associated with high BMI after supplementation

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
Significant viral load reduction	12	0.273	0.023
Absence of headache	12	0.225	0.063
Skin rashes	12	0.225	0.064
Absence of diarrhoea	12	0.265	0.028
Absence of weight loss	12	0.225	0.065
Absence of fatigue	12	0.387	0.001
Low WHO stage	0	0.228	0.060
High BMI	0	0.868	0.0001
Biochemical			
High HIV antibodies	0	0.229	0.058
Low ESR	0	0.300	0.012
Low ESR	12	0.296	0.014
High retinol	12	0.366	0.002
High retinol	8	0.275	0.029
High retinol	4	0.242	0.058
High zinc	12	0.242	0.049

High HIV antibody production at baseline (p = 0.058), low ESR (p = 0.012) and high serum retinol (p = 0.002) levels as was high serum zinc (0.049) were the biochemical parameters associated with high BMI after supplementation. Since viral load measurements are costly, it can be understood that an increase in BMI in HIV-seropositive subjects may be an indicator of some success in nutritional management of the subjects.

4.7.3.5 Predictors of high serum retinol at the 12th week

Several factors were associated with having high serum retinol in the subjects after intervention as presented in table 24.

Table 24: Predictors of high serum retinol levels after intervention

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
No diarrhoea	12	0.417	0.0001
No weight loss	12	0.257	0.029
No malaria parasitaemia	0	0.206	0.080
High BMI	0	0.312	0.007
High BMI	12	0.366	0.002
Advanced age	0	0.255	0.031
Biochemical			
Low $\log_{10}$ viral load	12	0.310	0.011
High CD4/CD8	0	0.217	0.073
High RBC	12	0.298	0.009
High Hb	8	0.498	0.0001
High Hb	12	0.462	0.0001
High serum zinc	8	0.316	0.009
High serum retinol	0	0.569	0.0001
High serum retinol	4	0.650	0.0001
High serum retinol	8	0.748	0.0001
Low ESR	0	0.542	0.0001
Low ESR	12	0.246	0.033
High serum copper	4	0.515	0.087

High serum retinol after intervention correlated with high BMI at baseline ($p = 0.007$) and after intervention ($p = 0.002$), high serum retinol concentration at baseline ($p = 0.001$), high serum zinc levels by the 8^{th} week ($p = 0.009$), advanced age ($p = 0.031$), low viral load after intervention ($p = 0.011$) and absence of diarrhoea by the 12^{th} week of intervention ($p = 0.0001$). Since high serum retinol concentration is associated with an up-regulated humoral immunity while high serum zinc level is correlated with cellular immunity, subjects associated with this outcome were nutritionally sound as demonstrated by the high BMIs (Palenicek et al., 1995; van Lettow *et al.*, 2004). Coincidentally, subjects with high serum retinol level after intervention had low HIV viral loads also. Since subjects with high serum retinol concentration up-regulated humoral immunity, it was notable that they were older, an observation that is consistent with the

fact that acquired immunity matures with age. These observations are supported by a study which showed that a single large dose of vitamin A to neonates improved survival by the 6th week in subjects who were HIV-seropositive by polymerase chain reaction (Humphrey *et al.*, 2006).

Absence of diarrhoea in subjects with big weight and high serum retinol levels suggests two things: (i) humoral immunity that is associated with high serum retinol level is critical in preventing diarrhoea and (ii) occurrence of diarrhoea in subjects with low BMI suggests that weight loss is probably partly due to the up-regulation of humoral immunity by mobilization of zinc from tissues/stores and subsequent excretion in diarrhoea. However, more research is required to establish the actual cause of diarrhoea during HIV infection. This immunological reality may explain observations that use of highly active anti-viral therapy (HAART) restores immunologic function but does not eliminate weight loss and wasting (Autran *et al.*, 1997; Wanke *et al.*, 2000; Tang *et al*., 2005). Consequently, some researchers have called for micro-nutrient supplements as adjunct therapy to HAART (Singhal and Austin, 2002; Lanzillotti and Tang, 2005; Baum *et al.*, 2010).

4.7.3.6 Factors associated with high serum zinc at the 12th week

Factors associated with high serum zinc levels after intervention are presented in table 25.

Table 25: Predictors of high serum zinc in all subjects after intervention

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
No use of TB drugs	12	0.306	0.009
Skin rashes	12	0.284	0.016
No oral thrush	12	0.321	0.006
No TB	12	0.257	0.030
No diarrhoea	0	0.334	0.004
No weight loss	0	0.308	0.008
No fever	0	0.253	0.031
No fatigue	0	0.233	0.048
No boils	0	0.250	0.033
High BMI	12	0.242	0.049
No history of TB	0	0.342	0.003
No malaria parasitaemia	0	0.226	0.054
Low WHO clinical staging of disease	0	0.346	0.003
HIV–seronegativity	0	0.449	0.0001
Biochemical			
High CD4	12	0.396	0.001
High RBC	12	0.314	0.006
Low CD8	12	0.212	0.02
Low blood total protein	0	0.610	0.009
High CD4/CD8	12	0.487	0.0001
High NK	12	0.247	0.034
High CD4	0	0.390	0.001
Low CD8	0	0.310	0.010
High CD4/CD8	0	0.468	0.0001
High NK	0	0.226	0.062
High serum retinol	8	0.344	0.004
High serum retinol	4	0.530	0.001
High serum retinol	0	0.315	0.007
High serum zinc	8	0.417	0.0001
Low serum copper	4	0.586	0.035
Low serum vitamin E	12	0.524	0.080

High serum zinc levels after intervention was associated with low WHO staging of disease at baseline ($p = 0.003$), HIV–seronegativity ($p = 0.001$), high NK cell count after intervention ($p = 0.034$), high serum retinol levels at baseline ($p = 0.007$), high serum zinc concentration by the 8^{th} week ($p = 0.0001$), low serum copper levels by the 4^{th} week ($p = 0.035$) and low serum alpha-

tocopherol levels after intervention (p = 0.080). Since high serum zinc concentration is associated with up-regulation of cell-mediated immunity, subjects with these levels of zinc probably had enhanced after intervention (Cantorna *et al.,* 1994, 1995; Prasad *et al.,* 1997; Bao *et al.,* 2003). Furthermore, the subjects had low WHO clinical staging of disease, low copper levels and were asymptomatic or HIV-seronegative despite having been exposed to HIV by diseased spouses. This is probably a pointer to the role micro-nutrient zinc could play in prevention of HIV infection and has been shown, immunological failure in subjects on HAART (Mbakaya *et al*., 2004a; Baum *et al*., 2010).

4.7.3.7 Predictors of high serum copper at the 12th week

Not many factors could be associated with high serum copper levels after intervention as shown in table 26.

Table 26: Predictors of high serum copper levels after intervention

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
Headache	0	0.629	0.021
No history of pneumonia	0	0.514	0.072
Biochemical			
High serum copper	0	0.756	0.003
High serum copper	4	0.602	0.030
High Hb	12	0.519	0.069
High WBC	0	0.512	0.074
High RBC	12	0.590	0.034
Low ESR	12	0.501	0.081
Low optical density of HIV antibodies	0	0.613	0.034

Only a few factors were associated with high serum copper levels after intervention. They include: high baseline serum copper level (p = 0.003), headache at baseline (p = 0.021) and low

optical density of HIV antibodies at baseline ($p = 0.034$). Due to the interference of zinc with copper nutriture, the low optical density of HIV antibodies associated with high serum zinc level at baseline and by extension an up-regulated cellular immunity, high serum copper concentration suggested stimulation of humoral immunity. Similarly, since copper is needed for synthesis of hemoglobin, the headache reported in the subjects at baseline suggests that elevated serum zinc concentration interferes with hemoglobin and the transportation of oxygen to the brain. The observation is supported by the absence of headache in subjects with high serum copper levels after intervention. Consequently, the headache that is one of the symptoms of HIV and AIDS infection may be due to reduced copper and hemoglobin levels because of increased serum zinc levels in support of up-regulation of cellular immunity. There are reports of copper deficiency caused by over-use of zinc supplementation (Rowin and Lewis, 2005; Igic *et al.*, 2002).

4.7.3.8 Predictors of high blood Hb levels at the 12th week

Factors associated with high Hb levels after intervention are summarized in table 27.

Table 27: Predictors of high Hb levels in all subjects after intervention

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
No use of septrin.	12	0.298	0.010
No TB drugs	12	0.299	0.010
No headache	12	0.246	0.036
No diarrhoea	12	0.341	0.003
No weight loss	12	0.294	0.012
No fever	12	0.224	0.057
No oral thrush	12	0.235	0.045
No fatigue	0	0.265	0.024
No boils	0	0.223	0.058
Low WHO clinical staging	0	0.281	0.016
HIV-seronegativity	0	0.252	0.032
Male	0	0.327	0.005
Advanced age	0	0.209	0.078
Biochemical			
High CD4	12	0.298	0.009
High total lymphocytes	12	0.233	0.044
High CD4/CD8	12	0.316	0.006
High NK	12	0.295	0.010
High CD4/CD8	0	0.252	0.037
Low ESR	0	0.583	0.0001
Low ESR	12	0.545	0.0001
High serum retinol	12	0.462	0.0001
High serum retinol	8	0.473	0.0001
High serum retinol	0	0.466	0.0001
High serum retinol	0	0.299	0.009
High serum zinc	8	0.364	0.002
High serum copper	12	0.519	0.069
High Hb	8	0.846	0.0001
High Hb	0	0.321	0.009
High RBC	12	0.724	0.0001
High RBC	0	0.257	0.028
Low neutrophils	12	0.224	0.052
High serum albumin	12	0.597	0.011
High BMI	0	0.346	0.003
High BMI	12	0.408	0.001

Several clinical factors, including diarrhoea after intervention (p = 0.003), low WHO clinical disease staging at baseline (p = 0.016) and being male (p = 0.005) were associated with high Hb level after intervention in the cohort. The biochemical factors associated with high Hb levels after intervention include high serum retinol levels at baseline (p = 0.0001), high serum retinol concentration after intervention (p = 0.0001), high serum zinc concentration after intervention (p = 0.009) and high CD4 cell count after intervention (p = 0.009). These observations suggest that high Hb level after intervention correlated with high serum zinc and retinol levels that are due to up-regulation of Th-1 and Th-2 immune response (Cantorna *et al.*, 1994; 1995; Prasad *et al.*, 1997; Bao *et al.*, 2003). High serum retinol levels at baseline and after intervention were associated with high Hb after intervention. This observation suggests that high serum retinol level is a critical factor in high Hb level. Furthermore, this suggests that when serum retinol concentration is high, humoral immunity is primed to fight HIV, resulting in the increase in CD4 cell count. Although high Hb level and by extension high circulatory iron is associated with deleterious events during HIV infection, this is not the case when serum retinol concentration is high, anchoring the importance of elevated serum retinol levels during HIV infection (Nimmagadda *et al.*, 1998; Semba and Gray, 2001).

4.7.4 Correlation with immunological parameters

4.7.4.1 Predictors of an increase in CD4 cell count at the 12th week

Several factors were associated with high CD4 cell count after intervention in HIV-seropositive subjects as presented in table 28.

Table 28: Predictors of high CD4 cell count after intervention

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
No oral thrush	12	0.274	0.020
No boils	12	0.323	0.006
No skin rashes	0	0.285	0.015
No diarrhoea	0	0.258	0.029
No fever	0	0.254	0.031
No oral thrush	0	0.334	0.004
Low WHO clinical staging of disease	0	0.487	0.0001
HIV-seronegativity	0	0.580	0.0001
Biochemical			
Low total protein	0	0.605	0.010
Low ESR	0	0.292	0.013
Low ESR	12	0.359	0.002
High serum retinol	8	0.300	0.014
High serum retinol	4	0.269	0.030
High serum retinol	0	0.396	0.001
High CD3	12	0.587	0.0001
High serum zinc	12	0.396	0.0001
High total lymphocytes	12	0.718	0.0001
High serum zinc	8	0.254	0.038
High CD4/CD8	12	0.035	0.0001
High Hb	12	0.298	0.009
High NK	12	0.345	0.002
High Hb	8	0.436	0.0001
High CD4	0	0.781	0.0001
High WBC	12	0.568	0.0001
Low CD8	0	0.315	0.009
High RBC	12	0.303	0.008
High CD4/CD8	0	0.819	0.0001
Low neutrophils	12	0.492	0.0001

Several factors were associated with high CD4 cell count after intervention. These included low WHO clinical staging of the disease at baseline ($p = 0.0001$), high serum zinc levels after intervention ($p = 0.0001$), high NK cell count after intervention ($p = 0.002$), low neutrophil count after intervention ($p = 0.0001$) and high serum retinol levels at baseline ($p = 0.001$). It has been reported that vitamin B_{12} deficiency impairs neutrophil function (Benedich and Cohen, 1988) as

zinc deficiency decreases lymphocyte concentrations (Fraker *et al.*, 2000). Similarly, selenium deficiency has been associated with impairment of neutrophil and T-lymphocyte responses (Ferencik and Ebringer, 2003). Since high CD4 cell count after intervention was associated with high baseline serum retinol levels and high after intervention serum zinc levels, it appears that the most appropriate strategy to supplement HIV and AIDS subjects would be to first provide them with carefully designed micro-nutrients containing vitamin A and thereafter follow this with provision of mega-doses of zinc. This approach is logical given that HIV-seropositive subjects first need to clear viral loads using humoral immunity that is up-regulated using vitamin A (Jason *et al*., 2002) before their cellular immunity can be primed using-mega doses of zinc to promote chances of HIV-seroconversion (Sprietsma, 1999). Furthermore, absence of this hierarchy in micro-nutrient administration may have resulted in conflicting outcomes and suggestions that micro-nutrient supplements may not always be beneficial to HIV-seropositive persons (Tang *et al*., 1993; 1996). In South Africa, a study administered 15 mg of zinc daily to HIV-infected children for 6 months, resulting in no gains in viral load reduction compared to the placebo-group (Bobat *et al*., 2005). Furthermore, the failure to increase CD4 cell count in HAART-treated HIV-infected patients with satisfactory virological suppression has been related to low CD4 T-cell product ion, high turnover and death. However, the relative contribution of these factors is still unclear, although studies suggest that CD4 T-cell repopulation during HAART is determined by CD4 T-cell activation and death (Massanella *et al*., 2010).

4.7.4.2 Predictors of significant viral load reduction at the 12th week

Several factors associated with significant viral load reduction are presented in table 29.

Table 29: Predictors of significant viral load reduction

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
Skin rashes	12	0.292	0.014
Skin rashes	0	0.224	0.035
Absence of weight loss	0	0.208	0.051
Advanced age	0	0.344	0.001
No oral thrush	0	0.232	0.025
No fatigue	0	0.256	0.015
High BMI	12	0.273	0.023
Arm 1	0	0.128	0.233
Biochemical			
High serum copper	8	0.491	0.088
High serum alpha-tocopherol	4	0.657	0.028
Balanced diet	0	0.186	0.083

The factors associated with significant viral load reduction include being older ($p = 0.001$), skin rashes at baseline ($p = 0.035$), skin rashes after intervention ($p = 0.014$); high serum alpha-tocopherol the 4^{th} week ($p = 0.028$) and eating 3 meals per day at baseline ($p = 0.083$). In a placebo-controlled trial where 29 HIV-seropositive patients received either 6 months of vitamin E supplements or placebo while simultaneously initiating HAART, no significant differences were observed in CD4 cell count, CD4/CD8 and viral load between the groups but a higher increase in lymphocyte viability was noted in the vitamin E – supplemented group (De Souza *et al.*, 2005). It is probably by up-regulating these functions that vitamin E repletion by the 4^{th} week of intervention is associated with significant viral load reduction in the subjects by the 12^{th} week of supplementation.

Being older, and particularly eating 3 meals per day, was associated with significant viral load reduction after micro-nutrient supplementation and suggests several things. One, being old meant that one had a more -developed acquired immune system that only needed proper nutrition

to be activated for enhanced reduction of viral load in the subjects. Second, consumption of 3 meals per day provided the necessary nutritional resources for producing assorted immune proteins that are needed in preventing HIV infection progressing to AIDS as observed by other researchers (Tang *et al*., 1993; 1996). Furthermore, significantly higher serum alpha-tocopherol levels by the 4^{th} week in subjects with substantial decrease in viral load suggests that use of mega-doses of vitamin E at the initial stages of supplementation, together with vitamin A may be preferable for reduction of viral load and increment of CD4 cell count. Lastly, if micro-nutrient administration to HIV and AIDS subjects was accompanied by development of skin rashes, one may expect a favourable outcome in viral load reduction. It is notable that skin rashes are among the most characteristic symptom of HIV and AIDS and are an indication of successful reduction of viral load.

4.7.4.3 Predictors of high CD8 cell count at the 12th week

High CD8 cell count was predicted by several factors as presented in table 30.

Table 30: Predictors of high CD8 cell count after intervention

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
High HIV antibody optical density	0	0.200	0.094
High HIV antibody optical density	12	0.236	0.043
Treatment for pneumonia	12	0.256	0.030
No malaria parasitaemia	0	0.553	0.0001
High WHO clinical staging	0	0.315	0.007
HIV-seropositivity	0	0.312	0.011
Biochemical			
High CD3	12	0.727	0.0001
High total lymphocytes	12	0.653	0.0001
Low CD4/CD8	12	0.379	0.001
High CD3	0	0.393	0.001
High CD8	0	0.593	0.0001
Low CD4/CD8	0	0.367	0.002
Low NK	0	0.369	0.002
Low serum retinol	4	0.245	0.038
Low serum zinc	12	0.292	0.012
Low serum zinc	8	0.259	0.034
Low serum zinc	0	0.489	0.0001
High WBC	12	0.280	0.017
Low neutrophils	12	0.507	0.0001
Low neutrophils	0	0.217	0.068
High total serum protein	0	0.427	0.087

Factors associated with high CD8 cell count after intervention included high total lymphocyte cell count after intervention ($p = 0.0001$), high CD8 cell count at baseline ($p = 0.0001$), low NK cell count at baseline ($p = 0.002$), low serum zinc at baseline ($p = 0.0001$), low serum zinc concentration at the 8th week ($p = 0.034$), low serum zinc concentration at the 12th week ($p = 0.012$), high WBC counts after intervention ($p = 0.0001$) and high WHO clinical staging of disease at baseline ($p = 0.038$). Consequently, low serum zinc levels at baseline, during and after

supplementation, significantly correlated with high CD8 cell count. Previous studies demonstrated that vitamin A deficiency reduces lymphocyte response (Semba, 1999) while vitamin C and selenium deficiency depresses cell-mediated immune response (Benedich, 1988) and impairment of neutrophil and T-lymphocyte responses, respectively (Ferencik and Ebringer, 2003). Therefore, the results indicate that provision of mega-doses of zinc alone to HIV-seropositive subjects may compromise their capacity to fight HIV. The findings are supported by reports that there is no scientific unanimity on the benefits of zinc supplementation to HIV-seropositive subjects (Mbakaya *et al.*, 2005). A randomized double-blind placebo-controlled trial for zinc supplementation with 10 mg daily dose of elemental zinc (as zinc sulphate) to children with HIV-1 infection in South Africa had no effect on viral load reduction between the groups by the 6^{th} month of intervention (Bobat *et al*., 2005). However, the only observed benefit was that children given zinc supplementation were less likely to get watery-diarrhoea than those given placebo ($p = 0.001$). Astudy on HIV-infected adults in Kenya showed that low serum zinc levels were the result of the inflammatory response and that overcoming this challenge during supplementation was a tall order (Mburu *et al*., 2010).

4.7.4.4 Predictors of high NK cell count at the 12th week

Factors associated with high NK cell count after intervention are presented in table 31.

Table 31: Predictors of high NK cell count after intervention

Factor	Time (Weeks)	Correlation Coefficient	p-Value
Clinical			
No diarrhoea	12	0.272	0.021
No boils	12	0.200	0.092
No weight loss	0	0.268	0.023
No fever	0	0.212	0.074
Low WHO clinical staging	0	0.388	0.001
Biochemical			
Significant increase in CD4	12	0.351	0.002
Low HIV optical density of antibodies	0	0.244	0.039
Low $\log_{10}$ viral load	12	0.296	0.015
Lower $\log_{10}$ viral load	0	0.333	0.006
High CD4	12	0.345	0.002
High total lymphocytes	12	0.338	0.003
High CD4/CD8	12	0.342	0.003
Low CD8	0	0.317	0.008
High CD4/CD8	0	0.289	0.017
High NK	0	0.423	0.0001
HIV-seronegativity	0	0.349	0.003
Low ESR	0	0.234	0.048
Low ESR	12	0.294	0.010
High serum retinol	4	0.293	0.018
High serum retinol	0	0.247	0.036
High Hb	12	0.295	0.010
High Hb	8	0.270	0.027
High WBC	12	0.243	0.036
High RBC	12	0.267	0.021
Low neutrophils	12	0.329	0.001

A significant increase in CD4 cell count after intervention ($p = 0.002$), low WHO clinical disease staging ($p = 0.001$), high NK cell count at baseline ($p = 0.001$), HIV-seronegativity ($p = 0.003$), high serum retinol at baseline ($p = 0.036$), and low neutrophils after intervention ($p = 0.004$) predicted high NK cell count after intervention. It would appear that high NK cell count after intervention was associated with HIV-seronegativity or low WHO clinical staging of disease.

Coincidentally, high serum retinol levels at baseline also predicted this outcome, implying that high baseline serum retinol level is critical in prevention of progression of HIV infection to disease. Since high serum retinol level is associated with enhanced integrity of humoral (antibody) immunity, the importance of the vitamin in combating HIV and AIDS cannot be over-emphasized (Jason *et al.*, 2002).

4.8 Intervention safety by Arm of study

Data on liver function tests performed on a sub-sample of the subjects in Arm1 and 2, as an evaluation of the safety of the interventions, is presented in table 32.

Table 32: Medians of liver function tests before and after intervention

Parameter	Baseline Median (IQR)	After 12 weeks Median (IQR)	p-value	Normal values
Arm 2				
ALT	11.0 (8.0-21.5)	19.0 (12.5-28.0)	0.010	5 – 60 IU/l
Bun	4.3 (2.8-5.1)	4.8 (3.9-6.6)	0.173	7 – 21 mg/l
Glucose	4.0 (3.4-5.1)	3.5 (2.7-4.6)	0.441	4 – 7 mmol/l
Total protein	87.0 (77.0-95.0)	87.0 (80.0-89.0)	0.594	65 – 82 g/l
Albumin	42.4 (37.4-44.5)	37.3 (36.2-45.0)	0.953	39 -50 g/l
Ferritin	82.6(44.6-135.6)	88.3(30.5-282.7)	0.273	13 – 400 ng/ml
Arm 1				
ALT	16.5 (5.5-31.3)	23.0 (13.3-28.0)	0.205	
Bun	4.3 (3.0-5.2)	4.1 (4.0-5.0)	0.484	
Glucose	4.1 (3.3-5.1)	3.7 (3.5 (4.7)	0.674	
Total protein	86.5 (75.8-95.0)	85.0 (80.0-93.5)	0.438	
Albumin	37.9 (33.5-41.2)	35.1 (29.1-42.0)	0.208	
Ferritin	36.2(8.7-135.7)	10.8(5.1-66.0)	1.000	

Arm 1, n = 8; Arm 2, n = 9;

To minimize costs, only a few randomly selected samples were considered in Arm 2 (n = 9) and Arm 1 (n = 8) for the safety tests before and after intervention. Significant reduction was noted in

the ALT values in Arm 1 ($p = 0.001$) after intervention, with a significant increase in Arm 2 ($p = 0.010$), while in Arm 1 the increase was marginal; suggesting use of citric acid in the formulations was safe. Most of the other parameters changed marginally in Arms 1 and 2, and were within the normal ranges as presented (table 32). Overall, the data suggests the interventions were safe and could be up-scaled for use in management of HIV and AIDS subjects in Kenya and beyond. In particular, the formulation in the mega-dose supplements could be used as it demonstrated greater immunological benefits to the subjects, but may have to be administered for slightly longer to also ensure beneficial outcomes in viral load reduction. Such an addition to the already on-going HAART program in Kenya are desirable considering that nutritional interventions are now associated with prevention of immunological failure in HIV and AIDS subjects on antiretroviral drugs (Baum *et al*., 2010).

CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Although both sexes had an equal chance of being included in the study, 70% were women probably because women are more willing to get help for issues affecting their health. Since only half the subjects consumed 3 meals per day, this may possibly be an underlying factor for their compromised immunity and by extension susceptibility to HIV and AIDS. There was a significant reduction in prevalence of signs and symptoms associated with HIV and AIDS in subjects on the mega-dose supplements including headache ($p = 0.049$), skin rash ($p = 0.012$), pneumonia ($p = 0.004$) and loss of weight ($p = 0.007$), suggesting that the intervention was effective in management of this disease.

As serum zinc levels increased and decreased during intervention, the trend with the other micro-nutrients was the reverse, suggesting that the levels serve different immune functions as reported in the literature. The immunity of subjects on mega-dose micronutrients increased more significantly than that of subjects in the control group on VIUSID^{TM} only. The viral load of subjects decreased substantially in both Arms while the serum zinc levels were not significantly different in these two groups probably due to use of citric acid in the formula that appeared to make zinc more bio-available in both Arms, narrowing the benefit gap due to the mega-dose supplement. However, a longer intervention period than the 12 weeks for which this study was conducted may be needed to obtain more significant results.

Significant correlation between low serum zinc levels and enhanced HIV antibody production and significant viral load reduction suggested that zinc levels were regulated physiologically, hence a preliminary re-definition of the cause of AIDS in the light of this finding. High serum retinol levels both at baseline and after intervention predicted significant viral load reduction, suggesting that this micro-nutrient has a role to play in modulating the immune system of HIV and AIDS patients.

The normal liver function tests of the subjects in both the study Arms suggested that the interventions were safe and could be used for a longer intervention time.

5.2 RECOMMENDATIONS

The study has shown that there are major gains in immune reconstitution as well as clearance of signs and symptoms of HIV and AIDS with use of mega-dose micro-nutrients. Therefore, more research should be undertaken to optimize results and eventually up-scale this strategy to delay use of ARVs in Kenya, considering their costs and challenges of resistance and compliance.

Based on correlations of micro-nutrient levels with viral load and immunity, more research should be done on programme-based supplementation approaches i.e. provide nutrients that help substantially reduce viral load and thereafter following this with those that improve cellular immunity of the subjects.

Development of new and innovative combinations of mega-doses of nutritional formulations of appropriate dosages for use in management of HIV and AIDS patients may result in the growth

of a new and vibrant nutraceutical industry in Kenya and enhance the role of chemistry in provision of societal solutions to stubborn public health problems in Kenya and beyond.

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Appendix I: Informed consent explanation and consent form

Project Title: EFFECTS OF FORTIFIED VIUSID™ ON SERUM ZINC, RETINOL AND IMMUNE STATUS OF HIV AND AIDS PATIENTS IN WESTERN KENYA

Purpose of the study

We all need food to stay alive and be able to perform our duties effectively. However, besides food, we also need other substances called vitamins and minerals. These substances are found in the foods we eat. Although these substances are very essential to our bodies, the volumes required are usually very small. Some of the foods we eat do not contain adequate amounts of the vitamins and minerals we require. In such situations we need these vitamins and minerals to be added to the foods we eat. The food supplements we are studying contain vitamins and minerals that are found to be deficient in the general Kenyan population and particularly amongst immuno-compromised persons. Studies from other parts of the world and even here in Kenya have identified VIUSID™ and the additional vitamins and minerals proposed in this study as the most beneficial nutrients to persons living with HIV and AIDS. We think that used in the proposed combination, we will be able to obtain even better management outcomes of HIV and AIDS patients in Kenya and in other parts of Sub-Saharan Africa where 70% of the global disease burden is to be found. In order for us to find out this, we have decided to give the supplements to some people and follow them for some time so that they may tell us what they think about the product. However, before we give you the supplements we need your permission. The study has been approved by the Scientific Steering Committee of KEMRI and the KEMRI/National Ethical Review Committee.

The clinical aspects of the study will be under Dr. Evans Amukoye, with assistance from Kisingu Wilfred whose performance during the pilot study was remarkable. Mr. Mbakaya Charles will take care of the immunochemical aspects of the study and overall project administration and coordination, having successfully done the same in the VIUSID™ pilot study.

Procedures to be followed

In this study, we shall see and examine you at this clinic on particular prearranged days. During this study, you will be given adequate supplies of the supplements and you will be instructed on how to use them. However, if you wish to participate in this study, we shall also take blood from you at the beginning of the study and on other days that you shall be informed in advance. You will be followed for a period of up to six months. Once the study is over, the results will be communicated to you, the community, and sponsors and in local and international scientific fora.

Risks

Following the results of the pilot study that demonstrated that VIUSID™ had no side effects to the study subjects, this follow-up study has no foreseeable risks associated to it and we shall monitor clients closely to ensure that no toxic or any harm is caused to them.

Benefits

The study that is being undertaken is expected to yield results on the effects of the supplements we shall give on the health of HIV andf AIDS clients in Kenya. Once the results become known, you will be informed and hopefully this will assist you in making important decisions about yourself in future. We shall also be in a better position in advising the healthcare workers in Kenya, especially, the National AIDS Control Council (NACC) on the best ways for caring for HIV and AIDS patients who are known to have nutritional problems. We particularly hope that through this study we shall be able to overcome key micronutrient deficiencies that could not be overcome when VIUSID™ was used as a mono-therapy; thereby realizing better management outcomes.

Confidentiality of the records

Your medical records that are related to this study will be maintained in confidence. The sponsor, may examine your medical records, as long as your name cannot be identified from the records. Technical reports from this study may be submitted by the sponsor to the relevant regulatory agency, though your name may not be identified from such reports. No identity of any specific patient in this study will be disclosed in any public reports or publications.

If problems develop

If any serious medical problems develop during the study, you will receive prompt medical attention from the doctors in-charge of the clinical aspects of this study, and if need be, you will be referred to the appropriate medical specialist for proper attention.

Obtaining additional information

You are encouraged to ask any questions to clarify any issues at any time or ask questions at any time during your participation in the study. You will be given a copy of this agreement for your own information. If you later think you need more information you may call 0722-846964 and ask for Mr. Charles Mbakaya, who is the project Principal Investigator, to mobilize any possible assistance. Also, Dr. Evans Amukoye of KEMRI may be called on 0722634383 for further information on any medical condition. Mr. Wilfred Kisingu (Public Health Nurse) who very ably looked after the patients in the pilot VIUSID™ study may also be contacted on 0722-603828 for any further information. The Medical Officer of Health nearest to your clinic may also be approached for any further necessary assistance.

Basis of participation

You are free to withdraw the consent to participate in the study at any time. If you choose to do so, your rights to continue attending health care at the clinic will not be affected for the remaining duration of the study.

Name of Patient:……..
Date of Birth…….. Age……..Yrs Sex…….
Address…… Telephone……..
E-Mail……. Fax…….
Signature

I have read the above information and have had the opportunity to ask questions and all of my questions have been answered satisfactorily. I consent to participate in the study as has been explained and as I have understood it. I have been given a copy of this consent form for my own records and future reference.

Signature…….. Date…….
Address of witness……..

I, the undersigned, have fully explained the relevant details of this study to the person named above. By virtue of my training and wealth of research experience in this field, I'm qualified to perform this role.

Signature…… Name of PI…… Date……..
Signature…… Name of Witness……. Date……..

Appendix II: Quality of life and clinical evaluation form

KEMRI/KU/NACC/World Bank VIUSID™ HIV and AIDS Project

Date: ……ID No:…….
Location……
Telephone…….

BIODATA
Name:……
Age:…….
Sex……
Level of education:…….
Occupation:……..
Marital status:……
Monthly financial Income……
Time of follow-up: (0) Baseline (1) 4th week (2) 8th Week (3) 12th Week
Weight:…….Kg Height……..M
Body Mass Index:……..Kg/m^2

VITAL SIGNS
Respiratory rate:……per minute
Pulse rate: ……..per minute Temperature:……..(°C)
SYSTEMATIC REVIEW
General (1) poor (2) Fair (3) Good
Pallor (1) Yes (2) No
Lymphadenopathy (1) Yes (2) No
Edema (1)Yes (2) No
URTI (1) Yes (2) No

SIGNS /SYMPTOMS (Check list)

SIGNS/SYMPTOMS	PRESENT	
	YES	NO
Headache	1	2
Skin rashes/infection	1	2
Diarrhoea(1month)	1	2
Loss of weight	1	2
Cough	1	2
Fever (1month)	1	2
Oral thrush	1	2
Herpes zoster	1	2
Kaposi's Sarcoma	1	2
PCP	1	2
Cryptoccocal	1	2
Loss of appetite	1	2

Fatigue	1	2
Pneumonia	1	2
Boils	1	2
TB	1	2
Piles	1	2
Genital lesions	1	2
Others	1	2
Night sweat	1	2
Staging-W.H.O	1 2	3 4

OTHER MEDICATIONS

MEDICATION	YES	NO
ARVs	1	2
Septrin	1	2
Anti –TB drugs	1	2
Other Antibiotics	1	2
Other Nutrients	1	2
Herbs	1	2
Others,Specify	1	2

Number of times treated for malaria in the last month:
1. None
2. 1-2 times
3.3-5 times
4. >5times

24 HOUR FOOD RECALL DATA
Meals taken for breakfast:……
Meals taken for lunch……
Meals taken for supper……..
Meals taken per day: 1. One meal 2. Two meals 3. Three meals 4.Four meals 5. Other, specify……..
Is diet balanced (contains carbohydrates, proteins, fruits and vegetables)?
1. Yes 2. No

In your opinion, are the supplementations beneficial to management of your health?
1. Yes 2. No

OTHER COMMENTS
……
……

Appendix III: Letter of ethical clearance of study

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI, Kenya,
Tel: (254) (020) 2722541, 2713349; 0722-205901, 0733-400003; Fax (254) (020) 2720030,
E-mail: kemri-hq@nairobi.mimcom.net; director@kemri.org Website: www.kemri.org

Forwarded
13/05/04 Dr. Kombe

11th May 2004

KEMRI/RES/7/3/1

Mr. C. F. Mbakaya,
CPHR,
NAIROBI.

Thro'
Director,
CPHR,
NAIROBI.

Dear Sir,

RE: SSC Protocol No. 839 (Revised) – Investigations on the effects of Zinc, Selenium and Vitamin A fortified VIUSID™ on nutritional and immunological status of people living with HIV/AIDS in Western Kenya, by C. F. Mbakaya et al (CPHR)

This is to inform you that during the 111th meeting of the KEMRI/National Ethical Review Committee held on 11th May 2004, the above protocol was tabled and discussed.

It was agreed that the earlier provisional approval which was granted by the Chairman be ratified. You may therefore go on with your study

G. A. O. SEKO,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE