

**MICRONUTRIENTS DEFICIENCIES (ZINC, RETINOL  
AND ALPHA-TOCOPHEROL), MORBIDITY  
PATTERNS AND DIETARY PRACTICES AMONG THE  
HIV POSITIVE AND NEGATIVE SUBJECTS IN BUSIA  
COUNTY, KENYA.**

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**Micronutrients Deficiencies (zinc, retinol and alpha-tocopherol),  
Morbidity Patterns and Dietary Practices among the HIV Positive and  
Negative Subjects in Busia County, Kenya.**

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**A thesis submitted in partial fulfillment for the degree of Master of  
Science in Public Health in the Jomo Kenyatta University of Agriculture  
and Technology**

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**DECLARATION**

This thesis is my original work and has not been presented for a degree in any other University.

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## **DEDICATION**

This thesis is dedicated to my parents Dr. Shem I.D. Khamadi and my late mother, Dorcas Ayuma Khamadi for instilling in me the value of education and hard work. Not forgetting my husband, Nyambu Mwalenga for his immense love and support. May the Almighty God in his abundant grace, bless you.

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## ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immuno-deficiency Syndrome
ARV	Anti-retroviral drugs
CBS	Central Bureau of Statistics
CD	.Compact disk
CPHR	Centre for Public Health Research
CTX	.Co-trimoxazole
GoK	Government of Kenya
EDTA	.Ethylenediaminetetraacetic acid
HAART	.Highly Active Anti-retroviral Therapy
HHH	.House hold head
HIV	Human Immune-deficiency Virus
KAIS	Kenya Aids Indicator Survey
MRI	.Kenya Medical Research Institute
KNASP	Kenya National AIDS Strategic Plan
NACC	National Aids Control Council
NASCOP	National AIDS and STI Control Programme
NGO	Non-governmental Organization

PCP	.Pulmonary Pneumonia
PLWHA	.People living with HIV/AIDS
RDA	. Recommended Daily Allowance
REEP	Rural, Extension and Enterprise Program
SSC	Scientific Steering Committee
ST	Sexually Transmitted Infections
TB	.Tuberculosis
UD	.Undetermined value
UNAIDS	United Nations Programme on HIV/AIDS
URTI	.Upper Respiratory Tract Infections
VAD	Vitamin A Deficiency
VicRes	Lake Victoria Basin Research
WHO	World Health Organization

## DEFINITION OF TERMS

<b>Boils</b>	Red, swollen, painful bumps under the skin. Often look like overgrown pimples.
<b>Cryptococcal meningitis</b>	Inflammation of the meninges (three membranous layers of the connective tissue that envelop the brain and spinal cord) due to infection with the fungal organism <i>Cryptococcus neoformans</i>
<b>Fever</b>	Body temperature above the normal of 98.6 degrees Fahrenheit. (37 degrees Celsius.). In practice a person is usually not considered to have a significant fever until the temperature is above 100.4 degrees Fahrenheit (38 degrees Celsius).
<b>Herpes zoster/Shingles</b>	Extraordinarily painful condition that involves inflammation of sensory nerves. It causes numbness, itching, or pain followed by the appearance of clusters of little blisters in a strip pattern on one side of the body.

**Kaposi Sarcoma**

Highly vascular ("angioblastic") tumor of the skin characterized by soft purplish plaques and papules that form nodules which typically start on the feet and ankles and then slowly spread across the skin of the legs, hands and arms. In AIDS patients, these tumors can also develop internally and cause severe internal bleeding

**Lymphadenopathy**

Painless enlarged lymph nodes >1 cm in two or more non-contiguous sites in the absence of known cause and persisting for three months or more.

**Oedema**

Observable swelling from fluid accumulation in body tissues. The swelling is the result of the accumulation of excess fluid under the skin in the spaces within the tissues.

**Oral thrush**

Yeast infection of the mouth characterized by white patches on the inside.

**Pallor**

Pale condition, especially associated with anaemia.

Unusual or extreme paleness, as from fear or ill health

**Piles**

A varicose condition of the external hemorrhoidal veins causing painful swellings at the anus



**Pneumocystispneumonia  
(PCP)**

Occurs in immunosuppressed individuals and in premature, malnourished infants. The symptoms include dyspnea (difficulty breathing), nonproductive cough, and fever.

**Pneumonia**

Inflammation of one or both lungs, with dense areas of lung inflammation. Symptoms may include fever, chills, cough with sputum production, chest pain, and shortness of breath

**Tuberculosis**

Infectious disease that's transmitted from person to person caused by the bacterium, Mycobacterium tuberculosis. Clinical symptoms and signs of pulmonary TB include fever, nightsweats, cough, hemoptysis (coughing up blood-stained sputum), weight loss, fatigue and chest pain.

**Upper Respiratory**

**Tract Infection**

Infection of any of the components of the upper airway i.e. the sinuses, nasal passages, pharynx, and larynx. Its varying symptoms range from runny nose, sore throat, cough and breathing difficulty.

## ABSTRACT

Use of micronutrients has been recognized as a low-cost sustainable intervention in the management of HIV/AIDS worldwide, yet there is little information on the micronutrient levels among the population in Kenya that can aid in making informed decisions. The main objective of this study was to determine the levels of zinc, retinol and  $\alpha$ -tocopherol among the HIV positive and HIV negative subjects in Busia County, Western Kenya, in a case control study. Blood samples were obtained from a total of 155 consenting study subjects for determination of haematological and biochemical characteristics. A physical medical examination was done to determine the prevalence of morbidity among the respondents and a structured questionnaire used to determine the dietary practices and the behavior risk factors in the stated study population. Data collected was coded and entered into a database using MS-Access and a clean dataset exported into a Statistical Package format (SPSS) where univariate, bivariate and multivariate analysis were done. In the results, the levels of Zinc, Retinol and  $\alpha$ -tocopherol were similar in both the HIV positive and negative subjects with more than 60% of the population manifesting deficiency in the three micronutrients. However, the study subjects deficient in CD4+ cell count were 24.4% and 3.9% in HIV positive and negative subjects, respectively ( $P < 0.001$ ). In terms of morbidity, the disease burden among the two groups was similar. Of the twenty-three signs and symptoms associated with HIV/AIDS that were evaluated, a significant difference was noted in lymphadenopathy (12.8% HIV positive, 3.1% HIV negative,  $P = 0.005$ ), Upper Respiratory Tract Infections (34.6% HIV positive, 19.5% HIV negative,  $P = 0.034$ ) and Skin rash (25.6% HIV positive, 7.8% HIV negative,  $P = 0.003$ ). The dietary practices and food intake patterns were similar among the two groups of respondents where comparison of consumption of the different food groups between the HIV positive and negative participants revealed no significant difference among the two groups. In conclusion, this study implies that even though there were slight differences in the levels of micronutrients (Zinc, Retinol and  $\alpha$ -Tocopherol), the morbidity patterns and the dietary practices among the HIV positive the HIV negative persons, the differences were not significant.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background

The Millennium Development Goal (MDG) number six (6) that addresses HIV and AIDS targets to have halted new infections by 2015 and begun to reverse the spread of HIV/AIDS and by 2010, have achieved universal access to treatment for HIV/AIDS for all those who need it. We are now in 2015, the deadline for the MDGs and the question remains; will we achieve our target of halting and reversing the spread of HIV/AIDS by the end of 2015? Over the years, the gloom and disappointments chronicled in the early editions of the UNAIDS Global report on the AIDS epidemic have given way to more promising tidings, including historic declines in AIDS-related deaths and new HIV infections and the mobilisation of unprecedented financing for HIV-related activities in low- and middle-income countries. AIDS remains an unfinished business, underscoring the need for continued and strengthened international solidarity and determination to address this most serious of contemporary health challenges (UNAIDS, 2013).

The number of people living with HIV in Kenya is increasing due to population increases and a decline in AIDS deaths. However, the number of new infections remains unacceptably high, with an estimated 104,137 Kenyans becoming infected in 2011 (NACC, 2012). Each year, roughly 0.5% of the Kenyan adult population (or 1 out of every 200) is newly infected. In 2011, more than 91,000 Kenyan adults became infected. It is estimated that 12,894 children under age 15 became newly infected with HIV in 2011, with the overwhelming majority contracting the virus during pregnancy or delivery or as a result of breastfeeding. Sexual transmission accounts for an estimated 93% of new HIV infections in Kenya, with heterosexual intercourse representing 77% of incident infections. Adults in stable, seemingly low-risk heterosexual relationships make up the largest share of new HIV infections (NACC, 2012). In addition, several key populations – namely, sex workers and their clients, men who have sex with men, and people who inject drugs – account for roughly one in three new HIV infections, a far larger share than previously understood (Gelmon, 2009). The epidemic varies widely between and

within provinces, with a 15-fold difference in HIV prevalence between the most heavily affected province (Nyanza) and the least affected (North Eastern). (Kenya National Bureau of Statistics, 2010). Nyanza Province alone accounts for one in four HIV-infected people in Kenya. HIV affects Kenyans from all socioeconomic strata. Highest HIV prevalence (7.2%) is among the top wealth quintile, The poorest Kenyans (lowest wealth quintile) are least likely to be living with HIV, with a prevalence of 4.6%. For sub-Saharan Africa generally, educational attainment is inversely correlated with HIV risk for women, at least according to surveys conducted over the last 10–15 years (Hargreaves et al., 2008). In Kenya, this pattern is not so clearly established. Although women with secondary education or higher have lower HIV prevalence (6.9%) than women who completed only primary education (8.9%), lowest HIV prevalence is reported among women with no education (5.8%) (Kenya National Bureau of Statistics, 2010).

The future of HIV in Kenya will in large measure be determined by success in preventing new infections among the millions of young people who will become sexually active in the next few years (Kenya National Bureau of Statistics, 2010). Apart from preventing new infections, there is also need to slow down progression of disease of those who are already infected with HIV, so that they can live a meaningful life. Although management of HIV/AIDS in Kenya has been on activities such as awareness creation, condom distribution, provision of anti-retroviral therapies and more recently on male circumcision, there is increasing evidence that micronutrient deficiencies play an important role in HIV transmission and progression (NACC, 2000). Although anti-retroviral drugs (ARVs) are currently available and at much reduced cost, there are a number of issues of concern. Challenges facing anti-retroviral therapies are availability, compliance, adverse affects to HIV/AIDS patients and drug resistance problems (Tang, 2005). It is necessary therefore to look into interventions that can delay HIV patients from progressing to the level where they require ARVs. However, the role of nutrition is increasingly recognized as evidenced by the development and adoption of various nutrition intervention such as counseling cards, guidelines on care and support and food recommendations for persons using ARV drugs (GoK, 2005; FANTA, 2004) but still more needs to done to incorporate the aspect of micronutrients in the management of HIV and AIDS.

## **1.2 Statement of the Problem**

Emerging evidence recognizes that nutritional management of HIV/AIDS patients is a viable option that should be tested and successful experiences up-scaled to the benefit of humanity. One major setback in Kenya for successful nutritional implementation is the lack of data; data on micronutrient levels in the local population, which can be used to plan for and implement viable, economic and sustainable nutritional interventions in HIV/AIDS management. In addition, the role of nutrition in preventing the occurrence of opportunistic infections needs to be well documented within the Kenyan population. Another gap that needs to be addressed is the establishment of knowledge on how and if the Kenya population consumes food to maximally utilize the nutrients in the food available.

## **1.3 Justification**

Deficiencies of micronutrients such as zinc, retinol (vitamin A) and  $\alpha$ -tocopherol (vitamin E), which are needed by the immune system to fight infections, are common in people living with HIV/AIDS (FANTA, 2004). This study endeavored to find out if these deficiencies truly occur in the HIV positive persons and if the deficiencies extend to the total population in the community including in the HIV negative population, and if so, was there a difference in the two groups. In addition, the study looked at two other factors related to micronutrient deficiencies. These were 1) morbidity patterns, in terms of occurrence of opportunistic infections, and 2) the dietary practices among the HIV positive persons and as a comparison of the same in their HIV negative counterparts. The relationship among the three factors being that whatever one consumes directly determines the level of micronutrients in one's body (Ruel, 2012 ; Torheim et al, 2004), which in turn determines one's level of immunity against occurrence of diseases.

This study was a sub-protocol of a larger study entitled "Assessment of Indigenous Nutritional Food Supplements in the Management of HIV/AIDS Patients in the Lake Victoria Basin" (SSC No. 369), whose study site had already been chosen as Busia County, Butula division. Busia was also the preferred choice of study site since during the time of study; it had the highest prevalence of HIV in Kenya at 33%. The data from this study will be used as a backbone of the larger study in determining how much food supplements will be recommended to boost one's

micronutrient levels to the desired levels during the implementation of the larger study. The data will also be used as reference to other similar studies that will be carried out in other parts of Kenya and beyond.

#### **1.4 Research Questions**

1. Deficiencies of micronutrients (zinc, retinol and  $\alpha$ -tocopherol) occur only in the HIV positive persons?
2. Is the pattern of morbidity similar among the HIV positive and negative study subjects?
3. Do the dietary practices vary among HIV positive and negative study subjects?

#### **1.5 Objectives**

##### **1.5.1 Broad objective**

To determine the occurrence of micronutrients deficiencies (zinc, retinol and  $\alpha$ -tocopherol), morbidity pattern and dietary practices among HIV positive and negative study subjects in Busia County, Western Kenya.

##### **1.5.2 Specific objectives**

1. To determine the occurrence of micronutrient deficiencies (zinc, retinol and tocopherol) among HIV positive and negative subjects in Busia County, Western Kenya.
2. To determine the morbidity pattern among HIV positive and negative study subjects in Busia County, Western Kenya
3. To determine dietary practices among HIV positive and negative study subjects in Busia County, Western Kenya

## CHAPTER TWO

### LITERATURE REVIEW

#### **2.1 Influence of HIV positivity on micronutrients levels in the body**

In the second decade of the human immunodeficiency virus (HIV) epidemic, research related to nutrition in HIV/AIDS, a condition associated with increased oxidative stress, began to focus on micronutrients, largely because of their role as immunomodulators and antioxidants (Allard et al, 1998; Monteiro et al, 2000). Micronutrient deficiencies are common in HIV-infected children and adults, particularly in developing communities where diets are frequently inadequate to meet the recommended daily requirements. They are also more pronounced in individuals with advanced disease, as a consequence of reduced nutrient intake due to AIDS and opportunistic infections, and excessive losses due to diarrhoea, malabsorption, and parasitic infections (Irlam et al, 2007). Observational studies have suggested that both protein energy malnutrition (PEM) and micronutrient deficiencies may hasten the progression of HIV infection, and that HIV worsens malnutrition. HIV infection and malnutrition therefore form a “vicious cycle” of immune dysfunction, infectious disease, and malnutrition (Semba, 1998)

Over 90% of HIV/AIDS cases show symptoms of malnutrition. HIV/AIDS causes a breakdown in the body immune system leading to overwhelming infection that result in diarrhea, vomiting, weight loss and skin inflammation. This results in the body being unable to absorb nutrients, leading to malnutrition in PLWHA. In a study done by Khalili et al (2008), on the nutritional status and serum zinc and selenium levels in Iranian HIV infected individuals, it was found that malnutrition was prevalent in Iranian human immunodeficiency virus infected individuals and low serum zinc and selenium levels were also common in this population. In another study done by Fufa et al (2009), on nutritional and immunological status and their associations among HIV infected adults in Addis Ababa, Ethiopia, a conclusion from the results was that compromised nutritional and micronutrient status begins early in the course of HIV-1 infection. Low serum zinc and vitamin A levels were observed in almost half of the subjects. Serum zinc levels were low ( $<10.7 \mu\text{m/L}$ ) in 53% of subjects, and serum retinol levels were low ( $< 30 \mu\text{g/dL}$ ) in 47% of subjects. Even in patients with HIV/AIDS who are on anti-retroviral therapy, certain

micronutrients seem to be deficient despite patients being on treatment. In a study done by Jones et al (2006) on the micronutrient levels and HIV disease status in HIV-infected patients on highly active antiretroviral therapy in the nutrition for healthy living cohort, it was found that low levels of retinol, alpha-tocopherol, and selenium occurred in HIV-infected subjects on HAART. Zinc deficiency remains common, however. Decreased retinol levels in women and in men with CD4 counts >350 cells/mm and increased zinc and selenium levels in both genders may be associated with improved virologic control.

Micronutrient deficiencies can profoundly affect immunity. Even in asymptomatic patients, micronutrient deficiencies are widely seen in people infected with HIV. In a review by Patrick, L. (2000) on nutrients and HIV, it was evidenced that direct relationships existed between deficiencies of specific nutrients, such as vitamins A and B12, and a decline in CD4 counts. Deficiencies appear to influence vertical transmission (vitamin A) and affect progression to AIDS (vitamin A, B12, zinc). Vitamin and mineral deficiencies play an important role in HIV transmission and progression for a number of reasons. First, HIV-patients are under oxidative stress from the infection and loss of CD4 counts (Schwarz, 1996). Second, a number of micronutrients are required for fighting infection (Grimble, 1997; Semba, 1998; and Nimmagadda, 1998). Third, a number of HIV-associated clinical conditions including mucosal lesions of the mouth, oesophagus, fever and malignancies, decrease appetite as well as diarrhoea and malabsorption conditions increase demand for nutrition (Carbonnel, 1997; Timbo, 1994 and McLaren, 1993). Several observational studies have reported the association between low micronutrient level and faster HIV-disease progression. While low serum or plasma vitamin A level has been described as a risk factor for mortality during HIV-infection independent of clinical stage of disease, high intakes of micronutrients have been associated with reduced progression to AIDS and improved survival (Semba, 1993; Semba, 1995; Tang, 1993 & Tang, 1996). But with the relationship between vitamin-A and progression to AIDS being U-shaped with an intake between 2 to 4 times the recommended dietary intake being associated with reduced progression and improved survival, any intake of zinc results in poorer survival. However, another study has shown that normalization of plasma levels of zinc among 108 homosexual men was associated with an estimated increase in CD4 counts (Baum et al, 1995). A preliminary study on the use of a nutritional preparation with enhanced antioxidant properties



in Kenya showed that HIV/AIDS patients experience reduced clinical signs and symptoms, viral loads and have improved immune status (Mbakaya et al, 2003).

### **2.1.1 Zinc**

Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (Shankar and Prasad, 1998). The central role of zinc in cell division, protein synthesis, and growth is especially important for infants, children, adolescents, and pregnant women. These groups suffer most from an inadequate zinc intake. Studies suggest that a low zinc status in children not only affects growth but is also associated with an increased risk of severe infectious diseases (Black, 1998)

Zinc deficiency is the most prevalent micronutrient abnormality seen in human immunodeficiency virus (HIV) infection. Low levels of plasma zinc predict a 3-fold increase in HIV-related mortality (Baum et al, 2003). In a study by Lai et al (2001) in Miami, Florida, U.S.A, zinc deficiency was also associated independently with an increased risk of mortality of HIV-1 infected homosexuals. The prospective cohort study of 121 HIV-1-positive homosexual men was conducted to evaluate the associations between plasma zinc and copper levels and mortality where plasma zinc and copper levels were measured at baseline and then at semiannual visits. Over the average course of the 3.3-year follow-up, 19 participants (16%) died of HIV-1-related causes. After adjustment for potential confounders, including low CD4+ cell counts and antiretroviral therapy, zinc inadequacy and copper:zinc ratio  $>1$  (i.e., plasma copper level greater than plasma zinc level) were associated with increased mortality (relative risks [RRs]; 95% confidence intervals [CIs], 4.98, 1.30-19.00 and 8.28, 1.03-66.58, respectively). A negative association was also observed between plasma zinc levels and mortality (RR 0.94; 95% CI, 0.91-0.98). Plasma levels of copper were not significantly associated with mortality. These results indicated that plasma zinc inadequacy or the plasma copper:zinc ratio were useful predictors of survival in HIV-1 infection where zinc appeared to be a stronger predictor. Zinc is lost during diarrheal diseases, and zinc deficiency induces intestinal morphology-altering inflammatory responses that zinc supplementation can correct (Rahman, et al, 2005). According to WHO statistics, a person is said to be zinc deficient when their plasma levels fall below 100 $\mu$ g/l

(WHO/FAO, 2004). The clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes (Hambridge et al, 1987).

Lean red meat, whole-grain cereals, pulses, and legumes provide the highest concentrations of zinc. Concentrations in such foods are generally in the range of 25–50mg/kg (380–760mmol/kg) raw weight. Processed cereals with low extraction rates, polished rice, and chicken, pork or meat with high fat content have moderate zinc content, typically between 10 and 25mg/kg (150–380 mmol/kg). Fish, roots and tubers, green leafy vegetables, and fruits are only modest sources of zinc, having concentrations <10mg/kg (<150mmol/kg) (Sandström B, 1989).

Zinc has been used with impressive success as an immunostimulatory agent in malnutrition, obesity, sickle cell anemia, and in children, adolescents, and elderly persons (Chandra & Kutty, 1990; Chiricolo et al, 1993; Golden & Golden, 1981; Licastro et al, 1992; Prasad & Cossack, 1984). Zinc supplementation has been demonstrated to increase the efficiency of the immune system in a number of studies, including those of infants with marasmus (Castillo-Duran et al, 1987), children recovering from malnutrition (Golden and Golden, 1981), patients with sickle cell anemia (Prasad and Cossack, 1984), and children with Down syndrome (Chiricolo et al, 1993; Licastro et al, 2001). Additionally, an improvement in immunological responses has been demonstrated with zinc treatment in obese children and adolescents (Chandra and Kutty, 1990). Zinc supplementation at a nutritional dose (20 mg/day) in elderly subjects produced restoration, at least partially, of nutritional and thymic status, with no adverse effects (Boukaiba et al, 1993). Zinc supplementation delayed HIV-1 disease progression and decreased the rate of opportunistic infections in a cohort composed mainly (70%) of illicit drug users, as well as a cohort of HIV-1–infected subjects who were receiving HAART (Mocchegiani et al, 1999; Mocchegiani et al, 1995). Furthermore, normalization of plasma zinc levels in HIV-1–infected patients who were zinc deficient has been associated with a significantly slower disease progression (Baum et al, 1995). Zinc supplementation, in amounts above the recommended daily allowance (RDA) level, has been linked with faster disease progression and increased mortality (Tang et al, 1993; Tang et al, 1996). Zinc supplementation above RDA levels may interfere with copper and iron

utilization and affect high-density lipoprotein cholesterol concentrations and monocyte function (Fosmire, 1990; Schlesinger et al, 1993). Zinc supplementation significantly reduces the risk of CD4 cell counts falling below the critical 200 cells/mm<sup>3</sup> level. Nutritional levels of zinc supplementation given to HIV-infected adults results in a 4-fold decrease in the likelihood of immunological failure (Baum, 2010). Normalization has been associated with significantly slower disease progression and a decrease in the rate of opportunistic infections. (Baum et al, 2003).

In a study conducted in Kenya (Mbakaya et al, 2005) where HIV/AIDS subjects were supplemented with 1 x RDA and 7 x RDA of the nutrients, zinc, selenium and vitamins A, E & C for twelve weeks, the arm with 7 x RDA micronutrients registered notable reductions in the prevalence of key signs and symptoms/illnesses associated with HIV/AIDS such as headache (P = 0.029); weight loss (P = 0.002); fatigue (P = 0.052); skin rash (P = 0.007) and pneumonia (P = 0.003); with a reduction of 0.3342 log<sub>10</sub> viral load copies/ml of blood (P = 0.337) and a CD4 cell count of 111 x 10<sup>6</sup> cells/L (P = 0.018). These results are supported by observation from a study on the safety and efficacy of zinc supplementation for children with HIV-1 infection in South Africa though the results were not as significant as those observed in the Kenyan study (Bobat et. al., 2005; Mbakaya et al, 2005). The amount of zinc supplementation in HIV infection appears to be critical, because deficiency, as well as excessive dietary intake of zinc, has been linked with declining CD4 cell counts and reduced survival. (Baum et al, 2003). Supplementation of zinc for 1-2 months restores immune responses, reduces the incidence of infections and prolongs survival. However, in every single individual, zinc supplementation of food has to be adjusted to the particular zinc status in view of the great variability in habitat conditions, health status and dietary requirements. (Ferencik et al, 2003). In vitro treatment with zinc of peripheral blood mononuclear cells leads to an enhancement of lymphoproliferative immune response and apoptosis' inhibition (Neves et al, 1998). In a similar study done by Mocchegiani (1995), on zinc supplementation as an adjunct to zidovudine (AZT) therapy, it was found that zinc sulphate supplementation of stage III and in stage IV C1 patients was followed by an increase or a stabilization in the body weight and an increase of the number of CD4+ cells and the plasma level of active zinc-bound thymulin. The frequency of opportunistic infectious episodes in the 24 months following entry into the study was reduced after zinc supplementation in stage IV C1

subjects (11 infections vs. 25 in controls) and delayed in stage III zinc-treated subjects (1 infection/24 months vs. 13 infections/24 months in controls). The effect of zinc on opportunistic infections was restricted to infections due to *Pneumocystis carinii* and *Candida*, whereas no variations were observed in the frequencies of cytomegalovirus and toxoplasma infections.

### **2.1.2 Retinol (Vitamin A)**

Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system, growth and development, maintenance of epithelial cellular integrity, immune function and reproduction. Vitamin A functions at two levels in the body: the first is in the visual cycle in the retina of the eye; the second is in all body tissues where it systemically maintains the growth and integrity of cells (WHO/FAO, 2004). The World Health Organization defines Vitamin A Deficiency as tissue concentrations of vitamin A low enough to have adverse health consequences even if there is no evidence of clinical xerophthalmia. In blood, tissues, and human milk, vitamin A levels are conventionally expressed in mg/dl or mmol/l of all-trans-retinol. Blood levels between 0.35 and 0.70mmol/l are likely to characterize subclinical deficiency (Wachtmeister, Diczfalusy, & Emami, 1988) but subclinical deficiency may still be present at levels between 0.70 and 1.05mmol/l and occasionally above 1.05mmol/l (Flores H et al, 1984). The prevalence of values below 0.70mmol/l is a generally accepted population cutoff for preschool-age children to indicate risk of inadequate vitamin A status (World Health Organization, 1996) and above 1.05mmol/l to indicate an adequate status. Vitamin A Deficiency can occur in individuals of any age. However, it is a disabling and potentially fatal public health problem for children under 5 years of age. VAD related blindness is most prevalent in children less than 3 years of age. In addition, adequate intake of vitamin A reduces the risk of catching respiratory and gastrointestinal infections (WHO/FAO, 2004).

In a review article by Mehta, Giovannucci, Mugusi, Spiegelman, Aboud, Hertzmark, E., ... & Fawzi. (2010). it was established that the role of vitamin A in HIV infection has received prominent attention. This is because of its well-known role in affecting child morbidity and mortality, as well as early observations that vitamin A status was associated with increased risk of mother-to-child-transmission (MTCT) of HIV (Semba et al, 1994), progression to AIDS

(Nduati et al, 1995; John et al, 1997; Tang et al, 1993), adult survival (Coodley et al, 1993; Semba et al, 1994; Nduati et al, 1995), infant morbidity (Coutsoudis et al, 1995) and mortality (Dushimimana et al, 1992). Vitamin A has been recognized as a likely potential co-factor (micronutrient) in HIV progression and disease expression (Beach et al, 1989). HIV positive persons may be at particular risk for vitamin A deficiency for some reasons. These include chronic and recurrent infections, chronic inflammatory conditions, poor intake, diarrhoea with or without malabsorption (Beach et al, 1989; Chaisson & Volberding, 1990). Vitamin A has remarkable positive effects including established roles in haematopoiesis, the maintenance of epithelial integrity and optimal function of the immune system. In developing countries, up to 60% of HIV-infected women are hyporetinaemic. In such women, the relative risk of peri-natal transmission may be increased more than fourfold (Nimmagadda et al, 1998). According to Watson (Watson, 1994) vitamin A deficiency is associated with decreased CD4 T-cell production, depressed cellular immunity and reduced humoral response to protein antigens.

The effects of vitamin A on the vagina mucosa raise the possibility that changes in vitamin A status may influence heterosexual and peri-natal transmission of HIV (Mejia et al, 1977; Gherardi et al, 1991; Watson, 1994). Retinoids have been found to influence a wide range of human immune cell functions in vitro. These include proliferation of thymocytes, B-cells, T-cells and the production of a number of cytokines (Goldfarb & Herberman, 1982; Soppi et al, 1982; Matikainen et al, 1990). Vitamin A-deficient children respond poorly to tetanus toxoid, are found to have low CD4<sup>+</sup>/CD8<sup>+</sup> ratios and suffer increased infection-related mortality. Vitamin A repletion is associated with increased CD4<sup>+</sup> counts and CD4<sup>+</sup>/CD8<sup>+</sup> ratios as well as improved antibody production (Semba et al, 1994; Matikainen et al, 1990). Increased infant mortality has also been observed in children born to HIV positive mothers with vitamin A deficiency (Dushimimana et al, 1992). These findings suggest strongly that vitamin A status is an important co-factor in HIV progression.

Most observational studies have found that low vitamin A levels are associated with increased risk of transmission of HIV from mother to child (Semba, 1993). This finding has not been supported by large randomized trials of vitamin A supplementation. On the contrary, some trials have found that vitamin A supplementation increases the risk of mother-to-child transmission

(MTCT), (Mehta & Fawzi, 2007). There are a number of potential mechanisms that might explain these contradictory findings. One is the issue of reverse causality in observational studies—for instance, advanced HIV disease may suppress release of vitamin A from the liver. This would lead to low levels of vitamin A in the plasma despite the body having enough vitamin A liver stores. Further, advanced HIV disease is likely to increase the risk of MTCT, and hence it would appear that low serum vitamin A levels are associated with increased MTCT. The HIV genome also has a retinoic acid receptor element—hence; vitamin A may increase HIV replication via interacting with this element, thus increasing risk of MTCT. Finally, vitamin A is known to increase lymphoid cell differentiation, which leads to an increase in CCR5 receptors. These receptors are essential for attachment of HIV to the lymphocytes and therefore, an increase in their number is likely to increase HIV replication (Mehta & Fawzi, 2007)

Vitamin A may be ingested either as Preformed Vitamin A or Provitamin A carotenoids. Preformed vitamin A is found almost exclusively in animal products, such as human milk, glandular meats, liver and fish liver oils (especially), egg yolk, whole milk, and other dairy products. Preformed vitamin A is also used to fortify processed foods, which may include sugar, cereals, condiments, fats, and oils (Rodriguez-Amaya, 1997). Provitamin A carotenoids are found in green leafy vegetables (e.g. spinach, amaranth, and young leaves from various sources), yellow vegetables (e.g. pumpkins, squash, and carrots), and yellow and orange non-citrus fruits (e.g. mangoes, apricots, and papayas). Red palm oil produced in several countries worldwide is especially rich in provitamin A carotenoids.

Vitamin A supplementation in HIV-infected children, on the other hand, has been associated with protective effects against mortality and morbidity, similar to that seen in HIV-negative children. The risk for lower respiratory tract infection and severe watery diarrhea has been shown to be lower in HIV-infected children supplemented with vitamin A. All-cause mortality and AIDS-related deaths have also been found to be lower in vitamin A-supplemented HIV-infected children (Mehta & Fawzi, 2007). In a study of 288 HIV-positive homosexual men in Baltimore, dietary and supplemental intake of vitamin A (9000-20000 IU/day was associated with slower progression to AIDS during a seven-year follow-up (Mejia et al, 1977; Watson, 1994). Kennedy (Kennedy-Oji et al, 2001) examined the effects of vitamin A supplementation

during pregnancy and early lactation on maternal weight among HIV-1 sero-positive South African women. The author observed a benefit on maintenance of post-natal weight in vitamin A deficient women. It was concluded that in a population for whom anti-retroviral therapy is not readily available or accessible, the finding that vitamin A improve post-partum weight lends some hope to a relatively inexpensive treatment. Vitamin A could thus be used for helping to ameliorate some weight loss that is common during HIV infection.

According to Nimmagadda (Nimmagadda et al, 1998), vitamin A supplementation may be especially useful in adjunctive therapy for HIV-infected pregnant women who live in the developing world. Vitamin A supplementation of women during pregnancy improved vitamin A status of mothers and of their infants (Muslimatun et al, 2001). It has also been demonstrated from recent studies that vitamin A supplementation during pregnancy enhanced the concentration of retinal levels in breast milk (Muslimatun et al, 2001). Coutsoudis et al (1995) carried out a double-blind randomized controlled trial of vitamin A supplementation on children of HIV-positive mothers in Durban, South Africa. It was demonstrated from the study that 28% of the supplemented group had reduction in the incidence of prolonged diarrhoea and 77% had reductions in hospital admissions for diarrhoea. Benefits from vitamin A supplementation include not only improved health and welfare for individuals and family, but also improved chances of prolonged survival for HIV infected persons (Kennedy-Oji et al, 2001; Muslimatun et al, 2001). In Tanzania, vitamin A supplementation of HIV-infected children reduced all-cause mortality by 63% among HIV-infected children aged six months to five years and was associated with a 68% reduction in AIDS-related deaths and a 92% reduction in diarrhoea-related deaths (Semba et al, 1993).

### **2.1.3 $\alpha$ – Tocopherol (Vitamin E)**

A vitamin is an organic compound required as a nutrient in tiny amounts by organisms (Lieberman et al, 1990). The term vitamin E describes a family of eight antioxidants: four Tocopherol (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

(Traber, 1999).  $\alpha$ -Tocopherol is the form of vitamin E that appears to have the greatest nutritional significance. Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defence system and is exclusively obtained from the diet. The major biological role of vitamin E is to protect polyunsaturated fatty acids (PUFAs) and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals (WHO/FAO, 2004). Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation — a series of chemical reactions involving the oxidative deterioration of PUFAs.. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions (Duthie, 1993).

There are many signs of vitamin E deficiency in animals, most of which are related to damage of cell membranes and leakage of cell contents to external fluids. Disorders provoked by traces of peroxidized PUFAs in the diets of animals with low vitamin E status include cardiac or skeletal myopathies, neuropathies, and liver necrosis (McLaren et al, 1993). Muscle and neurological problems are also a consequence of human vitamin E deficiency (Sokol, 1988). Early diagnostic signs of deficiency include leakage of muscle enzymes such as creatine kinase and pyruvate kinase into plasma, increased levels of lipid peroxidation products in plasma, and increased erythrocyte haemolysis. Vitamin E is naturally present in plant-based diets and animal products. Animal fats, vegetables, and meats each contribute about 10% to the total per capita supply and fruit, nuts, cereals, and dairy products each contribute about 4%. Eggs, fish, and pulses contribute less than 2% each. Vitamin E is an important antioxidant and anti-inflammatory that is often deficient in HIV-positive people. Vitamin E is necessary to ensure the optimum functioning of cell membranes.

Deficiency may interfere with efficient immune functions. A study of 310 men followed for nine years found that those with serum vitamin E levels above 23.5 $\mu$ m/l had a significantly reduced risk of disease progression. A strong correlation was noted in this cohort between intake of supplements containing vitamin E on entry into the study and high blood levels of vitamin E (Tang et al, 1997). Vitamin E plays a complex role in maintaining immune function. Severe vitamin E deficiency early in life has been associated with stunted cognitive development (Pollitt, 2000). Low levels of this micronutrient have been found in HIV-1-infected individuals



with both asymptomatic and advanced disease (Beach et al, 1992; Baum et al, 1997). A study among HIV-1-seropositive drug abusers by Baum et al (1997), reported that nutritional deficiencies, particularly vitamin E, were widespread with up to 86% of the drug users. Although immune parameters (CD4 count, CD8 count, beta2-microglobulin) were similar in the HIV-1-infected men and women, women had significantly poorer overall nutritional status, as measured by plasma proteins, which are considered to be sensitive markers of malnutrition. A comparison of individuals with advanced disease (CD4 count <200/mm<sup>3</sup>) revealed significantly greater deficiency of vitamin E ( $p < .05$ ) in women than in men.

Another study on specific nutrient abnormalities in asymptomatic HIV-1 infection by Beach et al (1992) revealed that prevalence of specific nutrient abnormalities was widespread among HIV-1-infected subjects with marginally low blood levels of vitamin E up to 27% being documented in HIV-1-seropositive subjects which was not observed among HIV-1-seronegative. In vitro, vitamin E has been reported to inhibit HIV-1 transcription and to suppress the activation of virus in latently infected cells (Hirano et al, 1998; Heredia et al, 2005). Generally, a protective effect in HIV-1 infected persons has been assumed (Semba & Tang, 1999). However, recent evidence suggests that vitamin E supplementation may increase CCR5 expression, which in turn could increase HIV-1 replication (Portales et al, 2004). In a recent study on whether pre-infection vitamin E levels are associated with higher mortality in HIV-1-infected Kenyan women by Graham et al (2007), results showed that after controlling for potential confounding factors, each 1 mg/L increase in pre-infection vitamin E was associated with 0.08 log<sub>10</sub> copies/mL (95% CI -0.01 to +0.17) higher set point viral load and 1.58-fold higher risk of mortality (95% CI 1.15–2.16). The association between higher pre-infection vitamin E and mortality persisted after adjustment for set point viral load (HR 1.55, 95% CI 1.13–2.13). This led to the conclusion that higher pre-infection vitamin E levels were associated with increased mortality (Graham et al, 2007).

In prospective studies of HIV-1-infected people, higher vitamin E levels and higher intake have been associated with a lower risk of progression to AIDS (Tang et al, 1997; Abrams et al, 1993). In a study by Tang et al (1997), on the association between serum vitamin A and E levels and HIV-1 disease progression, 311 HIV-seroprevalent homo-/bisexual men participated over a

subsequent period of nine years. From the results, men in the highest quartile of serum vitamin E levels ( $\geq 23.5$   $\mu\text{mol/l}$ ) showed a 34% decrease in risk of progression to AIDS compared with those in the lowest quartile [relative hazard (RH), 0.66; 95% confidence interval (CI), 0.41-1.06]. Concurrently, men who reported current use of multivitamin containing vitamin E or single **vitamin E** supplements had significantly higher serum tocopherol levels than those who were not taking supplements ( $P = 0.0001$ ). This led to the conclusion that high serum levels of vitamin E may be associated with slower HIV-1 disease progression.

In addition, vitamin E levels can decrease as HIV-1 infection progresses, and deficiencies found late in disease may result from advanced illness (Pacht et al, 1997). In a study by Pacht et al (1997), it was found out that serum vitamin E decreases in HIV-seropositive subjects over time. In the study, serum levels of vitamin E in 121 HIV seropositive subjects with no prior pulmonary complications were measured. Although the mean level was normal at  $9.0 \pm 0.5$   $\mu\text{g/ml}$ , 22.3% of the subjects had a deficient level of less than 5  $\mu\text{g/ml}$ . In addition, 42 subjects were studied longitudinally and serum vitamin E levels were determined at baseline and 12 months later. The mean serum vitamin E level in this group significantly decreased after 12 months compared with baseline levels ( $5.9 \pm 0.5$   $\mu\text{g/ml}$  compared with  $9.6 \pm 0.9$   $\mu\text{g/ml}$ ,  $p = 0.001$ ).

## **2.2 Morbidity patterns among the HIV positive**

Opportunistic infections are an important cause of morbidity and mortality in persons infected with HIV (Dankner et al, 2001). In 1990, after HIV/AIDS became a global concern, WHO developed a staging system for HIV Infection and Disease in Adults and Adolescents (WHO, 1990). This was to harmonize the identification of cases worldwide and in particularly in resource-poor countries and communities, where sometimes medical facilities and testing was unavailable, and it was not possible to decide the appropriate time to begin treatment on the basis of test results. The World Health Organization developed a disease staging system for HIV infection which is not dependent on testing.

WHO disease system for HIV Infection and Disease in Adults and Adolescents

According to WHO (1990).

Clinical Stage I:

Asymptomatic

Generalized lymphadenopathy

Performance scale 1: asymptomatic, normal activity

Clinical Stage II:

Weight loss, < 10% of body weight

Minor mucocutaneous manifestations (seborrheic dermatitis, prurigo, fungal nail infections, recurrent oral ulcerations, angular cheilitis)

Herpes zoster within the last five years

Recurrent upper respiratory tract infections (i.e. bacterial sinusitis)

And/or performance scale 2: symptomatic, normal activity

Clinical Stage III:

Weight loss, > 10% of body weight

Unexplained chronic diarrhoea > 1 month

Unexplained prolonged fever (intermittent or constant), > 1 month

Oral candidiasis (thrush)

Oral hairy leucoplakia

Pulmonary tuberculosis

Severe bacterial infections (i.e. pneumonia, pyomyositis)

And/or performance scale 3: bedridden < 50% of the day during last month

Clinical Stage IV:

HIV wasting syndrome<sup>i</sup>

Pneumocystis carinii pneumonia

Toxoplasmosis of the brain

Cryptosporidiosis with diarrhoea > 1 month

Cryptococcosis, extrapulmonary

Cytomegalovirus disease of an organ other than liver, spleen or lymph node (e.g. retinitis)

Herpes simplex virus infection, mucocutaneous (>1 month) or visceral

Progressive multifocal leucoencephalopathy

Any disseminated endemic mycosis

Candidiasis of esophagus, trachea, bronchi

Atypical mycobacteriosis, disseminated or lungs

Non-typhoid Salmonella septicemia

Extrapulmonary tuberculosis

Lymphoma

Kaposi's sarcoma

HIV encephalopathy<sup>ii</sup>

And/or performance scale 4: bedridden > 50% of the day during last month

**Footnotes:**

HIV wasting syndrome: weight loss of > 10% of body weight, plus either unexplained chronic diarrhoea (> 1 month) or chronic weakness and unexplained prolonged fever (> 1 month).

HIV encephalopathy: clinical findings of disabling cognitive and/or motor dysfunction interfering with activities of daily living, progressing over weeks to months, in the absence of a concurrent illness or condition other than HIV infection which could explain the findings

In a descriptive study on the clinical feature of HIV/AIDS at presentation at the Jos University Teaching Hospital by Akolo et al (2008), two hundred (200) newly diagnosed and laboratory confirmed adult cases of HIV infection without prior antiretroviral drug use were recruited and a comprehensive history taken with emphasis on the clinical symptoms and detailed physical examination performed. Of these, those with the major symptoms in the study population were: weight loss (65.5%), fever (41.5%), chronic cough (38.5%), diarrhea (32.0%), pruritus (13.0%) and body rash (12.5%). The major signs were pallor (25.0%), oral thrush (20.5%), wasting (20.0%), lymphadenopathy (18.0%), dermatitis (16.0%), hyperpigmented nails (13.5%) and finger clubbing (8.5%). It was thus concluded that the symptoms and signs of HIV/AIDS obtained were similar to those obtained by other workers from different parts of the world; however, the findings of hyperpigmented nails and finger clubbing have not been frequently reported for other populations.

In another study in South Nigeria by Gyuse et al (2010), whose objective was to determine the causes of death among human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) patients as a step to planning strategies to improve mortality, data were obtained from sexually transmitted infection/acquired immunodeficiency syndrome (STI/AIDS) clinic register, admissions and discharge/death registers as well as the patients' case records and the hospitals monthly mortality reviews. From the results, it was established that the total number of mortalities during the study period was 350,100 were HIV positive representing 28.6% of all deaths. While advanced HIV/AIDS disease was the leading cause of death in our study representing 27.0%, tuberculosis was the single leading cause of deaths in HIV/AIDS patients constituting about 24.0% of deaths. This was followed by sepsis and septicaemia (13.0%),

meningitis and encephalitis, and anaemia accounting for 11.0%, while respiratory diseases constituted 5.0% of the mortality burden. The highest number of deaths occurred in those aged between 21–50 years (82.0%).

### **2.3. Dietary practices among the HIV positive**

Poverty and other socio-demographic disadvantages can limit adequate feeding practices (Mullie et al, 2009, Aggarwal et al, 2011). Households in low economic strata are prone to food insecurity. Under such circumstance, poor households have limited choices for food with adequate nutritional values (Ivers & Cullen, 2011, Saaka and Shaibu, 2013). They usually adapt themselves to this situation by cutting down the number of basic meals or reducing the amount in each meal (Keenan et al, 2001, Anema et al, 2009). In a typical household affected by food insecurity, children are usually less afflicted during its early stage. At this stage, other members of the household tend to reduce amount and frequency of food for themselves for the sake of children. However, children are more likely to be affected by extreme forms of food insecurity and later, hunger. Feeding frequency might also be affected in a similar way. In a study by Sunguya et al (2014), an independent association was detected between feeding frequency among households affected by food insecurity and poverty. Results from focus group discussions among caregivers of HIV-positive children also showed that households with food insecurity were more prone to low feeding frequency of their children. Because of the lack of food, children ate twice a day on most occasions. Similar results were observed in Ethiopia among adolescents with unknown serostatus (Belachew et al, 2013) and HIV-positive adults (Tiyou et al, 2012). However, these areas have been known to have long periods of hunger and food insecurity, characteristics that differ from our study.

Socio-economic disadvantages and the cycle of poverty are also associated with poor education. Children of poorly educated caregivers succumb to various forms of undernutrition (Saloojee et al, 2007). The possible link could be through poor feeding practices (Mpontshane et al, 2008). Through high agricultural yield or high purchasing power, food availability can reduce a household's food insecurity (FAO, 2010). However, even when sufficient food is available, its consumption may not be adequate. This is because the consumption of diverse types of foods in

adequate quality, quantity, and frequency may depend on nutrition knowledge of caregivers (Sunguya et al, 2013, Choi et al, 2008). This may also be a reason behind the high rates of undernutrition among children in the general populations of many developing countries, including Tanzania, even where food productivity is high (ICF Macro, NBS 2011, NBS, ORC-Macro 2010). In the study by Sunguya et al (2014), feeding practices, and in particular feeding frequency, were also associated with the caregiver's education level. In this case, even when food is available at the household level and other factors are controlled for; caregivers with low education levels may not provide foods to their children at the recommended frequency. More than one fifth of the caregivers of HIV-positive children in this population were not educated. In the qualitative study, too, caregivers had little knowledge of nutrition and how to feed their children. There were many misconceptions based on local beliefs and traditions that also led to wrong choices of foods. The level of education may determine nutrition knowledge (Muti et al, 2011). When caregivers are given such knowledge, they improve feeding practices, including feeding frequency and dietary diversity, of their children (Sunguya et al, 2013, Tiyou et al, 2012, Palwala et al, 2009, Muti et al, 2012).

In developing countries, if children are affected by HIV/AIDS in a household, such household is more likely to have lower potential to provide them with food of adequate quality, quantity, and in the required frequency (Anema et al, 2009; Saloojee et al, 2007; Mpontshane et al, 2008; Weiser et al, 2011). Furthermore, HIV-positive children have special nutritional needs, different from their HIV-negative counterparts (WHO, 2009). The World Health Organization (WHO) recommends a 10% increase in energy intake for the HIV-positive child growing well on antiretroviral therapy (ART) above the normal requirement of an otherwise HIV-negative child of the same age (WHO, 2009). A 20–30% increase in energy intake is required to sustain an HIV-positive child with HIV-related symptoms including TB, chronic lung infections, or persistent diarrhea. HIV-positive children need an extra 50–100% of energy intake compared to an otherwise normal child if they have severe malnutrition or severe failure to thrive, regardless of their ART status (WHO, 2009). These energy requirements are supposed to be met from the foods consumed daily. To achieve adequate nutrition, an HIV-positive child is supposed to eat at least five times a day (WHO, 2009). Such meals have to be balanced, diverse, and adequate in amount. Poor feeding practices were associated with a burden of other diseases. For example,

low feeding frequency was associated with the chance of having had acute respiratory tract infections at the same time. Such acute diseases are especially common among children with HIV/AIDS and under nutrition (Berkley et al, 2009). Results from the qualitative study by Sunguya et al (2014) may help to explain this phenomenon. Because of low immunity, HIV-positive children who are also affected by under nutrition are prone to other infections including upper respiratory tract infections. Children with these conditions improved upon proper feeding in adequate amount and frequency.

Similar findings from a study in Kibera, Kenya by Chege et al (2010), established that dietary practices of pre-school children in Kibera were poor as was shown by consumption of three or less meals per day by over 55% of the pre-school children. This was short of the five to six meals recommended per day (FANTA, 2004) and was attributed to lack of snacks between the major meals. There was no significant difference between the dietary practices (number of meals per day, amount of kilocalories and other nutrients consumed per day and frequency of consumption of foods) for the HIV/AIDS infected and non-infected pre-school children. The nutrition status of both the HIV/AIDS infected and non-infected pre-school children was poor (Chege et al, 2010) This was due to inadequate food consumption as a result of the poor economic status. There was a significant difference between the nutrition status for the HIV/AIDS infected and non-infected pre-school children. The HIV/AIDS infected children had a poorer nutrition status due to the fact that the HIV/AIDS infected children suffered from more illness, which persisted for a longer time thus aggravating the condition than for the non-infected pre-school children. Diseases increases nutrients needs while compromising nutrient intake and at the same time they lead to nutrient losses through diarrhea and vomiting. There was a significant difference between the number of sick children among the HIV/AIDS infected and non-infected pre-school children. This could have been due to reduced immunity in the body of the infected group as a result of HIV/AIDS. Cough persisted for an average of 5 days for the HIV/AIDS infected and 2 days for the non-infected pre-school children. For those who had malaria, it persisted for an average of 7 days for the HIV/AIDS infected and 4 days for the non-infected pre-school children. Cold persisted for an average of 6 days for the HIV/AIDS infected and 5 days for the non-infected pre-school children while diarrhea persisted for an average of 4 days for the HIV/AIDS infected and 2 days for the non-infected pre-school children. The various diseases persisted for 2-7 days



and generally longer for the HIV/AIDS infected group (average of 7 days). These illnesses manifested themselves as fever, loss of appetite, diarrhea and vomiting. Except for diarrhea, these symptoms were more among the HIV/AIDS infected than for the non-infected pre-school children. Diarrhea was common for all the children due to the poor hygienic and sanitary conditions in the slum (Chege et al 2010).

## CHAPTER THREE

### METHODS AND MATERIALS

#### 3.1 Study design

This was a case control study design where comparison was made among HIV positive and negative study subjects to determine differences between selected micronutrients (Zinc, Retinol and alpha-tocopherol), disease occurrence and dietary practices in Busia County, Kenya.

#### 3.2 Study Site

The study was carried out in Butula division, in the then Busia District (which is currently Busia County) in Western Province of Kenya, from August to October, 2009. Busia County borders Kakamega County to the east, Bungoma County to the north, Busia District, Uganda to the west and Lake Victoria to the south. The county had a total population of about 400,000 (Kenya National Bureau of Statistics, 2002). It had four constituencies: Nambale, Butula, Funyula and Budalangi. Butula constituency, whose headquarters was Butula, had a population of about 100,000, which was the highest of the four constituencies with an urban population of about 5,000 (Kenya National Bureau of Statistics, 2002). Busia County had a HIV prevalence of 33% (NACC, 2009). The prevalence in the urban centers was about 30 percent while in the rural areas was about 16 percent. The Kenya Aids Indicator Survey results also showed, among other issues, that 23% of primary students (10-14 year old) had had sexual intercourse. Risk perception was high although only 9% used condoms. The main economic activity in Busia County was trade with neighbouring Uganda, with Busia town - the county headquarters - being a cross-border centre. Away from town, the district economy was heavily reliant on fishing and agriculture, with cassava, millet, sweet potatoes, beans and maize being the principal cash crops (Kenya National Bureau of Statistics, 2002). Busia county represented a microcosm in its own right but also featured significantly in the pattern of spread of the disease in Kenya. Busia town is the most westerly point of the Trans - Africa High-way in Kenya. The Highway begins at Mombasa seaport and cut its way inland to serve six land-locked countries. Everywhere the Highway passes, HIV prevalence has been reported to be high and the same patterns appeared to hold

along other significant road net-works. A thriving commercial sex business made the people of Busia County vulnerable, especially to HIV/AIDS. The town is often the last stop for truck drivers who sometimes arrive there after weeks on the road. The fishermen from nearby Lake Victoria also contributed to the prostitutes' trade. Many of the commercial sex workers infected men who lived in Busia town, who in turn infected their female partners. This was coupled by the inherent practice of wife inheritance. Recent statistics showed that there seems to be a grave in nearly every yard in Busia county and in almost every house in the Butula Division there was someone living with AIDS virus (Kenya National Bureau of Statistics, 2002)

### **3.3 Study population**

#### **3.3.1 Definition of cases**

- Men and women who were above 18 years and below 60 years
- (Respondents above 60 years were excluded so as to minimize the occurrence of diseases that tend to occur due to old age among the participants.)
- Men and women who know their HIV status as either HIV positive or HIV negative
- (This was confirmed with a HIV test in the laboratory)
- Subjects who were not using anti-retrovirals (ARVs)
- Those who signed a written informed consent (Appendices I and II)

#### **3.3.2 Definition of controls**

- Men and women who were below 18 years and above 60 years
- Those who did not know their HIV status and did not agree to be tested
- Those who were using anti-retrovirals (ARVs)
- Those who did not agree to sign a written informed consent

### **3.4 Sample size and sampling**

Selection of the respondents from the eligible population was done by simple purposive sampling where recruitment of cases and controls was done in three (3) selected health centers in

Butula division. These three health facilities namely, Bumala A Health Centre, Bumala B Health Centre and Bukhalarire Health Centre, were selected from a total of seventeen (17) health facilities in Butula division on the basis that they were the only health facilities offering support services to the HIV positive persons at the time of study within the division. Sample size for the cases (HIV positive subjects) was derived using the formula shown below; (Lemeshow et al, 1990)

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

Where,

$$s \text{ to } n = 21\sigma^2 / d^2$$

Where

$$\sigma^2 = \text{variance and}$$

$$d = \text{difference to be detected} = \mu_1 - \mu_2 = 10 \mu\text{g/dL serum and } \sigma^2 = 18^2.$$

Therefore,

$$n = 68$$

Thus, 68 PLWHA were recruited and since it was a case control study, an equal number of HIV negative respondents were recruited as the control group. The study subjects in the control group were matched to the cases according to their age-groups at the beginning of the fieldwork – data collection. This brought the total number of respondents to 136 study subjects. Allowing for 12% non response, the sample size was adjusted upwards to 77 per arm, giving a total sample size of 154 study subjects. During data collection, one extra HIV positive respondent was interviewed and the researcher found it best not to waste such valuable information. This addition however did not seem to have a significant effect on the data analysis. Thus, a total sample of 155

participants was recruited for the study, 78 study subjects in the HIV positive arm and 77 study subjects in the HIV negative arm.

All the study subjects were recruited as they visited the health centers for treatment or for other healthcare services. The cases (the HIV positive) were recruited as they came and as long as they met the selection criteria and were willing to take part in the study. As for the controls (the HIV negative), systematic sampling was applied where every other 3<sup>rd</sup> person visiting the centers was selected. This was based on the fact that according to the Kenya AIDS Indicator Survey, 2007 (NASCO, 2009), about 33% of the population was infected with HIV/AIDS, which translated to 3 out of 10 persons in the division likely to be living with HIV/AIDS. In total, the study subjects recruited were; HIV positive 78 (50.3%) and HIV negative 77 (49.7%).

### **3.5 Data collection**

#### **3.5.1 Questionnaire (Appendix III)**

A detailed questionnaire was used to interview subjects once they had been recruited and consent given. The questionnaire was divided into four sections; Section A collected the demographic data for age, sex, highest level of education attained and one's marital status. Section B endeavored to establish the pattern of morbidity of each respondent. The questions were answered after a physical examination had been carried out by the qualified physician. Clinical information collected included signs and symptoms mainly associated with HIV (Literature Review Pg 22 – 23). In addition, information on whether the respondents were on any form of drugs, herbs or nutritional supplements at the time of the study was collected. Drugs inquired as being used included Septrin, Anti-TB drugs, other anti-biotics. The performance activity scale of each study subject was also determined by finding out if the respondent was actively involved in their day-to-day activities (Stage 1), if they were involved in their day-to-day activities but experiencing less than 10% weight loss (stage 2), if they had been bedridden for less than 50% days in the previous month due to sickness (stage 3) or if they had been bedridden for more than 50% of the days the previous month (stage 4). (WHO, 1990)

In section C, the dietary habits and food intake patterns of the participants in this study were determined by analyzing the frequency of meals per day and by use of food frequency analysis . Information was collected on each participant's food consumption from different food groups on a weekly basis. The foods were grouped according to their richness in various nutrients, i.e.; foods rich in carbohydrates (Cereals, Roots and tubers and fats and oils), foods rich in protein (Animal proteins, legumes, pulses and nuts), vegetables and fruits. The food frequency analysis was categorized into the number of times a week the food was consumed i.e. Daily (seven times a week), Frequently (5 – 6 times a week), Moderately (3 – 4 times a week), Occasionally (1 – 2 times a week) and Never (the food was not consumed any time during the week). Then, the consumption was compared among the HIV positives and the HIV negatives study subjects.

### **3.5.2 Collection of blood samples**

Blood samples were taken once at baseline after consent was given by the research subject. Five millimeters (5 ml) of venous blood was obtained by a trained phlebotomist using a 21-gauge needle and put into two 4 ml plain and EDTA vacutainers. The samples in the plain vacutainers were allowed to settle, spinned in a centrifuge at 3000 rpm and serum transferred using pipette tips into two 4 ml acid washed vials covered in aluminum foil. These samples were kept frozen at – 80 degrees Celsius until analyzed for serum zinc, retinol and  $\alpha$ -tocopherol at KEMRI laboratories. The samples in the EDTA tubes were immediately analyzed at KEMRI laboratories on arrival for CD4+ cell counts and confirmation of HIV status.

### **3.5.3 Laboratory procedures**

#### **Confirmation of HIV status**

Confirmation of the respondents' HIV status was done using Roche kits (Amplicor version 1.5) to perform both rapid and Eliza tests. Blood samples of half a millimeter (0.5 ml) were collected for the Rapid test and another 0.5 ml for the Eliza test in EDTA vacutainers and delivered at the Centre for Virus Research (CVR) of KEMRI for the analysis.

#### **Determination of CD4+ Cell Counts**

Determination of CD4<sup>+</sup> Cell Counts was done using Cytometry (FacsCalibur supplied by Becton & Dickson, Belgium). Blood samples of one millimeter (1 ml) were collected in EDTA vacutainers and analyzed at the Centre for Biotechnology Research and Development (CBRD), KEMRI. The normal absolute CD4 count in adolescents and adults ranges from 500 to 1500 cells per ml<sup>3</sup> of blood. In this study low CD4<sup>+</sup> Cell count was defined as any amount less than 350 cells/ml<sup>3</sup> (WHO, 2007).

### **Determination of Serum Zinc**

Serum zinc was determined using Flame Atomic Absorption Spectrometer (FAAS), at the Centre of Public Health Research (CPHR) at KEMRI. (Dawson & Walker, 1969). One millimeter (1 ml) of the blood samples that had been centrifuged and frozen was separated and analyzed for serum zinc determination. The cut-off of serum zinc levels in this study was 100µg/dl with reference to the WHO guidelines (WHO/FAO, 2004). Any participant with levels below that was termed as being deficient. (Detailed experimental procedures are provided in Appendix IV).

### **Determination of Serum Retinol**

Serum retinol was determined using a High Performance Liquid Chromatography (HPLC) (Miller et al. 1984). One millimeter (1 ml) of the blood samples that had been centrifuged and frozen was separated and analyzed for serum retinol determination. This was carried out at the Centre of Public Health Research (CPHR) at KEMRI. The cut-off level for Serum retinol levels was 0.7µg/dL (Russell, 2000; WHO/FAO, 2004). Any participant with levels below these was termed as being deficient. (Detailed experimental procedures are provided in Appendix IV).

### **Determination of Serum $\alpha$ -Tocopherol**

Determination of serum  $\alpha$ -Tocopherol was also determined using a High Performance Liquid Chromatography (HPLC) (Russell et al., 1986). One millimeter (1 ml) of the blood samples that had been centrifuged and frozen was separated and analyzed for serum  $\alpha$ -Tocopherol determination. The cut-off level for serum  $\alpha$ -Tocopherol levels was 9.0µg/dL where any

participant with levels below these was termed as being deficient (WHO/FAO, 2004). (Detailed experimental procedures are provided in Appendix IV).

### **3.6 Data Management and Analysis**

#### **3.6.1 Data management**

The quantitative data from the field was coded and double entered into a computer database designed using MS-Access application. Files Back-up was regularly done to avoid any loss or tampering. Data cleaning and validation was performed in order to achieve a clean dataset that was exported into a Statistical Package format (SPSS) for analysis. All the questionnaires were

#### **3.6.2 Data Analysis**

Data analysis was conducted using IBM SPSS version 20.0 statistical software. Exploratory data techniques were done at the initial stage of analysis to uncover the structure of data and identify outliers or unusual entered values. Univariate analysis was done on descriptive statistics such as proportions to summarize categorical variables while measures of central tendency such as mean, SD, median and ranges for continuous variables. Bivariate Analysis by Pearson's Chi-square or Fisher Exact Tests were used to test for the strength of association between categorical variables. All exposure variables (Independent factors) were associated with the dependent variable (HIV sero-status) to determine which ones had significant association. Odds Ratio (OR) and 95% Confidence Interval (CI) were used to estimate the strength of association between independent variables and the dependent variable. The threshold for statistical significance was set at  $p < 0.05$ .

Multivariate analysis was performed to identify independent predictors of HIV sero-status. Zinc, Vitamin A, and Vitamin E levels were fitted as the core-model. The core-model together with other independent factors associated with HIV sero-status at  $P < 0.1$  during bivariate analysis were considered for multivariate analysis. This was performed using Binary logistic regression where backward conditional method was specified in order to identify confounders and/or effect modifiers. Adjusted odds Ratio (AOR) with corresponding 95% Confidence Interval (CI) were



used to estimate the strength of association between the retained independent predictors of ‘HIV positive sero-status’.

### **3.7 Ethical considerations**

Before commencement of the study, the study proposal was approved by the National KEMRI Ethical Review Committee. The purpose of the study and the role of the study subject were explained clearly to the subjects, both in English and Kiswahili, before signing of the consent form. To minimize stigma, the recruitment of both the HIV positive study subjects and the HIV negative study subjects was done during the same time at the same places. The subjects were given codes for identification so as to ensure confidentiality of the personal information obtained. Any study subject who was unwell at the time of examination was given free consultation and in cases like depression that hindered the subject from adequately answering the questions, counseling was provided by the medical team and the interview rescheduled to a later time/date.

## CHAPTER FOUR

### RESULTS

#### 4.1 Demographic and Socioeconomic Characteristics

**Table 4.1: Demographic and Socioeconomic characteristics of the participants**

<b>Variables</b>	<b>N=155</b>	<b>%</b>
<b>Age in years</b>		
<30 years	28	18.1
30 - 39 years	58	37.4
40 - 49 years	37	23.9
50 or more years	32	20.6
<b>Gender</b>		
Male	29	18.7
Female	126	81.3
<b>Level of education</b>		
<Secondary	112	72.3
>=Secondary	43	27.7
<b>Occupation</b>		
Business	29	18.7
Farming	121	78.1
Employed	5	3.2
<b>Marital status</b>		
Single	8	5.2
Monogamous married	93	60.0
Polygamous married	21	13.5
Separated/divorced	9	5.8
Widowed	24	15.5

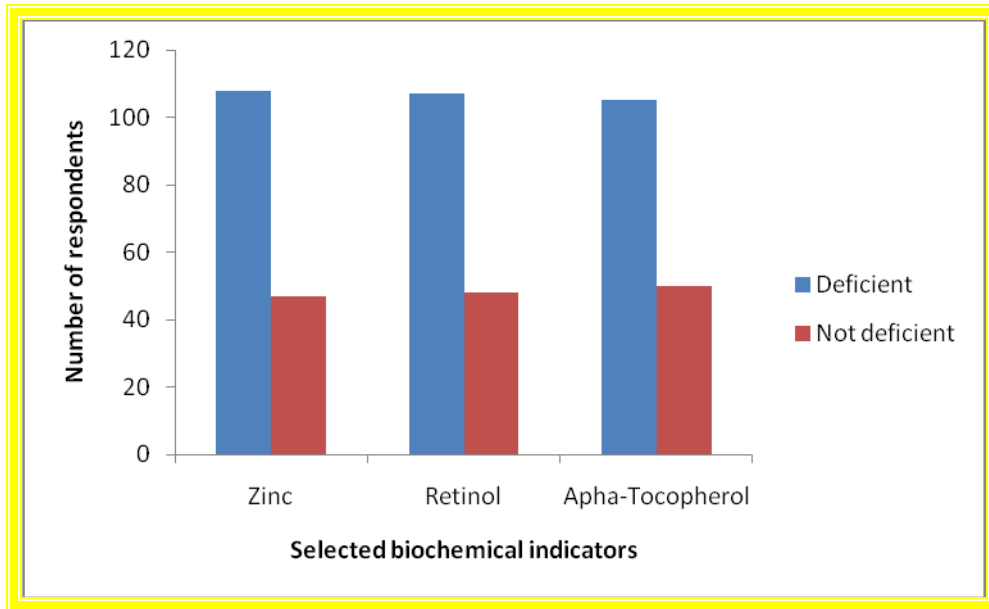
A total of 155 study participants with comparable distribution of HIV sero-status (50.3% (78) HIV positive and 49.7% (77) HIV negative) were interviewed. Information on their selected demographic and socioeconomic characteristics was collected as shown in Table 4.1. Mean age was 36 years (range 18 to 60 years). Gender distribution revealed a high proportion of females (81.3%; 126) than males (18.7%; 29). A relatively high proportion of the participants (72.3%; 112) had not obtained secondary school education. Majority (78.1%; 121) were practicing

farming with a very small proportion (3.2%; 3) working as Community health workers. Most of the participants (73.5%; 114) were married comprising 60.0% (93) Monogamous, and 13.5% (21) Polygamous.

**Table 4.2: Demographic and socioeconomic characteristics in relation to HIV status**

Variables	HIV Positive (n=78)		HIV Negative (n=77)		OR	95% CI		P value
	n	%	n	%		Lower	Upper	
<b>Age in years</b>								
<30 years	10	12.8	18	23.4	0.93	0.32	2.66	0.886
30 - 39 years	33	42.3	25	32.5	2.20	0.91	5.33	0.081
40 - 49 years	23	29.5	14	18.2	2.74	1.03	7.27	<b>0.043</b>
50 or more years	12	15.4	20	26.0	Reference			
<b>Gender</b>								
Male	17	21.8	12	15.6	1.51	0.67	3.42	0.322
Female	61	78.2	65	84.4	Reference			
<b>Level of education</b>								
<Secondary	55	70.5	57	74.0	0.84	0.41	1.70	0.625
>=Secondary	23	29.5	20	26.0	Reference			
<b>Marital status</b>								
Single	5	6.4	8	10.4	0.51	0.14	1.93	0.325
Monogamous married	39	50.0	37	48.1	0.87	0.38	2.01	0.741
Polygamous married	10	12.8	10	13.0	0.82	0.27	2.54	0.735
Separated/divorced	7	9.0	8	10.4	0.72	0.21	2.48	0.604
Widowed	17	21.8	14	18.2	Reference			
<b>Occupation</b>								
Business	19	24.4	10	13.0	0.48	0.05	4.84	0.530
Farming	55	70.5	66	85.7	0.21	0.02	1.92	0.166
Community health worker	4	5.1	1	1.3	Reference			

Table 4.2 shows the distribution of the study subjects according to selected demographic and socioeconomic characteristics in relation to their HIV sero-status. Mean age among the HIV positives was 37 compared to the HIV negatives, 35. Distribution of age varied between HIV positives and HIV negatives, with a significant difference observed in age category 40 – 49 years (P=0.043). There were no significant differences with regards to sex, level of education, marital status, and occupation.



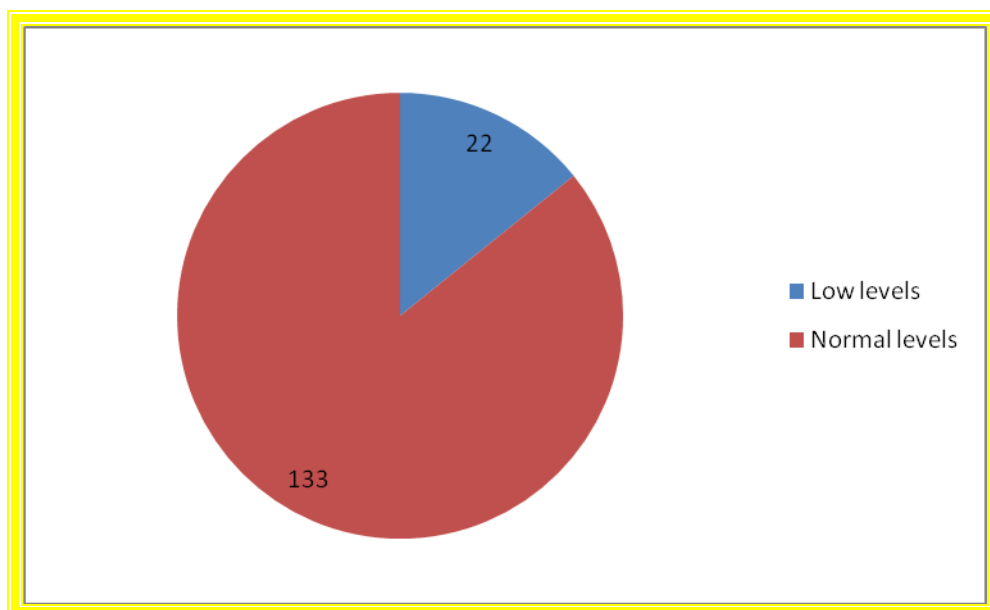
**Figure 4.1: Selected Biochemical indicators among study participants**

## 4.2 Selected Biochemical and Immunological indicators

### 4.2.1 Selected Biochemical indicators

Mean serum zinc level was 85 µg/dL, ranging from 34 to 242 µg/dL. As shown in Figure 4.1, Zinc deficiency was found in 69.7% (108) of the respondents. The cut-off level for serum zinc deficiency was 100 µg/dL (WHO/FAO, 2004). Mean vitamin A level was 0.244 µg/dL, ranging from 0.044 to 1.643 µg/dL. Vitamin A (Retinol) deficiency was observed in 69.0% (107) of all the participants, the cut-off level for retinol deficiency being 0.7 µg/dL (WHO/FAO, 2004). Mean vitamin E level was 6.05 µg /dl ranging from 1.19 to 33.65 µg /dl. The level of vitamin E

deficiency was observed in 67.7% (105) of all the participants, the cut-off level for  $\alpha$ -tocopherol deficiency being 9.0 $\mu$ g/dL (WHO/FAO, 2004).



**Figure 4.2: CD<sub>4</sub> cell count among study participants**

#### **4.2.2 Selected Immunological Indicator – CD<sub>4</sub> cell count**

Mean CD<sub>4</sub> count was 729 counts/mm<sup>3</sup>, ranging from 6 to 2455 counts/mm<sup>3</sup>. Low levels of CD<sub>4</sub><sup>+</sup> cell counts were found in 14.2% (22) of the participants. Any respondent with levels below 350 cells/mm<sup>3</sup> was termed to have low CD<sub>4</sub><sup>+</sup> levels (WHO, 2007).

**Table 4.3: Selected Biochemical and immunological indicators in relation to HIV status**

Variables	HIV Positive (n=78)		HIV Negative (n=77)		OR	95% CI		P value
	n	%	n	%		Lower	Upper	
	<b>Selected Biochemical indicators</b>							
<b>Zinc level</b>								
Deficient	55	70.5	53	68.8	1.27	0.57	2.81	0.555
Not deficient	23	29.5	24	31.2	Reference			
<b>Retinol level</b>								
Deficient	51	65.4	56	72.7	0.71	0.36	1.41	0.323
Not deficient	27	34.6	21	27.3	Reference			
<b>A-Tocopherol level</b>								
Deficient	56	71.8	49	63.6	1.45	0.74	2.86	0.277
Not deficient	22	28.2	28	36.4	Reference			
<b>Selected immunological indicator</b>								
<b>CD4 level</b>								
Low	19	24.4	3	3.9	7.94	2.24	28.14	<0.001
High	59	75.6	74	96.1	Reference			

Assessment of the selected Biochemical and immunological indicators in relation to HIV status was done as presented in Table 4.3. None of the biochemical indicators was significantly associated with HIV sero-status. Zinc deficiency was observed in a higher number of HIV positive respondents (70.5%; 55) as compared to the HIV negative respondents (68.8%; 53), but the difference was not statistically significant. Vitamin E deficiency was also observed in a

higher number of HIV positive respondents (71.8%; 56) as compared to the HIV negative respondents (63.6%; 49), but the difference was also not statistically significant. Deficiency in vitamin A however, was slightly lower (65.4%; 51) in HIV positives compared to HIV negatives (72.7%; 56), but the difference was also not statistically significant.

For the selected immunological indicator - CD4 cell count, low CD4 cell count was significantly higher (24.4%; 19) in the HIV positive respondents compared to the HIV negative respondents (3.9%; 3), (OR= 7.94; 95% CI: 2.24 – 28.14; P<0.001).

### 4.3 Pattern of morbidity

**Table 4.4: Pattern of morbidity of the participants**

<b>Variables</b>	<b>N=155</b>	<b>%</b>
Fatigue	75	48.4
Loss of weight	64	41.3
Headache	59	38.1
Cough	57	36.8
Fever	55	35.5
Night sweat	47	30.3
URTI	42	27.1
Loss of appetite	40	25.8
Skin rash	26	16.8
Pallor	24	15.5
Genital Lesions	20	12.9
Lymphadenopathy	11	7.1
Diarrhoea	7	4.5
Piles	6	3.9
Boils	5	3.2
TB	5	3.2
Oral thrush	4	2.6
Oedema	3	1.9
Herpes zoster	3	1.9
Pneumonia	2	1.3
Kaposi Sarcoma	1	0.6
PCP	1	0.6
Cryptococcus	1	0.6



Twenty-three signs/symptoms were reported in the course of the clinical review of the study participants (Table 4.4). The most commonly reported signs/symptoms included; Fatigue (48.4%; 75), Loss of weight (41.3%; 64), Headache (38.1%; 59), Cough (36.8%; 57), Fever (35.5%; 55), Night sweat (30.3%; 47), URTI (27.1%; 42) Loss of appetite (25.8%; 40), Skin rash (16.8%; 26), Pallor (15.5%; 24) and Genital Lesions (12.9%; 20).

Assessment of the relationship between HIV sero-status and occurrence of signs and symptoms was done as presented in Table 4.5. Out of twenty-three reported signs and symptoms, occurrence of skin rash was significantly associated with positive HIV sero-status. Presence of skin rash showed a significant difference (25.6%; 20) in the HIV positive respondents compared to the HIV negative respondents (7.8%; 6), (OR= 4.08; 95% CI: 1.54 – 10.83; P=0.003). Similarly, presence of lymphadenopathy was also significantly associated with HIV positive sero-status (OR= 11.18; 95% CI: 1.39 – 89.60; P=0.005). Occurrence of URTI was also associated with positive HIV sero-status (34.6%; 27) as compared to the HIV negative respondents (19.5%; 15), (OR= 2.19; 95% CI: 1.05 – 4.55; P=0.034).

**Table 4.5: Selected signs and symptoms in relation to HIV status**

Variables	HIV Positive (n=78)		HIV Negative (n=77)		OR	95% CI		P value
	n	%	n	%		Lower	Upper	
<b>Pallor</b>								
Yes	13	16.7	11	14.3	1.20	0.50	2.87	0.682
No	65	83.3	66	85.7	Reference			
<b>Lymphadenopathy</b>								
Yes	10	12.8	1	1.3	11.18	1.39	89.60	<b>0.005</b>
No	68	87.2	76	98.7	Reference			
<b>Oedema</b>								
Yes	2	2.6	1	1.3	2.00	0.18	22.52	1.000
No	76	97.4	76	98.7	Reference			
<b>URTI</b>								
Yes	27	34.6	15	19.5	2.19	1.05	4.55	<b>0.034</b>
No	51	65.4	62	80.5	Reference			
<b>Headache</b>								
Yes	31	39.7	28	36.4	1.15	0.60	2.21	0.665
No	47	60.3	49	63.6	Reference			
<b>Skin rash</b>								
Yes	20	25.6	6	7.8	4.08	1.54	10.83	<b>0.003</b>
No	58	74.4	71	92.2	Reference			
<b>Diarrhoea</b>								
Yes	5	6.4	2	2.6	2.57	0.48	13.66	0.442
No	73	93.6	75	97.4	Reference			
<b>Loss of weight</b>								
Yes	39	50.0	25	32.5	2.08	1.08	3.99	0.916
No	39	50.0	52	67.5	Reference			
<b>Fever</b>								
Yes	27	34.6	28	36.4	0.93	0.48	1.79	0.820
No	51	65.4	49	63.6	Reference			
<b>Oral thrush</b>								
Yes	3	3.8	1	1.3	3.04	0.31	29.89	0.620
No	75	96.2	76	98.7	Reference			
<b>Pneumonia</b>								
Yes	1	1.3	1	1.3	0.99	0.06	16.07	1.000
No	77	98.7	76	98.7	Reference			

### 4.3.1 Disease burden

Analysis of the disease burden revealed that out of the 155 participants, 135 (88.1%) reported at least one form of illness. Median number of illnesses was 4 (range 0 to 16). 34.2% (53) reporting more than four illnesses as shown in Figure 4.3 below.

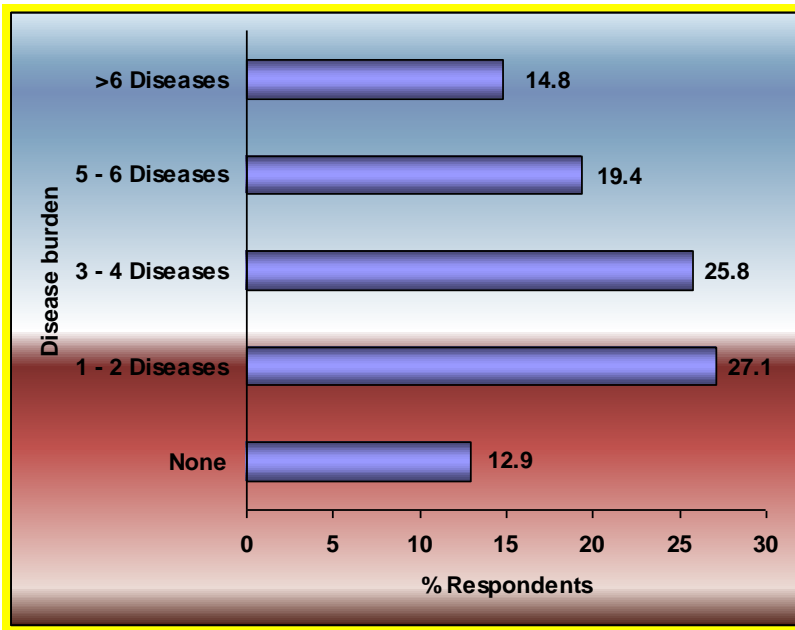
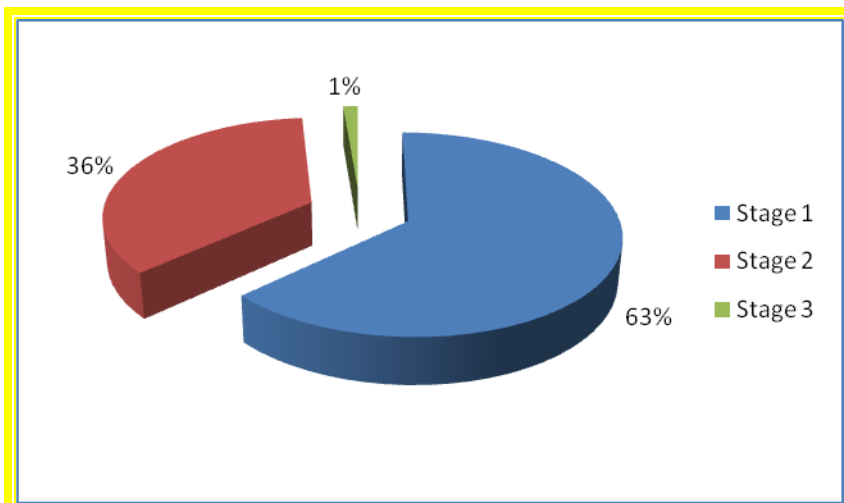


Figure 4.3: Disease burden among the study participants

### 4.3.2 Performance Activity Scale

Assessment of the performance activity scale also revealed that majority (63.2%; 98) had a normal performance activity (stage 1) while 35.5% (55) had normal activity but with weight loss < 10% (stage 2) as shown below in Figure 4.4.



**Figure 4.4: Performance Activity Scale of the study participants**

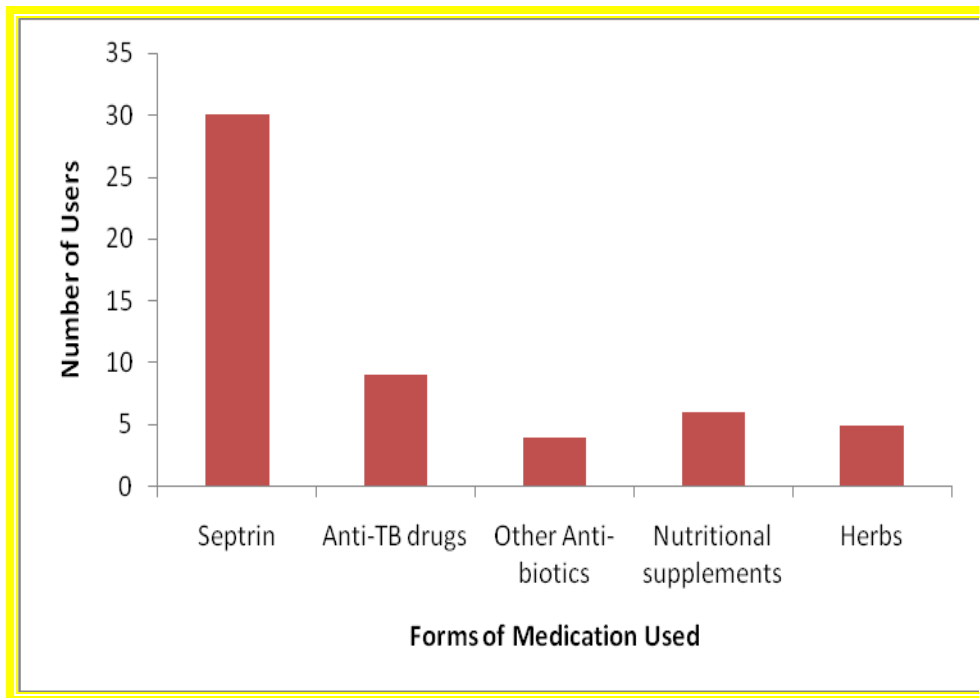
Table 4.6 shows disease burden and the performance activity scale in relation to HIV status. There was a significant difference among participant in Stage 2 and above, HIV positives (51.3%) and HIV negatives (22.1%) (OR= 3.70; 95% CI: 1.85 – 7.69; P<0.001).

**Table 4.6: Disease burden and Performance Activity Scale in relation to HIV status**

Variables	HIV Positive (n=78)		HIV Negative (n=77)		OR	95% CI		P value
	n	%	n	%		Lower	Upper	
	<b>Disease burden score</b>							
>6 Diseases	17	21.8	6	7.8	2.83	0.79	10.17	0.110
5 - 6 Diseases	16	20.5	14	18.2	1.14	0.37	3.55	0.817
3 - 4 Diseases	21	26.9	19	24.7	1.11	0.38	3.24	0.855
1 - 2 Diseases	14	17.9	28	36.4	0.50	0.17	1.48	0.211
None	10	12.8	10	13.0	Reference			
<b>Performance Activity Scale</b>								
Stage 2 or more	40	51.3	17	22.1	3.70	1.85	7.69	<b>&lt;0.001</b>
Stage 1	38	48.7	60	77.9	Reference			

### 4.3.3 Use of Medication

About one third of the participants (34.8%; 54) used at least one form medication, drugs, herbs or nutritional supplements. Among the drugs used, Septrin was the most commonly used (55.5%; 30) as shown in Figure 4.5 below.



**Figure 4.5: Use of Medication among study participants**

Analysis of use of Medication in relation to HIV status was assessed as presented in Table 4.7. Overall assessment on use of any form of Medication revealed significant association with the HIV positive respondents. Use of any form of drugs, herbs and nutritional supplements was significantly higher (53.8%; 42) in the HIV positive respondents compared to the HIV negative respondents (9.1%; 7), (OR= 11.67; 95% CI: 4.76 – 28.57;  $P < 0.001$ ). Use of septrin was also significantly associated with HIV positive sero-status (OR= 15.68; 95% CI: 3.55 – 69.32;  $P < 0.001$ ) with those who were HIV positive showing a significantly higher rate of use ( $P < 0.001$ ) than those who were HIV negative.

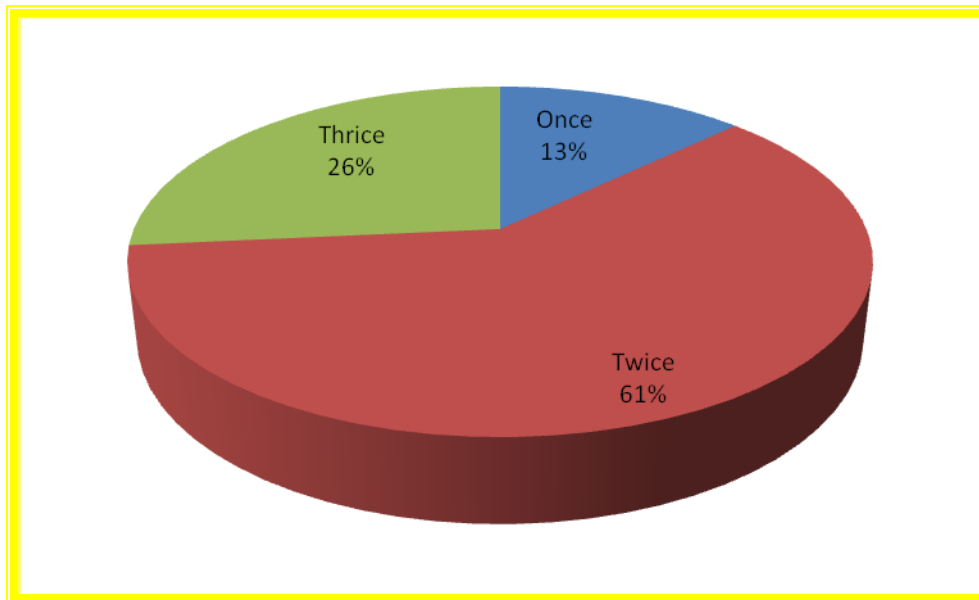
**Table 4.7: Use of Medication in relation to HIV status**

Variables	HIV Positive (n=78)		HIV Negative (n=77)		OR	95% CI		P value
	n	%	n	%		Lower	Upper	
	<b>Use of Medication</b>							
Yes	42	53.8	7	9.1	11.67	4.76	28.57	<0.001
No	36	46.2	70	90.9	Reference			
<b>Septtrin</b>								
Yes	28	35.9	2	2.6	21.00	4.79	92.11	<0.001
No	50	64.1	75	97.4	Reference			
<b>Anti -TB drugs</b>								
Yes	7	9.0	2	2.6	3.70	0.74	18.40	0.167
No	71	91.0	75	97.4	Reference			
<b>Other Anti -biotics</b>								
Yes	3	3.8	1	1.3	3.04	0.31	29.89	0.620
No	75	96.2	76	98.7	Reference			
<b>Nutritional supplements</b>								
Yes	6	7.7	0	0.0	UD	UD	UD	0.028
No	72	92.3	77	100.0	Reference			
<b>Herbs</b>								
Yes	3	3.8	2	2.6	1.50	0.24	9.24	1.000
No	75	96.2	75	97.4	Reference			

#### 4.4 Dietary habits and Food intake patterns

##### Frequency of meals

Close to two-third of the participants (60.6%) consumed two meals per day as compared with only 26.5% who consumed three meals as presented in Figure 4.6.



**Figure 4.6: Frequency of meals among study participants**

Analysis of the frequency of meals in relation to HIV sero-status was assessed and no statistical significant difference was noted as shown in Table 4.8

**Table 4.8: Frequency of meals in relation to HIV status**

Variables	HIV Positive		HIV Negative		OR	95% CI		P
	n	%	n	%		Lo	U	
Number of meals per day								
ONCE	9	11.5	11	14.3	0.78	0.27	2.28	0.649
TWICE	48	61.5	46	59.7	0.99	0.48	2.07	0.987
THRICE	21	26.9	20	26.0	Reference			

Table 4.9 presents the consumption of foods rich in carbohydrates among the study participants on a weekly basis. Most of the carbohydrates rich foods were consumed either moderately (3 – 4 times weekly) or occasionally (1 – 2 times weekly). A few were consumed frequently (5 – 6 times weekly) namely millet, sorghum, irish potatoes and pumpkins but by only less than 3% of the targeted population. Some of the study participants never consumed some of the carbohydrates rich foods for a period of one week. The carbohydrates rich foods that were never consumed by a high percentage of the study participants included; Irish potatoes (50.3), Pumpkin (49.6%), Millet (46.5%), Matoke (41.4%) and Wheat products (41.3%). None of the carbohydrates rich foods was consumed daily.



**Table 4.9: Weekly consumption of foods rich in carbohydrates among the study participants**

SNO.	FOOD	DAILY		5-6 TIMES		3-4 TIMES		1-2 TIMES		NEVER	
				FREQUENT		MODERATE		OCCASIONALLY			
1	Maize	0	0%	0	0%	8	5.16%	145	93.54%	2	1.30%
2	Rice	0	0%	0	0%	101	65.20%	30	19.40%	24	15.50%
3	Cassava	0	0%	0	0%	106	68.40%	29	18.70%	20	12.90%
4	Millet	0	0%	4	2.56%	46	29.64%	33	21.30%	72	46.50%
5	Sorghum	0	0%	4	2.56%	24	15.50%	95	61.30%	32	20.64%
6	Sweet Potatoes	0	0%	0	0%	41	26.45%	99	63.87%	15	9.68%
7	Irish Potatoes	0	0%	3	1.94%	65	42%	9	5.80%	78	50.26%
8	Pumpkin	0	0%	4	2.56%	65	42%	9	5.80%	77	49.64%
9	Matoke	0	0%	0	0%	78	50.26%	13	8.39%	64	41.35%
10	Wheat products	0	0%	0	0%	68	43.87%	23	14.84%	64	41.30%
11	Fats & Oils	0	0%	0	0%	6	3.87%	121	78.10%	28	18.03%
12	Sugar	0	0%	1	0.66%	26	16.80%	106	68.34%	22	14.20%

Assessment of consumption of food rich in proteins on a weekly basis was analysed as presented in Table 4.10. Most of the animal and plant protein rich foods were consumed either moderately (3 – 4 times weekly) or occasionally (1 – 2 times weekly). A few were consumed frequently (5 – 6 times weekly) namely eggs (by 1.3% of the targeted population), simsim seeds (10%) and peas (1.3%). Some of the study participants never consumed some of the protein rich foods for a period of one week. The protein rich foods that were never consumed by a high percentage of the study participants included; Peas (66.5%), Simsim seeds (61.6%), Nuts (49.6%), Eggs (26.5%) and Milk (23.2%). None of the protein rich foods were consumed daily.

**Table 4.10 Weekly consumption of proteins rich foods among the study participants**

SNO.	FOOD	DAILY		5-6 TIMES		3-4 TIMES		1-2 TIMES		NEVER	
				FREQUENT		MODERATE		OCCASIONALLY			
<b>ANIMAL PROTEINS</b>											
13	Beef	0	0%	0	0%	133	85.86%	13	8.34%	9	5.80%
14	Chicken	0	0%	0	0%	134	86.48%	5	3.20%	16	10.32%
15	Fish	0	0%	0	0%	40	25.80%	110	71.00%	5	3.20%
16	Milk	0	0%	0	0%	30	19.34%	89	57.46%	36	23.20%
17	Eggs	0	0%	2	1.29%	61	39.35%	51	32.90%	41	26.46%
<b>PLANT PROTEIN</b>											
18	Beans	0	0%	0	0%	84	54.20%	38	24.50%	33	21.30%
19	Simsim seeds	0	0%	16	10%	34	22.00%	10	6.45%	95	61.55%
20	Peas	0	0%	2	1.30%	44	28.38%	6	3.87%	103	66.45%
21	Nuts	0	0%	0	0%	50	32.30%	28	18.10%	77	49.60%

Table 4.11 presents the consumption of fruits among the study participants on a weekly basis. Most of the fruits were consumed either moderately (3 – 4 times weekly) or occasionally (1 – 2 times weekly). One fruit, the Avocado was consumed daily by one participant (0.7%). A few fruits were consumed frequently (5 – 6 times weekly) namely watermelon (by 7.7% of the targeted population), jackfruit (3.9%), oranges (1.3%) and avocado (0.65%). Some of the study participants never consumed some of the fruits for a period of one week. The fruits that were never consumed by a high percentage of the study participants included; Watermelon (74.9%), jackfruit (74.1%), lemon (64.5%), and pawpaw (32.9%).

**Table 4.11: Weekly consumption of fruits among the study participants**

SNO.	FOOD	DAILY		5-6 TIMES		3-4 TIMES		1-2 TIMES		NEVER	
				FREQUENT		MODERATE		OCCASIONALLY			
<b>FRUITS</b>											
22	Oranges	0	0%	2	1.30%	60	38.70%	64	41.30%	29	18.70%
23	Pawpaw	0	0%	0	0%	61	39.40%	43	27.70%	51	32.90%
24	Mangoes	0	0%	0	0%	90	58.10%	27	17.40%	38	24.50%
25	Ripe Bananas	0	0%	5	0%	56	36.10%	72	46.50%	27	17.40%
26	Avocado	1	1%	1	0.65%	65	42.00%	71	45.80%	17	10.90%
27	Guava	0	0%	0	0%	63	40.64%	43	27.76%	49	31.60%
28	Lemon	0	0%	0	0%	41	26.50%	14	9.00%	100	64.50%
29	Jackfruit	0	0%	6	3.90%	26	16.80%	8	5.20%	115	74.10%
30	Watermelon	0	0%	12	7.70%	22	14.20%	5	3.20%	116	74.90%

Assessment of consumption of vegetables on a weekly basis was analysed as presented in Table 4.12. Most of the vegetables were consumed either moderately (3 – 4 times weekly) or occasionally (1 – 2 times weekly) similar to the other kind of foods above. Quite a number were consumed frequently (5 – 6 times weekly) namely cassava leaves (by 11.6% of the targeted population), bean leaves (6.5%), spinach (4.5%), carrots (3.9%), tomatoes (0.7%), pumpkin leaves (0.7%) and mrenda/jute mallow (*Corchorus olitorius*) (0.7%). Some of the study participants never consumed some of the vegetables for a period of one week. The vegetables that were not consumed by a high percentage of the study participants included; Cassava leaves (84.5%), Bean leaves (76.1%), Spinach (67.7%), Carrots (54.5%) and Cabbage (54.2%). None of the vegetables were consumed daily.

**Table 4.12: Weekly consumption of vegetables among the study participants**

SNO.	FOOD	DAILY		5-6 TIMES		3-4 TIMES		1-2 TIMES		NEVER	
				FREQUENT		MODERATE		OCCASIONALLY			
<b>VEGETABLES</b>											
31	Kales	0	0%	0	0%	5	3.20%	145	93.60%	5	3.20%
32	Spinach	0	0%	7	4.50%	40	25.80%	3	2.00%	105	67.70%
33	Carrot	0	0%	6	3.90%	43	27.70%	6	3.90%	100	64.50%
34	Cabbage	0	0%	0	0%	38	24.50%	33	21.30%	84	54.20%
35	Tomatoes	0	0%	1	0.65%	2	1.30%	142	91.60%	10	6.50%
36	Pumpkin	0	0%	1	0.65%	20	13.00%	85	55.00%	49	31.35%
	leaves										
37	Kunde	0	0%	0	0%	4	2.60%	139	89.70%	12	7.70%
	leaves										
38	Mrende	0	0%	1	0.65%	24	15.50%	102	65.75%	28	18.10%
39	Amaranth	0	0%	0	0%	30	19.40%	74	47.70%	51	32.90%
40	Saka	0	0%	0	0%	53	34.20%	58	37.40%	44	28.40%
41	Miro	0	0%	0	0%	34	22.00%	85	54.80%	36	23.20%
42	Managu	0	0%	0	0%	39	25.20%	51	32.80%	65	42.00%
43	Cassava	0	0%	18	11.60%	4	2.60%	2	1.30%	131	84.50%
	leaves										
44	Bean leaves	0	0%	10	6.50%	20	12.90%	7	4.50%	118	76.10%
45	Onions	0	0%	0	0%	0	0%	126	81.30%	29	18.70%

Assessment of the relationship between HIV sero-status and the consumption of foods from the different food groups was done as presented in Appendix VI. Most of the foods in the different food groups showed no significant difference in consumption between the HIV positive and negative study subjects apart from chicken and pumpkin. Chicken, which is rich in animal protein, was consumed significantly more by the HIV negative as compared to the positive participants at a P value of 0.042 while pumpkin, a vegetable, was significantly consumed more by the HIV positive participants as compared to the positive ones at a P value of 0.023.

## 4.6 Multivariate analysis

Multivariate analysis of the five factors that were retained in the final model was as shown below;

**Table 4.13: Predictors of HIV positive status**

Variables	AOR	95% CI		p value
		Lower	Upper	
<b>Vitamin A level</b>				
Deficient	0.39	0.15	1.01	0.052
Not deficient	Reference			
<b>CD4 level</b>				
Low	4.69	1.12	19.65	<b>0.034</b>
High	Reference			
<b>Lymphadenopathy</b>				
Infected	20.79	1.88	230.39	<b>0.013</b>
Not infected	Reference			
<b>Performance Activity Scale</b>				
Normal Activity (Stage 1)	Reference			
Normal Activity but with weight loss < 10% (Stage 2)	6.09	2.36	15.73	<b>&lt;0.001</b>
<b>Use of Medication</b>				
Yes	11.58	3.93	34.13	<b>&lt;0.001</b>
No	Reference			

Adjusting for other factors, there was an increased risk of low CD4 level due to HIV positive sero-status (AOR= 4.69; 95% CI: 1.12 – 19.65; P=0.034). A person with HIV positive sero-status has approximately 5-fold risk of having low CD4 count. The relative risk of Lymphadenopathy for the incidence of HIV positive sero-status was statistically significant

(AOR= 20.79; 95% CI: 1.88 – 230.39; P=0.013). HIV positive sero-status predisposes a person to approximately 21-fold risk of having Lymphadenopathy. Normal Activity but with weight loss < 10% (Stage 2) was significantly associated with HIV positive sero-status (AOR= 6.09; 95% CI: 2.36 – 15.73; P<0.001). A person with HIV positive sero-status had approximately 6-fold risk of having Normal Activity but with weight loss < 10% (Stage 2). HIV positivity was highly associated with use of medication (AOR= 11.58; 95% CI: 3.93 – 34.13; P<0.001) and use of condoms with casual sex partner/s (AOR= 4.62; 95% CI: 1.53 – 13.92; P=0.007).

## CHAPTER FIVE

### DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

#### 5.1. Discussions

##### 5.1.1 Biochemical and immunological indicators of the respondents

Serum zinc, serum retinol and serum  $\alpha$ -tocopherol levels were found to be similar among the HIV positive and negative respondents. Actually, more than 60% of the population was deficient in all the above indicators, with no significant difference in terms of deficiency between the HIV positive and negative respondents. These were; Zinc, 69.7%; Vitamin A, 69%; and Vitamin E, 67.7%. Only the CD4+ cell count levels showed a significant difference among the two groups; 24.4% deficiency among the HIV positive group and 3.9% deficiency among the HIV negative group ( $P < 0.001$ ).

In a similar study by Monteiro et al, 2009, on HIV positive and negative children, it was found that low concentrations of retinol, beta-carotene, and vitamin E are very common in children exposed to HIV living in Brazil, Argentina, and Mexico, regardless of HIV-infection status. This was contrary to their hypothesis that the rates of low concentrations of these micronutrients would be higher in the HIV-infected group than those in the HIV-exposed, uninfected group. Rates of low concentrations were 74% for retinol, 27% for beta-carotene, and 89% for vitamin E. The rates of low serum concentrations observed were explained to be partly due to factors related to in utero HIV exposure, including possible genetic defects in vitamin transfer proteins, fat metabolism disorders and oxidative stress which could alter vitamins requirements (Azzi et al, 2004).

Cunningham-Rundles et al, 1996 reported that 70% of North American infants perinatally exposed to HIV were retinol deficient in the first months of life whereas infants with various other disorders had normal retinol levels. The overall rates of low serum concentrations were in the range of those previously reported in pediatric populations without HIV exposure or infection. Although definitions of retinol deficiency vary across studies, reports of retinol

deficiency in pre-school children show rates up to 74.5% in Brazil (Ferraz et al, 2006), 30% in Argentina (Escobal et al, 2001), and 46.3% in Mexico (Valencia et al, 1999). These studies support the idea of poor intake as the main cause for the high prevalence of retinol deficiency. Similarly, while definitions of vitamin E deficiency vary across studies, ranging from 7 to 28  $\mu\text{mol/l}$  without adjustment for serum cholesterol (Ford & Sowell, 1999), reports of vitamin E deficiency show rates as high as 69% in apparently healthy Latino children in the United States (Kim et al, 2006) and 70% in Mexican preschoolers (Allen et al, 2000). In addition, vitamin E deficiency was found in 70% of HIV- infected and uninfected South African women and no significant difference by HIV status was observed (Papathakis et al, 2007). Poor nutritional intake is likely the main factor contributing to the low serum values of micronutrients in these children (Monteiro et al, 2009). Maybe the same can be said of the study subjects in Busia County from the above arguments that either most of them were born already deficient in the said micronutrients or as that poor nutritional intake was likely the main factor contributing to the low serum values of micronutrients in their bodies.

Similar results were also observed in a study done in Addis Ababa, Ethiopia by Fufa et al, 2009, where low serum zinc and vitamin A levels were observed in almost half of the subjects.. Low serum zinc ( $< 10.7$  micro mol/l) was observed in 53% of the study subjects and low retinol levels ( $< 30$  microg/dl) were observed in 47% of the study subjects. CD4+ cell counts as well ( $< 200\text{mm}^3$ ) were observed in only 21% of the study subjects. Similar results have also been found by Ndagije et al, 2007, where there was no significant difference in levels of selenium, zinc and copper between HIV-infected and uninfected children in a study done among Rwandan children. In another study on levels of micronutrients in pregnant and non-pregnant women in Gondar, Northwest Ethiopia by Kassu et al, 2008 it was established that pregnant women in developing countries are vulnerable to multiple micronutrient deficiencies. But more interestingly was the finding that except for selenium, which was significantly lower in HIV-seropositive pregnant women ( $P < 0.05$ ), the mean serum concentrations of zinc, copper, calcium, and magnesium were not significantly different between pregnant women by HIV serostatus.



Even in HIV positive patients on HAART, zinc deficiency remains common. This was concluded by Jones et al, 2006 in a study where out of 171 men and 117 women, forty percent of men and 36% of women had low zinc levels from their serum samples (zinc <670 microg/L). Low retinol, alpha-tocopherol, and selenium were found to be uncommon in HIV-infected subjects on HAART but zinc deficiency remained common, however. The findings of the studies resonate well with the findings of this study carried out in Kenya. There other studies that negate these findings. Khalili et al, 2008, in the study on the nutritional status and serum zinc and selenium levels in Iranian HIV infected individuals, noted that compared with the healthy control group, serum level of zinc and selenium in the human immunodeficiency virus infected subjects were significantly lower ( $P = 0.01$  and  $P = 0.02$  respectively). In another study by Okwara et al, 2012 in Nigeria, results showed that all minerals (selenium, zinc and magnesium) were significantly lower in HIV patients ( $P < 0.05$ ). Interestingly, when comparisons were made in terms of gender, it was noted that all minerals were significantly lower in male HIV patients than male controls while there were no significant differences in respective micro-mineral level between female controls and female HIV patients. It was thus concluded that depletion was predominant in males possibly due to better health seeking behavior of females than males causing early presentation in females.

The significant difference in CD4 levels among the two study arms can easily be attributed to the low immunity among the HIV positive individuals, where a low immunity is equated to low levels of CD4s in one's body. In general, the CD4 (%CD4+ or absolute count) progressively decreases as HIV disease advances. According to WHO guidelines, the pathogenesis of HIV infection is largely attributable to the decrease in the number of T cells (a specific type of lymphocyte) that bear the CD4 receptor (CD4+). Progressive depletion of CD4+ T cells is associated with progression of HIV disease and an increased likelihood of opportunistic infections and other clinical events associated with HIV, including wasting and death (WHO, 2007). Thus, as anticipated, the PLWHA were significantly more deficient in CD4+ cell counts as compared to their HIV negative counterparts. This was also found true in Rwandese children in a study by Ndagije et al, 2007, where in severely malnourished children with HIV infection, low CD4+ levels were associated mainly with HIV infection. The mean (SD) CD4+ count was 1054 in the HIV-infected and 1579 in the uninfected group ( $p=0.001$ ). The CD4+ count was also

significantly lower in the HIV-infected group than in the uninfected group for the ages <12 mths (p=0.09), 12-24 mths (p=0.045) and >36 mths (p=0.001).

In a Walter Reed project carried out by Kibaya et al in Kericho Kenya (2008) on 1239 HIV seronegative adults, a median CD4+ T cell count for the group was 810 cells/microl which was comparable to this study where the median CD4 count was 729 counts/mm<sup>3</sup>, ranging from 6 to 2455 counts/mm<sup>3</sup> among both the HIV positive and negative study subjects. The findings in this study can also justify why there was no significant difference between the HIV positive and HIV negative respondents in terms of disease burden (section 4.4). It should be noted here that prior to the study, it was anticipated the HIV positive respondents will definitely show a greater disease burden when compared to their HIV negative counterparts. But the results showed no significant difference between the two groups and this can be attributed to the high median count of the CD4 cells that are equated to a high immunity in both groups.

## **5.2. Pattern of morbidity of the respondents**

Most of the clinical signs and symptoms associated with HIV reviewed in this study were found to be similar also among the HIV positive and HIV negative study subjects except for three, which showed a significant difference between the two study groups. These were lymphadenopathy, (12.8% HIV positive, 3.1% HIV negative, P = 0.005), Upper Respiratory Tract Infections, (34.6% HIV positive, 19.5% HIV negative, P = 0.034), and Skin rash, (25.6% HIV positive, 7.8% HIV negative, P = 0.003). These three symptoms are normally associated with an immuno-compromised immune system resulting from an infection with the HIV virus (WHO, 1996) and might have been the reason they were found to be more prevalent among the HIV positive as compared to the HIV negative respondents. Particularly the swelling of the lymph nodes and skin infection, commonly appear in HIV infected individuals in the first and second stages of AIDS. According to the WHO clinical staging of HIV/AIDS for adults and adolescents with confirmed HIV infection, lymphadenopathy is a characteristic of Clinical stage 1 which is mainly asymptomatic. In the multivariate analysis of this study, HIV positive serostatus predisposes a person to approximately 21-fold risk of having lymphadenopathy.

In a similar study in Uganda by Komwa et al, 2010, similar results were observed where 56 HIV-infected participants reported significantly higher rates of diarrhoea in the past month (21.4% vs 11.3%,  $p=0.028$ ) than the participants who did not state that they had HIV infection; there were no differences in the rates of tuberculosis (5.4% vs 2.3%,  $p=0.458$ ), pneumonia (7.1% vs 9.8%,  $p=0.568$ ), malaria (73.2% vs 71.1%,  $p=0.776$ ), or fever (46.4% vs 47.0%,  $p=0.809$ ). Even though in this study the occurrence of diarrhoea was not significantly different in the two groups (HIV positive 6.4% vs HIV negative 2.6%,  $p=0.442$ ) as it was in the above mentioned Uganda study, the rest of the symptoms were similar as in the Ugandan study, that is; tuberculosis (3.8% vs 2.6%,  $p=1.000$ ), pneumonia (1.3% vs 1.3%,  $p=1.000$ ) and fever (34.6% vs 36.4%,  $p=0.820$ ).

Other than the above three symptoms, another indicator that showed a significant difference among the HIV positive and negative respondents was the Performance Activity Scale in stage 2 and above HIV positives (51.3%) and HIV negatives (22.1%) ( $P<0.001$ ). This indicator, as explained earlier in the methodology (Section 3.5.2), was a measure of how the respondents were actively involved in their day-to-day activities without experiencing either weight loss and/or being bedridden due to sickness. The significant difference resulted in stage 2 and above of the performance indicator scale, where more of the HIV positive respondents were less involved in their day-to-day activities due various illnesses as compared to their HIV negative counterparts. This result was generally anticipated since due to the heavy disease burden experienced by the HIV positive respondents, they were expected to be more sick, which led them to seek more rest and hence be less able to go about their daily activities.

This is contrary to a study by Komwa et al, 2010 in Uganda on HIV/AIDS-associated beliefs and practices relating to diet and work in southeastern Uganda, where it was found out that there were no significant differences in work-hours reported by HIV status when analyzing working time as either a continuous or a categorical variable. Even those who reported illness in the past month did not report significantly fewer hours, except for participants who had been diagnosed in the past month with pneumonia ( $p=0.028$ ) or had tuberculosis ( $p=0.011$ ). The similarity in work-hours in the Ugandan study was therefore attributed to misclassification of undiagnosed individuals with HIV who had illnesses that forced them to work fewer hours than healthier adults, a bias that would cause an artificially-low difference in the reported work-hour estimates

between the groups. The results could also be the result of the sample population under-representing the healthiest or the sickest members of the community, which may have led to the failure to detect potentially real differences in work-hours for HIV-infected and other participants. Therefore, the results seem to indicate that although it is commonly believed that if it is possible to work fewer hours a person who has tested positive for HIV should rest more often, the reality is that few households can afford to allow a healthy person with HIV to work less.

This study of subjects in Busia County, Kenya did not take into account the rates of mortality in relation to the performance scale of the subjects, but results from a study by Bhatta et al, 2013 in Nepal, have shown that patients with baseline performance scale of bedridden <50% were two times more likely to die compared to patients with normal performance scale at start of treatment (HR 2.05; 95% CI: 1.19- 3.52). However, the risk of mortality increased 3.4 times when the patients had baseline performance scale of bedridden >50% compared to patients with normal baseline performance scale (HR 3.41; 95% CI: 1.67- 6.98). A study in Ethiopia showed similar findings, where bedridden performance status (not able to perform activities of daily living) was significantly associated with mortality (Biadgilign et al, 2012). Another study found no significant association, but when lost to follow-up patients were counted as death cases significant association was found (Worku, 2009).

Despite the increasingly wider availability of antiretroviral therapy (ART), some people living with HIV (PLHIV) and eligible for treatment have opted to adopt self-care practices. It should be noted that the study subjects in this study who were HIV positive were not using ARVs, which leads us to the two other clinical indicators that showed a significant difference in this study between the HIV positive and negative respondents i.e. the use of drugs, herbs and nutritional supplements (53.9% HIV positive, 9.1% HIV negative,  $P = <0.001$ ) and the use of Septrin (29.5% HIV positive, 2.6% HIV negative,  $P = <0.001$ ). Generally, use of any type of drugs or herbs or nutritional supplements among the participants revealed a significant association with those who were HIV sero-positive. The specific drugs used were Septrin, Anti-TB drugs and other Antibiotics. It can be deduced from the findings that the HIV positive respondents seemed

to really value their health and thus used various methods to treat their opportunistic infections and also boost their immune system in order to prevent further ill-health.

Of the medications used, use of septrin was also highly associated with HIV positive sero-status (OR= 15.68; 95% CI: 3.55 – 69.32; P<0.001). Septrin was the commonly used drug among the HIV positive respondents, whether it was by prescription of a qualified physician or self prescribed by the individuals, was a fact that was not established. It can also be noted here that the use of traditional herbs and nutritional supplements was found to be utilized exclusively by the HIV positive individuals, although the exact nature of the herbs and supplements was also not established.

In comparison to other similar studies, results from the Kenya AIDS Indicator Survey, 2007 done by the National Aids Control Council of Kenya showed similar findings. Among HIV- infected adults who knew their HIV status, a large majority (12%) were taking septrin. Apparently, doctors recommend that all HIV-infected persons take daily Septrin to prevent common infections. (NACC, 2009). In the same 2007 KAIS findings, among HIV-infected adults who knew they were infected with HIV, 7.3% reported taking one or more daily caloric supplements and 4.6% reported taking immune boosters. The most common supplement taken was a daily multivitamin, with 36.4% of HIV infected persons who knew they were infected taking multivitamins on a daily basis. There was no significant difference in the use of multivitamins by age, sex, rural/urban residence, educational level, wealth index or marital status. In addition, results from KAIS 2012 showed that use of the drug Co-trimoxazole (CTX) among those aware of their HIV infection was as high as 89%. CTX is an antibiotic that is recommended for everyone diagnosed with HIV that reduces the risk of early mortality and rates of hospitalisation, malaria, diarrhoea, and pneumonia (NASCOP, 2011). This was an increase from 2007, where CTX use was 76% among HIV-infected persons.

What we are seeing from the above findings is that some of the PLWHA even though they are not on ARVs, they still make a lot of effort to take care of their health and mitigate any associated opportunistic infections. In a qualitative study by Musheke et al, 2013, conducted in urban Zambia to gain insights into PLHIV self-care practices and experiences, it was found out

that acutely aware of their fledgling health and limitations of other self care strategies, some PLWHA did not completely cut-off ties with the formal health system. In the absence of herbal medication or whenever symptoms did not abate, some PLWHA sought formal health care, but only to use conventional medicine to treat opportunistic infections. Skin infections, respiratory infections and gastrointestinal tract infections were the most commonly reported HIV-related ailments.. Antibiotics such as septrin (co-trimoxazole prophylaxis), flagyl, amoxicillin, anti-diarrhoeal medication were the most reported conventional drugs used. Some PLWHA reportedly used antibiotics used by their spouses on ART. Previous studies also indicate that patient retention in ART care is problematic. For instance, in sub-Saharan Africa (SSA), only an estimated 60% of patients were retained in ART programmes after 2 years of starting treatment. This is despite the proven efficacy of ART in reducing AIDS-related deaths. The reasons for patient attrition from ART care and failure to initiate treatment were varied and include financial costs associated with accessing treatment, fear of side effects, fear of drug toxicity and long-term harm to the body and feeling healthy. Other barriers were stigma, belief in faith healing, use of traditional medicine and perceived burden of being on life-long treatment (Musheke et al, 2013).

### **5.3. Dietary practices and food intake pattern of the respondents**

Food and nutrition security are fundamentally important for the prevention, care, treatment, and mitigation of HIV and AIDS. A study by Gillespie and Kadiyala (2005), showed that a programme of care without a nutritional component is likely to crumble, and the efficacy of ART may be compromised by malnutrition. Similarly, since access to and availability of food are affected by the impact of HIV, any strategy to improve nutrition of those affected must prioritize enhancing appropriate nutritional knowledge, attitudes, and use of the little available food.

The analysis of food frequency showed that close to two-third of the participants (60.6%) consumed two meals per day as compared with only 26.5% who consumed three meals. This can be interpreted that the socio-economic status of the majority of the study participants was not high enough to enable them afford more than two meals per day. In a similar study by Bukusuba et.al, 2010 in Uganda, only 21.8% of the participants consumed three or more meals per day and this was greatly attributed to changes in prices of food being a major contributing factor. The

frequency of meals has been shown per se to serve as a proxy indicator of consumption of macronutrients (Swindale & Bilinsky, 2005; Hoddinott, 1999). Following that line of reason, it can be assumed in this study that the participants who consumed three meals per day would have higher amounts of the micronutrients in question as compared to the participants who consumed only two meals per day. It can also be argued that since the majority of the participants (60.6%) consumed two meals per day which equates to inadequate consumption of nutrients, it is therefore no surprise that more than 60% of the study participants were deficient in the three micronutrients in question in this study.

Studies done by Ruel, 2012 and Torheim et al, 2004 have shown that dietary diversity measured by the number of food-groups consumed was a potential 'proxy' indicator of adequacy of nutrients. In this study the participants consumed quite a number of foods from different categories of carbohydrate-rich foods, protein-rich foods, fruits and vegetables. Most of the foods from any of the food groups in the study have been shown to be consumed either moderately (3 – 4 times per week) or occasionally (1 – 2 times per week) by the study participants. Only one fruit, the avocado, was consumed everyday by only one participant. No other food in any other category was shown to be consumed daily. A few foods in each category were consumed frequently (5 – 6 times per week) by the study participants showing no particular favorite kind of food. Interestingly, some of the foods that were frequently consumed were the same ones that were never consumed by the majority of the participants within the one week period. Some examples include; Millet, Irish potatoes and Pumpkin, carbohydrate rich foods that were frequently consumed but not also consumed by 46.5%, 50.3%, 49.6% respectively. Eggs and Peas were the protein-rich foods that were frequently consumed but also not consumed by 26.5% and 66.5% of the participants respectively. Jackfruit and Watermelon were the some of the frequently fruits but also not consumed by majority of the participants 74.1% and 74.9% respectively. Spinach, Carrot, Cassava leaves and Bean leaves were among the vegetables that were frequently consumed but also not consumed by 67.7%, 64.5%, 84.5% and 76.1% respectively by most of the participants.

Analysis of the nutritional content of the foods that were never consumed by majority of the study population revealed that they are the very foods that were rich in the micronutrients in

question in this study. For example, Avocado fruit is high in zinc and rich in Vitamin E. Pumpkin is rich in Zinc, Vitamin A and Vitamin E. Green Peas are rich in both Zinc and Vitamin A. Both Jackfruit and Watermelon are rich in Vitamin A. Spinach is high in all Zinc, Vitamin A and Vitamin E while Carrots are high in Vitamin A. If most of the study population did not consume the above named different categories of food, then it explains the high deficiency of most of the study population (up to 60%) in the micronutrients Zinc, Vitamin A and Vitamin E.

A comparison of consumption of the foods in the different food categories between the HIV positive and the negative study participants showed no significant difference among the two groups. Chicken, which was consumed more among the HIV negative and Pumpkin leaves which were consumed more by the HIV positives, were the only foods that showed a significant difference among the two groups at a P value of 0.042 and 0.023 respectively. This could possibly explain why the levels of serum zinc, retinol (vitamin A) and  $\alpha$ -tocopherol (vitamin E) were not significantly different in terms of deficiency between the HIV positive and negative respondents. It can be argued here that since micronutrients are derived from the food we consume and if the respondents had equal access to and consumed similar variety of foods regardless of one's HIV sero-status, then the respondents ended up having similar levels of micronutrients regardless of their individual HIV status.

Other studies have shown that the importance of nutrition is well-established in HIV infection (Kim et al, 2006; Coodley et al, 1983). Poor nutritional status is a strong predictor of mortality. Even after controlling for CD4+ cell counts, a weight loss of >66% of ideal body-weight was linked to the timing of death in AIDS patients (Süttmann et al, 1995; Semba et al, 1995; Kotler et al, 1989). In a comparable study in Uganda by Komwa et al, (2010), the PLWHA were found to consume different varieties of food from the others unlike in this study. People with HIV infection reported eating more special foods, especially fruits ( $p=0.020$ ) and vegetables ( $p=0.012$ ). People with HIV also reported eating different varieties of foods than uninfected members of the community, which indicates a conviction that good nutrition is an essential component of maintaining health when infected with HIV (Komwa et al, 2010).



### **5.1.2 Conclusion**

Deficiency in the micronutrients Zinc, Retinol (Vitamin A) and  $\alpha$ -Tocopherol (Vitamin E), seemed to be a common occurrence among the study population of Busia County. Most of the study population (more than 60%) was deficient in these indicators whether one had an HIV infection or not. Thus, being HIV positive did not necessarily translate into one being deficient in Zinc, Retino or  $\alpha$ -Tocopherol.

The pattern of morbidity was also similar as shown by the occurrence of signs and symptoms related to HIV/AIDS among the study participants. Being HIV positive did not make one experience more illnesses, neither did it make one less active in their day-to day activities as shown by comparison of the Performance Indicator scale among the two study groups. But being HIV positive predisposed one to more frequent use of Medication as compared to being HIV negative.

Dietary practices were also similar among the HIV positive and HIV negative study subjects. Most of the HIV positives consumed two meals per day and ate a wide variety of foods from the different food groups examined in the study just as the HIV negatives did.

### **5.3 Recommendations**

More research is needed to establish the reason behind the widespread deficiency of the micronutrients zinc, retinol and  $\alpha$ -tocopherol in the population of Busia County despite the availability of a wide variety of food.

More research studies need to be carried out to determine why the HIV positives do not experience more HIV related illnesses and are equally active as their HIV negative counterparts yet they are not on Anti-retroviral Therapy. This discovery could help other HIV positive populations to live better lives without relying on Anti-retroviral medication.

Lastly, there is need to promote and encourage the consumption of foods rich in Zinc, Vitamin A and Vitamin D, which will not only help the PLWHA to prolong their lives more, but will also boost the immunity of the whole population at large.



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## **Appendix I: Informed Consent Explanation and Consent Form**

PROJECT TITLE: Micronutrient deficiencies (zinc, retinol and  $\alpha$ -tocopherol ), morbidity patterns and dietary practices among the HIV positive and negative subjects in Busia County, Kenya.

### **Purpose of Study**

We all need food to stay alive and be able to perform our duties effectively. However, besides food, we also need other substance 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 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 that use of additional vitamins and minerals such as Zinc, Vitamin A and Vitamin E, proposed in this study as the most beneficial nutrients to persons, especially those living with HIV& AIDS. In order for us to find out this, we must first estimate the amount of these micronutrients in our bodies before deciding to intervene with additional amounts. However, before we estimate the amounts of micronutrients in one's body, we need your permission. The study has been approved by the KEMRI/National Ethical Review Committee.

#### **1.1) Procedure's to be followed**

In this study, you will be asked questions on your basic data, illnesses that affect you and your dietary practices. Also, blood shall be taken from you at the beginning of the study and taken to the laboratory in order to be able to determine your micronutrient levels in your body.

#### **1.2) Risks**

This study has no foreseeable risks associated to it.

### **1.3) Benefits**

The study that is being undertaken is expected to yield results on the micronutrients (Zinc, Vitamin A and Vitamin E) levels in one's body. Once the results become known, you will be informed and hopefully this will assist you in making important decisions about yourself in future. These useful findings will also provide a baseline upon which other interventions will be done using indigenous nutritional food supplements

### **1.4) 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. No identity of any specific participant in this study will be disclosed in any public reports or publications.

#### Obtaining additional information

You are allowed to ask any questions that occur to you at this time or any time in the course of your contact with the investigator, the laboratory specialist or the research assistant. You will also be given a copy of this agreement for your own information. If you desire any more information at a later date, you may directly contact or call Mmbone Vivian on 0720 389439.

### **1.6) Basis of participation**

You are free to withdraw the consent to participate in this study at any time.

### **1.7) Declaration**

#### The Participant

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.

Participant's code number.

.....

Participant's signature or thumbprint ..... Date .....

The Investigator

I, the undersigned have fully explained the relevant details of this study to the participant named above. I am qualified to perform the role of principal investigator in this study.

**Investigator:** Mmbone Vivian

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

Witness: .....

Address of Witness: .....

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



## **Appendix II: Informed Consent Form In Kiswahili**

### **MAELEZO YA KUTAFUTA IDHINI KUTOKA KWA WATU WANAOTARAJIWA KUSHIRIKI KATIKA UTAFITI**

KICHWA CHA UTAFITI: Utafiti wa Madini ya Zinc, Vitamini A na Vitamini E, kama kigeuzi cha madhara yatokanayo na Ukimwi katika eneo la Butula, wilaya ya Busia, Kenya.

#### **1.0) Lengo la utafiti:**

Sote twahitaji vyakula ili tuendeleo kuishi na kuendesha shughuli zetu kiadilifu. Hata hivyo, pamoja na vyakula, tunahitaji aina nyingine za lishe kama vitamini na madini. Vitamini na madini haya huwa vinapatikana katika vyakula tunavyokula lakini ijapokuwa hivi ni muhimu sana kwa miili yetu, huwa vyahitajika kwa kiasi kichache sana. Vyakula vyengine tulavyo huwa havina kiasi cha kutosha cha vitamini ama madini ambayo ni haba katika makundi fulani ya wakaaji Kenya na haswa walio na upungufu wa kinga mwilini. Utafiti uliofanywa sehemu nyingine za dunia na hata hapa Kenya umegundua kwamba matumizi ya vitamini na madini ya ziada, ambayo mradi huu umekusudia, yana manufaa zaidi kwa watu hasa wale walio na ukimwi/virusi vya ukimwi. Ili kuthibitisha haya, inafaa ya kwanza kujua kipimo cha hizi vitamin na madini katika miili yetu kabla ya kuamua kuongeza. Hata hivyo, kabla ya kupima viwango hivi katika mwili wako, tutakuomba utupe idhini yako. Utafiti huu umeruhusiwa na Taasisi ya Utafiti ya Kenya (KEMRI) ikiwa pia ndio kamati ya kitaifa inayo simamia njia bora za utafiti inchini.

#### **1.1) Kanuni Zitakazofuatwa:**

Katika uchunguzi huu, tutapenda kukuuliza maswali kadha kuhusu ili kukuelewa zaidi, hasa juu ya magonjwa yanaokusumbuwa mara kwa mara na vyakula unavyo vila kila siku. Hata hivyo, tutachukua vipimo fulani vya damu utakapojiunga na utafiti huu ambavyo vitafanyiwa uchunguzi ili kujua vipimo vya vitamini na madini katika mwili wako.

#### **1.2) Madhara ya Kushiriki katika Mradi Huu.**

Hatutazamii kuwa kutatokea madhara yoyote kwa watakao shiriki katika mradi huu.

### **1.3) Manufaa ya Kushiriki katika Mradi Huu.**

Matokeo ya utafiti huu yatakunufaisha, ila ukishaambiwa, utaweza kuyawaza na kuyatumia kunufaisha maisha yako, kiafya na kula vyema zaidi. Matokeo pia yatatumiwa na watafiti wengine kusaidia wale ambao wana vipimo vya chini vya vitamin na madini mwilini kutumia vyakula vya kitamaduni kumudu afya yao, hasa wanaougua ugonjwa wa Ukimwi.

### **1.4) Hifadhi ya Nakala ya Habari Utakazotoa:**

Habari zote zitakazo kusanywa na watafiti zitahifadhiwa kama siri na kutumiwa tu katika utafiti huu. Majina ya watu binafsi wanaoshiriki hataandikwa pahali popote wakati wowote. Habari zote zitafungiwa katika makabati maalum wakati wote wa utafiti utakapokuwa ukiendelea. Habari hizi zitapowekwa katita komputa zitatumiwa tu na watafiti watakaotumia kitambulisho cha siri ili kufikia habari hizi. Tunasisitiza usiri huu katika kusimamia habari unazo tupa ili kuzuia kujulikana kwa watakao shiriki katika utafiti huu.

### **1.5) Maswali na Utata:**

Uwapo na swala lolote kuhusu utafiti tafadhali afikiana na: Mmbone Vivian, mkuu wa utafiti huu. Nambari ya simu 0720-389439.

### **1.6) Ni Hiari yako Kushiriki katika Utafiti huu**

Uko huru kuamua kutojibu maswali ama kukatiza kushiriki kwako katika mradi huu wakati wowote ule kama utakavyoamua mwenyewe

### **1.7) Idhini Yako na Sahihi:**

Ukiwa umesoma maelezo haya (ama umesomewa, ama hata kuelezwa na mtu) yakutaka idhini yako na umeelwa ulivyo elezwa na kwamba umekubali kwa hiari yako mwenyewe kushiriki katika mradi huu, tafadhali soma kwa makini yafuatayo hapa chini kabla ya kutia sahihi ama

kuweka alama yakok yoyote ya kukutambulisha. Lolote utakalo amua halitaathiri haki zako kwa njia yoyote;

Ya kwamba nimepewa nafasi tosha ya kuuliza maswali niliyokuwa nayo na nimetosheka na majibu ya maswali hayo yote.

Ya kuwa ninajua kwamba nakala ya habari zote nitakazotoa zitahifadhiwa kisiri na pia ninaweza kukatisha kushiriki kwangu katika utafiti huu wakati wowote nipendapo.

Ya kwamba nimeelezwa na pia kuandikiwa jina, nambari ya simu na anwani ya mtu ninayeweza kuafikiana naye kunapotokea hali ya taharuki.

Nunakubali kushiriki kwa kujitolea mwenyewe katika utafiti huu

Sahihi ya Mshiriki ..... Tarehe.....

Sahihi ya Mtafiti..... Tarehe .....

Sahihi ya Shahidi ..... Tarehe ...

### **Appendix III: Questionnaire**

#### Section A: Demography

Name of interviewer.....

Date: .....

Serial Number of Interviewee:.....

Location.....

Sub-location.....

Village .....

Telephone.....

Age in years

18 – 30 years

30 – 39 years

40 – 49 years

Above 50 years

Sex

2.1 Male

2.2 Female

3. Marital Status

3.1 Single adult

3.2 Monogamous married

3.3 Polygamous married

3.4 Separated/divorced

3.5 Widowed

4. Highest level of education

4.1 Never went to school

4.2 Primary school

4.3 Secondary school

4.4 College/Polytechnic

4.5 University

5. Occupation/ Source of Income

5.1 Employed

5.2 Farming

5.3 Business

5.4 Other

Section B: Clinical Evaluation

**6. VITAL SIGNS**

6.1. Respiratory rate:.....per minute

6.2. Pulse rate: .....per minute

6.3. Temperature :.....(°C)

7. SIGNS /SYMPTOMS (Check list)

SIGNS/SYMPTOMS	PRESENT	
	YES	NO
7.1 Headache		
7.2 Skin rash/infection		
7.3 Diarrhoea		
7.4 Loss of weight		
7.5 Cough		
7.6 Fever		
7.7 Oral thrush		
7.8 Pallor		
7.9 Lymphadenopathy		
7.10 Oedama		
7.11 URTI		
7.12 Herpes zooster		
7.13 Karposis Sarcoma		
7.14 PCP		
7.15 Cryptococcal		
7.16 Loss of appetite		
7.17 Fatigue		
7.18 Pneumonia		
7.19 Boils		
7.20 TB		
7.21 Piles		
7.22 Genital Lesions		
7.23 Night sweat		

## 8. MEDICATION

MEDICATION	YES	NO
8.1 Septrin		
8.2 Anti –TB drugs		
8.3 Other Anti –biotics		
8.4 Nutritional Supplements		
8.5 Herbs		

## 9. PERFORMANCE ACTIVITY SCALE

9.1 Normal Activity (Stage 1)

Normal Activity but with weight loss < 10% (Stage 2)

Bedridden < 50% days in past month (Stage 3)

9.4 Bedridden > 50% of the days (Stage 4)

### Section C: Nutritional Information

How many meals do you have per day?

One

Two

Three

Four

10. Tell me how often do you consume the following foods?

Food	Number of times per week		
	1/2/3/4/5/6	Everyday	None
<b>Foods rich in Carbohydrates</b>			
10.1 Maize			
10.2 Rice			
10.3 Cassava			
10.4 Millet			
10.5 Sorghum			
10.6 Sweet Potatoes			
10.7 Irish Potatoes			
10.8 Pumpkin			
10.9 Plantain (Matoke)			
10.10 Wheat Products			
10.11 Fats And Oils			
10.12 Sugar			
10.13 Others (List)			
<b>Foods rich in Proteins</b>			
10.14 Beef			
10.15 Chicken			
10.16 Fish			
10.17 Milk			
10.18 Eggs			
10.19 Beans			
10.20 Seeds e.g. pumpkin Sim sim			
10.21 Peas			
10.22 Nuts			
10.23 Other (List)			
<b>Fruits</b>			
10.24 Orange			
10.25 Paw paw			
10.26 Mangoes			
10.27 Ripe bananas			
10.28 Avocado			
10.29 Guava			
10.30 Lemon			
10.31 Water Melon			
10.32 Other (List)			
	<b>Number of times per week</b>		
<b>Food (Cont'd)</b>	<b>1/2/3/4/5/6</b>	<b>Everyday</b>	<b>None</b>



<b>Vegetables</b>			
10.33 Sukuma			
10.34 Spinach			
10.35 Carrot			
10.36 Cabbage			
10.37 Tomatoes			
10.38 Pumpkin Leaves			
10.39 Kunde Leaves			
10.40 Mrende			
10.41 Saka			
10.42 Miro			
10.43 Managu			
10.44 Cassava Leaves			
10.45 Bean Leaves			
10.46 Onions			
10.47 Other (list)			

11. Was yesterday a normal days pattern

1=Yes      2=No

12. If no please explain how it was different from your normal daily patterns

.....

## **Appendix IV: Laboratory experimental procedures**

### **1: ANALYSIS OF SERUM ZINC LEVELS**

(Butzow and Eichhorn, 1975; Kornberg A, 1982; Prasad A, 1995; Sandstead, 1994; Sandstrom, 1997).

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. 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. Clinical effects of zinc deficiency can be present in the absence of abnormal laboratory indices. Clinicians consider risk factors (such as inadequate caloric intake, alcoholism, and digestive diseases) and symptoms of zinc deficiency (such as impaired growth in infants and children) when determining the need for zinc supplementation.

### **ANALYSIS**

#### **Procedures for cleaning containers**

Soak the containers in hot water containing detergent for about two hours then clean thoroughly with a brush.

Rinse thoroughly with tap water then with De-ionized water.

Soak them in 50% nitric acid for the glassware and 2 % for the plastic bottles for 18 hours.

Pour out the acid and rinse twice with cold De-ionized water.

Fill the containers with hot De-ionized water and let stand for one hour then do more rinsing with hot De-ionized water.

Dry the tubes in an oven.

## **Calibration standards for zinc**

### **100 mg/l of zinc**

10ml of 1000 ppm. Zinc solution (Zinc standard for AAS) is pipette into 100 ml volumetric flask and made to the mark with de-ionized water. Transfer into a plastic bottle.

### **10 mg/l of zinc**

10 ml of 100 ppm. Zinc solution above (1) is pipette into 100 ml volumetric flask and made to the mark with de-ionized water. Transfer into a plastic bottle.

### **100 µg/100 ml of zinc**

10ml of 10 ppm. Zinc solution (2) is pipette into 100ml volumetric flask and made to the mark with de-ionized water. Transfer into a plastic bottle.

## **Preparations for the seronorm solutions**

### **Dilution 1:9 (x10)**

0.125 ml of the reconstituted seronorm is pipette into a plastic tube containing 1.125 ml of De-ionized water. This is prepared in triplicate. Vortex the mixture for 30 seconds and let to settle for 2 minutes before being aspirated into the AAs.

### **Dilution 1:9 (x20)**

0.25 ml of the reconstituted seronorm is pipette into a plastic tube containing 2.25 ml of de-ionized water. This is prepared in triplicate. Vortex the mixture for 30 seconds and let to settle for 2 minutes before being aspirated into the AAS.

## **Calibration of the instrument**

The AAS is calibrated according to the following parameters:

The lamp current used is usually 3 mA

Delay time is zero

Sample introduction is manual

Measurement time is 1

Replicates 2

Using the coarse knob adjust the wavelength to 213.9 nm.

Set the slit width to 1.0.

Optimization- using the fine knob and the coarse knob align the lamp in order to obtain the lowest voltage produced by the lamp.

Zero the machine.

Open the gas valve, both air and 1.5 ml of acetylene gas.

Light flame.

Aspirate the standards followed by the samples.

## **2: ANALYSIS OF SERUM RETINOL LEVELS**

(Becker et al, 2007; Bjelakovic et al, 2008; Saltzman and King, 2007; Swami et al, 2007).

### **Preparation of standard curve**

To correct for any variation in extraction from the plasma matrix, an internal standard curve is prepared (retinal acetate that has extraction and chromatographic properties similar to those of retinol standards that are added to a matrix of pooled plasma)

### Standard curve

Retinol concentration ( $\mu\text{g}/\text{mg}$ )	0	0.1	0.2	0.4	0.6
Vitamin A deficient plasma (ml)	250	250	250	250	250
Retinol( $2\mu\text{g}/\text{ml}$ ) standard( $\mu\text{l}$ )	0	25	50	100	150
Retinal acetate( $2\mu\text{g}/\text{ml}$ )std( $\mu\text{l}$ )	62.5	62.5	62.5	62.5	62.5
Methanol ( $\mu\text{l}$ )	187.5	162.5	137.5	87.5	37.5

Vortex intermittently every 15 seconds for 1 minute. Extract and analyze as described for the samples below from step 3.

### Sample extraction

This is to be performed at room temperature in dim light

Pipette 250  $\mu\text{l}$  of homogenized serum into aluminum covered centrifuge tube (with a Teflon-sealed screw cap).

Add 250  $\mu\text{l}$  of 0.5  $\mu\text{g}/\text{ml}$  Retinal acetate internal standard and vortex at intermittent intervals of 15seconds for 1 minute.

Add 1.5 ml of HPLC grade hexane. Vortex as above.

Centrifuge at 3000 rpm for 2 minutes.

Remove upper layer with a pasture pipette and save into second aluminum covered tube.

Re-extract the lower phase (as in steps 3-5) and pool the two extracts together.

Evaporate extracts under gentle stream of N<sub>2</sub> in a water bath at 37°C.

Reconstitute extract (residue) in 100 µl of the mobile phase.

Inject 30 µl into the HPLC reverse phase column.

### **Calculation of results**

Retinol concentrations in unknown samples are 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) are plotted against the final retinol concentration for the spike pool standards.

Regression analysis of the data is performed for the retinol and the Y (vertical) axis intercept value is 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 yields a regression formula for calculating a scientific calculator or a computer can be used to perform this task.

### **3: ANALYSIS OF SERUM $\alpha$ -TOCOPHEROL**

(Traber M.G, 1999; Russell et al, 1986).

#### **$\alpha$ -Tocopherol assay**

Vitamin E is assayed using the High Performance Liquid Chromatography (HPLC). This assay can also be done simultaneously with vitamin A if some certain parameters are set. An HPLC method utilizing a UV and a fluorimetric detector linked in series is described. By use of a simple integrator-controlled time-switched relay, analysis of serum vitamin A and E is accomplished on the same chromatogram and at optimum sensitivity for each detector. A single internal standard (retinyl acetate) monitored only by the UV detector permits measurement of both vitamins over a wide linear range. Precision of the assays is satisfactory, both on a within-

day and on a day-to-day basis. Recoveries of both vitamins are virtually 100% whilst sensitivity is 2 µg/l (retinol) and 0.05 mg/l (α-Tocopherol), (Russell et al.,1986).

### Preparation of standard curve

The standard calibration curve is prepared by plotting a standard addition curve, where a series of known concentration of α-Tocopherol are used. The standard used is α- Tocopheryl acetate. If the assay is being done simultaneously with retinol, then an internal standard (retinyl acetate) is required in addition to the standard addition. The internal standard is used to calculate the peak ratio of the retinol concentration.

### Sample of a standard curve if the assay is simultaneously done with retinol

Retinol conc.(µg/mg)	0	0.1	0.2	0.4	0.6
Vitamin E and A deficient plasma (µl)	250	250	250	250	250
Retinol (2µg/ml)standard (µl)	0	25	50	100	150
Retinol acetate(2µg/ml)std(µl)	62.5	62.5	62.5	62.5	62.5
Methanol(HPLC Grade) (µl)	187.5	162.5	137.5	87.5	37.5

Vortex intermittently for 15 seconds for 1 minute. Extract and analyze as described for the samples below from step 3.

### Sample extraction

**NOTE:** This is to be performed at room temperature in dim light

Pipette 250 µl of homogenized serum into aluminum covered centrifuge tube (with a Teflon-sealed screw cap).

Add 250  $\mu\text{l}$  of 0.5  $\mu\text{g}/\text{ml}$  retinyl acetate internal standard and vortex at intermittent intervals of 15seconds for 1 minute.

Add 1.5 ml of HPLC grade hexane. Vortex as above.

Centrifuge at 3000 rpm for 2 minutes.

Remove upper layer with a pasture pipette and save into second aluminum covered tube.

Re-extract the lower phase (as in steps 3-5) and pool the two extracts together.

Evaporate extracts under gentle stream of  $\text{N}_2$  in a water bath at  $37^\circ\text{C}$ .

switch on the HPLC machine and allow it to warm up for about 30 minutes before using it, then set al the required parameters:-

Prepare the mobile phase (75 ml acetonitrile and 25 ml methanol) then degas to remove air bubbles.

Wavelength- 292 nm

Retention time (RT) - guided by the standard but it is normally around 8-10 minutes.

Temperature- regulated automatically by the machine and ranges from  $0\text{-}350^\circ\text{C}$ .

Column- (Silica C18 waters)

Reconstitute extract (residue) in 100  $\mu\text{l}$  of the mobile phase.

Inject 30  $\mu\text{l}$  into the HPLC reverse phase column.

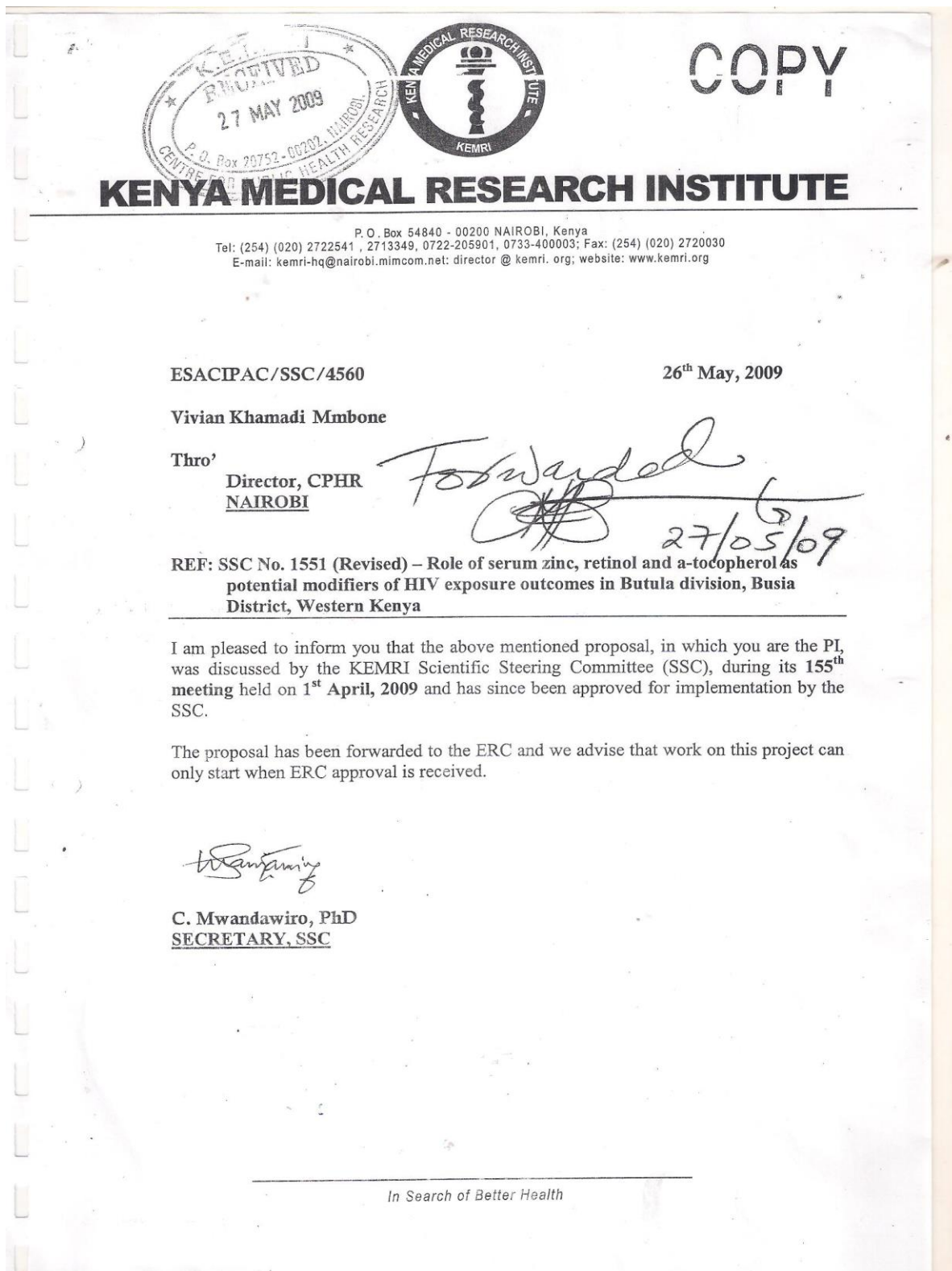
### **Calculation of results**



$\alpha$ -Tocopherol concentrations in unknown samples are determined from a standard curve prepared by the standard addition ( $\alpha$ -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 has known concentrations and values for the  $\alpha$ -Tocopherol.**

Appendix V. SSC approval



**Appendix VI. ERC approval**



## Appendix VII. Publication approval



# KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/AJHS/358/2013

23<sup>rd</sup> January, 2014

Vivian Mmbone Khamadi  
Institute of Tropical Medicine and Infectious Diseases,  
Jomo Kenyatta University of Agriculture and Technology,  
Box 62000-00200  
**NAIROBI**

Dear Madam,


AJHS/2013/358 – Serum Zinc, Retinol and á - Tocopherol in the HIV Positive and Negative subjects in Busia County, Kenya, by Vivian M. Khamadi et al

This is to inform you that the above manuscript has been approved for publication in the African Journal of Health Sciences.

It was noted that your study was on Serum Zinc, Retinol and á - Tocopherol in the HIV Positive and Negative subjects in Busia County, Kenya,

Thank you for taking interest in the journal.

Yours faithfully,

  
DR. HUDSON NLODENYO  
EDITOR-IN-CHIEF  
**AFRICAN JOURNAL OF HEALTH SCIENCES**

## Appendix VIII. Manuscript

21/01/2016

The Editor-In-Chief,

African Journal of Health Sciences,

P.O Box 54840-00200,

NAIROBI

Dear Sir,

Please find enclosed a manuscript entitled: ‘‘Micronutrient Deficiencies (Zinc, Retinol and  $\alpha$ -Tocopherol), Morbidity patterns and Dietary practices among the HIV positive and negative subjects in Busia County, Kenya’’, which is being submitted for exclusive consideration of publication as an article in the African Journal of Health Sciences

By submitting the manuscript to the African Journal of Health Sciences, the authors understand that the material presented in this paper has not been published before nor has it been submitted for publication to another scientific journal or being considered for publication elsewhere. I attest that this work has been approved by all the authors. The authors also understand that should the submitted material be accepted for publication in the journal, we will automatically transfer the copyright to the publisher.

I hope that the reviewing process finds the manuscript acceptable for publication in the journal.

Sincerely Yours,

Vivian Mmbone Khamadi,

P.O Box 19084 - 00100,

NAIROBI.

Mobile: 0720 389439

Email: [mmbone2014@gmail.com](mailto:mmbone2014@gmail.com)

This manuscript has been submitted for consideration of publication as an article in the African Journal of Health Sciences with our approval as University Supervisors.

## **COPYRIGHT STATEMENT**

We, Vivian Mmbone Khamadi, Charles F.L. Mbakaya, Yeri Kombe and Anselimo Makokha, the undersigned, who are the authors of the manuscript titled ‘‘Micronutrient Deficiencies (Zinc, Retinol and  $\alpha$ - Tocopherol) among the HIV positive and negative subjects in Busia County, Kenya’’, transfer all copyright ownership of this manuscript to the African Journal of Health Sciences, in the event that the manuscript is published in the Journal. We give guarantee that the content of the manuscript is original, and is not currently being considered for publication by another Journal.

### **Micronutrient Deficiencies (Zinc, Retinol and $\alpha$ - Tocopherol) among the HIV positive and negative subjects in Busia County, Kenya**

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## **SUMMARY**

**Introduction:** Although management of HIV/AIDS in Kenya has been on activities such as awareness creation, condom distribution, provision of anti-retroviral therapies and more recently on male circumcision, there is increasing evidence that micronutrient deficiencies play an important role in HIV transmission and progression (NACC, 2000). This study endeavored to fact find if these deficiencies truly occur in the HIV positive persons and if the deficiencies extend to the total population in the community including the HIV negative population, and if so, was there a difference in the two groups. In addition, the study looked at two other factors related to micronutrient deficiencies. These were 1) morbidity patterns, in terms of occurrence of

opportunistic infections, and 2) the dietary practices among the HIV positive persons and as a comparison of the same in their HIV negative counterparts.

**Methods:** A case control study which was carried out within 3 selected health centers in Butula division within Busia County from August to October, 2009. Cases (HIV positive persons) were recruited for the study as they came in to seek treatment from the facilities and met the selection criteria while controls (HIV negatives) were recruited by systematic sampling. A total of 155 study subjects (78 HIV positive, 77 HIV negative) took part in the study.

**Results:** The levels of Zinc, Retinol and  $\alpha$ -tocopherol were similar in both the HIV positive and negative subjects with more than 60% of the population manifesting deficiency in the three micronutrients. However, the study subjects deficient in CD4+ cell count were 24.4% and 3.9% in HIV positive and negative subjects, respectively ( $P < 0.001$ ). In terms of morbidity, the disease burden among the two groups was similar. Of the twenty-three signs and symptoms associated with HIV/AIDS that were evaluated, a significant difference was noted in lymphadenopathy (12.8% HIV positive, 3.1% HIV negative,  $P = 0.005$ ), Upper Respiratory Tract Infections (34.6% HIV positive, 19.5% HIV negative,  $P = 0.034$ ) and Skin rash (25.6% HIV positive, 7.8% HIV negative,  $P = 0.003$ ). The dietary practices and food intake patterns were similar among the two groups of respondents where comparison of consumption of the different food groups between the HIV positive and negative participants revealed no significant difference among the two groups.

**Conclusion:** Even though there were slight differences in the levels of micronutrients (Zinc, Retinol and  $\alpha$ -Tocopherol), the morbidity patterns and the dietary practices among the HIV positive the HIV negative persons, the differences were not significant. Thus, more work is needed to elucidate these differences in the HIV seropositive and seronegative subjects in Kenya and beyond.

## **Introduction**

Control of HIV/AIDS remains a major challenge in Kenya. High prevalence of HIV with regional variations, low levels of HIV testing, HIV discordance within couple relationships and concurrent epidemics of other sexually transmitted infections (STI) make management of the HIV epidemic difficult and complex [1] Management of HIV/AIDS has been on activities such as awareness creation, condom distribution and provision of anti-retroviral therapies though there is increasing evidence that micronutrient deficiencies play an important role in HIV transmission and progression [2] . The role of nutrition is increasingly recognized as evidenced by the development and adoption of various nutrition interventions such as counseling cards, guidelines on care and support and food recommendations for persons using ARV drugs [3, 4]

Deficiencies of micronutrients such as zinc, retinol (vitamin A) and  $\alpha$ -tocopherol (vitamin E), which are needed by the immune system to fight infections, are common in people living with HIV/AIDS [4]. Observational studies have suggested that both protein energy malnutrition (PEM) and micronutrient deficiencies may hasten the progression of HIV infection, and that HIV worsens malnutrition. HIV infection and malnutrition form a “vicious cycle” of immune

dysfunction, infectious disease, and malnutrition [5]. Zinc deficiency is the most prevalent micronutrient abnormality seen in human immunodeficiency virus (HIV) infection. According to WHO statistics, a person is said to be zinc deficient when their plasma levels fall below 100µg/dL. Low levels of plasma zinc predict a 3-fold increase in HIV-related mortality, whereas normalization has been associated with significantly slower disease progression and a decrease in the rate of opportunistic infections [6]. Zinc supplementation significantly reduces the risk of CD4 cell counts falling below the critical 200 cells/mm<sup>3</sup> level. Nutritional levels of zinc supplementation given to HIV-infected adults results in a 4-fold decrease in the likelihood of immunological failure [7].

**Most observational studies have found that low vitamin A** levels are associated with increased risk of transmission of HIV from mother to child [8]. Vitamin A supplementation in HIV-infected children, on the other hand, has been associated with protective effects against mortality and morbidity, similar to that seen in HIV-negative children. The risk for lower respiratory tract infection and severe watery diarrhea has been shown to be lower in HIV-infected children supplemented with vitamin A. All-cause mortality and AIDS-related deaths have also been found to be lower in vitamin A-supplemented HIV-infected children [9]. Vitamin E is an important antioxidant and anti-inflammatory that is often deficient in HIV-positive people. Vitamin E is necessary to ensure the optimum functioning of cell membranes. Deficiency may interfere with efficient immune functions. A study of 310 men followed for nine years found that those with serum vitamin E levels above 23.5µm/l had a significantly reduced risk of disease progression. A strong correlation was noted in this cohort between intake of supplements containing vitamin E on entry into the study and high blood levels of vitamin E [10].

## Materials and Methods

### Study Area

This study was carried out in the then Busia district (which is currently Busia County) in the Western Province of Kenya from August to October, 2009. Busia County is the most westerly point of the Trans - Africa High-way in Kenya, with a HIV prevalence of 33% [1] and a thriving commercial sex business that made the people of Busia County vulnerable to HIV/AIDS.

### Sample size and Sampling

Selection of the respondents from the eligible population was done by simple purposive sampling where recruitment of cases and controls was done in three (3) selected health centers in Butula division. These three health facilities namely, Bumala A Health Centre, Bumala B Health Centre and Bukhalarire Health Centre, were selected from a total of seventeen (17) health facilities in Butula division on the basis that they were the only health facilities offering support services to the HIV positive persons at the time of study within the division. Sample size for the cases (HIV positive subjects) was derived using the formula shown below; [11]

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



Where,

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

Reduces to  $n = 21\sigma^2 / d^2$

Where  $\sigma^2$  = variance and

$d$  = difference to be detected =  $\mu_1 - \mu_2 = 10 \mu\text{g/dL}$  serum and  $\sigma^2 = 18^2$ .

Therefore,  $n = 68$

Thus, 68 PLWHA were recruited and since it was a case control study, an equal number of HIV negative respondents were recruited as the control group. The study subjects in the control group were matched to the cases according to their age-groups at the beginning of the fieldwork – data collection. This brought the total number of respondents to 136 study subjects. Allowing for 12% non response, the sample size was adjusted upwards to 77 per arm, giving a total sample size of 154 study subjects. During data collection, one extra HIV positive respondent was interviewed and the researcher found it best not to waste such valuable information. This addition however did not seem to have a significant effect on the data analysis. Thus, a total sample of 155 participants was recruited for the study, 78 study subjects in the HIV positive arm and 77 study subjects in the HIV negative arm.

All the study subjects were recruited as they visited the health centers for treatment or for other healthcare services. The cases (the HIV positive) were recruited as they came and as long as they met the selection criteria and were willing to take part in the study. As for the controls (the HIV negative), systematic sampling was applied where every other 3<sup>rd</sup> person visiting the centers was selected. This was based on the fact that according to the Kenya AIDS Indicator Survey, 2007 (NASCO, 2009), about 33% of the population was infected with HIV/AIDS, which translated to 3 out of 10 persons in the division likely to be living with HIV/AIDS. In total, the study subjects recruited were; HIV positive 78 (50.3%) and HIV negative 77 (49.7%).

### **Data collection**

A detailed questionnaire was used to interview subjects once they had been recruited and consent given. The questionnaire was divided into three sections; Section A collected the basic demographic data; age, sex, highest level of education attained and one's marital status. Section B obtained data on the pattern of morbidity including signs and symptoms associated with HIV infection of each respondent after a physical examination was done by a qualified physician. Use of any form of medication at the time of the study was established in addition to determination of one's performance activity regarding how actively the respondent was involved in their day-to-day activities. The questionnaire also determined the participants' dietary habits in section C, by assessing their weekly food consumption.

### **Laboratory analysis**

Blood samples were taken once at baseline after consent was given by the research subject. Five millimeters (5 ml) of venous blood was obtained by a trained phlebotomist using a 21-gauge needle and put into two kinds of vacutainers; 3 ml of blood were put in the 4 ml plain vacutainers and 2 ml of blood was put in the EDTA vacutainers. The samples in the plain vacutainers were allowed to settle, spun in a centrifuge at 3000 rpm and serum transferred using pipette tips

into two 4 ml acid washed vials covered in aluminum foil. These samples were kept frozen at – 80 degrees Celsius and were later analyzed for serum zinc (1 ml), retinol (1 ml) and  $\alpha$ -tocopherol (1 ml) at KEMRI laboratories. The samples in the EDTA tubes were immediately analyzed at KEMRI laboratories on arrival for CD4+ cell counts (1 ml) and confirmation of HIV status (1 ml).

## **Statistical analysis**

The quantitative data from the field was coded and double entered into a database designed using MS-Access application. Data analysis was conducted using IBM SPSS version 20.0 statistical software. Univariate analysis was done for descriptive statistics such as proportions to summarize categorical variables and measures of central tendency such as means for continuous variables. Bivariate Analysis was used to test for the strength of association between categorical variables. Odds Ratio (OR) and 95% Confidence Interval (CI) were used to estimate the strength of association between independent variables and the dependent variable. The threshold for statistical significance was set at  $p < 0.05$ . Multivariate analysis was performed to identify independent predictors of HIV sero-status.

## **Results**

### **1. Demographic and Socioeconomic Characteristics**

A total of 155 study participants with comparable distribution of HIV sero-status 50.3% (78) HIV positive and 49.7% (77) HIV negative) were interviewed. Median age was 36 (Range 18 to 60 years.) Gender distribution revealed a high proportion of females 81.3% (126) than males 18.7% (29). A relatively high proportion of the participants 72.3% (112) had not obtained secondary school education. Majority 78.1% (121) were practicing farming with a very small proportion 3.2% (3) were employed. Most of the participants 73.5% (114) were married comprising 60.0% (93) Monogamous, and 13.5% (21) Polygamous.

Table 1 shows the distribution of HIV positive and negative study subjects according to selected demographic and economic characteristics. Mean age among the HIV positives was 37 compared to the HIV negatives, 35. Distribution of age varied between HIV positives and HIV negatives, with a statistical significant difference observed in age category 40 – 49 years ( $P=0.043$ ). Distribution of other characteristics (i.e. level of education, marital status, and occupation) was not significantly different between the HIV positives and negatives.

**Table 1: Demographic and Socioeconomic characteristics in relation to HIV status**

Variables	HIV Positive (n=78)		HIV Negative (n=77)		OR	95% CI		p value
	n	%	n	%		Lower	Upper	
<b>Age in years</b>								
<30 years	10	12.8	18	23.4	0.93	0.32	2.66	0.886
30 - 39 years	33	42.3	25	32.5	2.20	0.91	5.33	0.081
40 - 49 years	23	29.5	14	18.2	2.74	1.03	7.27	<b>0.043</b>
50 or more years	12	15.4	20	26.0	Reference			
<b>Gender</b>								
Male	17	21.8	12	15.6	1.51	0.67	3.42	0.322
Female	61	78.2	65	84.4	Reference			
<b>Level of education</b>								
<Secondary	55	70.5	57	74.0	0.84	0.41	1.70	0.625
>=Secondary	23	29.5	20	26.0	Reference			
<b>Marital status</b>								
Single	5	6.4	8	10.4	0.51	0.14	1.93	0.325
Monogamous married	39	50.0	37	48.1	0.87	0.38	2.01	0.741
Polygamous married	10	12.8	10	13.0	0.82	0.27	2.54	0.735
Separated/divorced	7	9.0	8	10.4	0.72	0.21	2.48	0.604
Widowed	17	21.8	14	18.2	Reference			
<b>Occupation</b>								
Business	19	24.4	10	13.0	0.48	0.05	4.84	0.530
Farming	55	70.5	66	85.7	0.21	0.02	1.92	0.166
Community health worker	4	5.1	1	1.3	Reference			

## 2. Selected Biochemical and immunological indicators

Assessment of Biochemical and immunological indicators in relation to HIV status was done as presented in Table 2. Zinc: Median serum zinc level was 85ug/dl for all the subjects ranging from 34 to 242 ug/dl. Zinc deficiency was found in 69.7% (108) of the respondents (cut-off 100ug/dl). Zinc deficiency was observed in a higher number of HIV positive respondents (35.9%) as compared to the HIV negative respondents (29.9%), but the difference was not statistically significant. Retinol (Vitamin A): Median vitamin A level was 0.244µg/dl for all the subjects ranging from 1.19 to 33.65 µg /dl. Vitamin A deficiency was observed in 69.0% (107) of the participants (cut-off 9.0µg/dl). Deficiency in vitamin A however was slightly lower (65.4%) in HIV positives compared to HIV negatives (72.7%), but the difference was also not statistically significant

α-Tocopherol (Vitamin E): Median vitamin E level was 6.05 µg /dl for all the subjects ranging from 1.19 to 33.65 µg /dl. The level of vitamin E deficiency was observed in 67.7% (105) of the participants (cut-off 9.0µg/dl). Vitamin E deficiency was observed in a higher number of HIV

positive respondents (71.8%) as compared to the HIV negative respondents (63.6%), but the difference was not statistically significant. CD4+ cell counts: Median CD4 count was 729 counts/mm<sup>3</sup> for all the subjects ranging from 6 to 2455 counts/mm<sup>3</sup>. Low CD4 count was found in 14.2% (22) of the participants (cut-off 350 cells/mm<sup>3</sup>). Low CD4 count was statistically significant (24.4%) in the HIV positive respondents compared to the HIV negative respondents (3.9%), (OR= 7.94; 95% CI: 2.24 – 28.14; P<0.001).

**Table 2: Selected Biochemical and immunological indicators in relation to HIV status**

Variables	HIV Positive (n=78)		HIV Negative (n=77)		OR	95% CI		P value
	n	%	n	%		Lower	Upper	
<b>Zinc level</b>								
Deficient	55	70.5	53	68.8	1.27	0.57	2.81	0.555
Not deficient	23	29.5	24	31.2	Reference			
<b>Vitamin A level</b>								
Deficient	51	65.4	56	72.7	0.71	0.36	1.41	0.323
Not deficient	27	34.6	21	27.3	Reference			
<b>Vitamin E level</b>								
Deficient	56	71.8	49	63.6	1.45	0.74	2.86	0.277
Not deficient	22	28.2	28	36.4	Reference			
<b>CD4 level</b>								
Low	19	24.4	3	3.9	7.94	2.24	28.14	<0.001
High	59	75.6	74	96.1	Reference			

### 3. Morbidity patterns

Assessment of the relationship between HIV sero-status, occurrence of signs and symptoms and performance activity scale was done as presented in Table 3. Twenty-three (23) signs and symptoms were reported in the course of clinical evaluation. Out of these, only three (3) were statistically significantly associated with HIV status. Presence of skin rash showed a significant difference (25.6%) in the HIV positive respondents compared to the HIV negative respondents (7.8%), (OR= 4.08; 95% CI: 1.54 – 10.83; P=0.003). Similarly, presence of lymphadenopathy was also significantly associated with HIV positive sero-status (OR= 11.18; 95% CI: 1.39 – 89.60; P=0.005). Occurrence of Upper Respiratory Tract Infection (URTI) showed a significant difference (34.6%) in the HIV positive respondents compared to the HIV negative respondents (19.5%), (OR= 2.19; 95% CI: 1.05 – 4.55; P=0.034). Analysis of the performance activity scale in relation with HIV sero-status revealed that the proportion of participants in stage 1 was significantly high in HIV negatives (77.9%) compared to HIV positives (48.7%), (OR= 0.27; 95% CI: 0.13 – 0.54; P<0.001).

**Table 3: Signs and symptoms and Performance Activity Scale in relation to HIV status**

Variables	HIV Positive (n=78)		HIV Negative (n=77)		OR	95% CI		P value
	n	%	n	%		Lower	Upper	
<b>Skin rash</b>								
Yes	20	25.6	6	7.8	4.08	1.54	10.83	<b>0.003</b>
No	58	74.4	71	92.2	Reference			
<b>Lymphadenopathy</b>								
Yes	10	12.8	1	1.3	11.18	1.39	89.60	<b>0.005</b>
No	68	87.2	76	98.7	Reference			
<b>URTI</b>								
Yes	27	34.6	15	19.5	2.19	1.05	4.55	<b>0.034</b>
No	51	65.4	62	80.5	Reference			
<b>Performance Activity Scale</b>								
Stage 1	38	48.7	60	77.9	0.27	0.13	0.54	<b>&lt;0.001</b>
Stage 2 or more	40	51.3	17	22.1	Reference			

**3.1 Use of Medication**

Analysis of use of medication and other nutritional supplements in relation to HIV sero-status was assessed as presented in Table 4. Overall assessment on use of any type of medication and other nutritional supplements revealed significant association with HIV sero-status, where a significant difference was observed in the HIV positive respondents (53.8%) compared to the HIV negative respondents (9.1%), (OR= 11.67; 95% CI: 4.76 – 28.57; P<0.001). Use of septrin was also significantly associated with HIV positive sero-status (35.9%) compared to the HIV negatives (2.6%) (OR= 15.68; 95% CI: 3.55 – 69.32; P<0.001). Similarly, use of nutrient supplements was observed in the HIV positive respondent only (7.7%) compared to the HIV negatives (0.0).

**Table 4: Use of medication in relation to HIV status**

Variables	HIV Positive (n=78)		HIV Negative (n=77)		OR	95% CI		P value
	n	%	n	%		Lower	Upper	
<b>Use of medication</b>								
Yes	42	53.8	7	9.1	11.67	4.76	28.57	<b>&lt;0.001</b>
No	36	46.2	70	90.9	Reference			
<b>Septtrin</b>								
Yes	28	35.9	2	2.6	21.00	4.79	92.11	<b>&lt;0.001</b>
No	50	64.1	75	97.4	Reference			
<b>Anti -TB drugs</b>								

Yes	7	9.0	2	2.6	3.70	0.74	18.40	0.167
No	71	91.0	75	97.4	Reference			
<b>Other Anti -biotics</b>								
Yes	3	3.8	1	1.3	3.04	0.31	29.89	0.620
No	75	96.2	76	98.7	Reference			
<b>Nutrient supplements</b>								
Yes	6	7.7	0	0.0	UD	UD	UD	0.028
No	72	92.3	77	100.0	Reference			
<b>Herbs</b>								
Yes	3	3.8	2	2.6	1.50	0.24	9.24	1.000
No	75	96.2	75	97.4	Reference			

#### 4. Dietary habits and Food intake patterns

##### . Frequency of meals

Close to two-third of the participants (60.6%) consumed two meals per day as compared with only 26.5% who consumed three meals. Analysis of the frequency of meals in relation to HIV sero-status was assessed and no statistical significant difference was noted as shown in Table 5.

**Table 5: Frequency of meals in relation to HIV status**

P	HIV Positive		HIV Negative		OR	95% CI	
	(n=78)		(n=77)			Lower	Upper
Variables	n	%	n	%			
<b>Number of meals per day</b>							
Once 0.649	9	11.5	11	14.3	0.78	0.27	2.28
Twice 0.987	48	61.5	46	59.7	0.99	0.48	2.07
Thrice	21	26.9	20	26.0	Reference		

Food frequency analysis was done on consumption of foods from the different food groups on a weekly basis among the study participants. Consumption of foods rich in carbohydrates among the study participants on a weekly basis revealed that millet, sorghum, irish potatoes and pumpkins were consumed frequently (5 – 6 times weekly) and some carbohydrates rich foods that were never consumed by a high percentage of the study participants included; Irish potatoes (50.3), Pumpkin (49.6%), Millet (46.5%), Matoke (41.4%) and Wheat products (41.3%). Assessment of consumption of food rich in proteins on a weekly basis revealed that most of the animal and plant protein rich foods were consumed either moderately (3 – 4 times weekly) or occasionally (1 – 2 times weekly). A few were consumed frequently (5 – 6 times weekly) namely eggs (by 1.3% of the targeted population), simsim seeds (10%) and peas (1.3%). Some of the study participants never consumed some of the protein rich foods for a period of one week. The protein rich foods that were never consumed by a high percentage of the study participants included; Peas (66.5%), Simsim seeds (61.6%), Nuts (49.6%), Eggs (26.5%) and Milk (23.2%).

Analysis of consumption of fruits weekly showed that One fruit, the Avocado was consumed daily by one participant (0.7%). A few fruits were consumed frequently (5 – 6 times weekly) namely watermelon (by 7.7% of the targeted population), jackfruit (3.9%), oranges (1.3%) and avocado (0.65%). Some of the study participants never consumed some of the fruits for a period of one week. The fruits that were never consumed by a high percentage of the study participants included; Watermelon (74.9%), jackfruit (74.1%), lemon (64.5%), and pawpaw (32.9%). Analysis of weekly consumption of vegetables showed that quite a number were consumed frequently (5 – 6 times weekly) namely cassava leaves (by 11.6% of the targeted population), bean leaves (6.5%), spinach (4.5%), carrots (3.9%), tomatoes (0.7%), pumpkin leaves (0.7%) and mrenda/jute mallow (*Corchorus olitorius*) (0.7%).. The vegetables that were not consumed by a high percentage of the study participants included; Cassava leaves (84.5%), Bean leaves (76.1%), Spinach (67.7%), Carrots (54.5%) and Cabbage (54.2%).

## **Discussion**

### **Selected Biochemical and Immunological indicators of the respondents**

The biochemical and immunological indicators carried out in this study included determination of serum zinc levels, retinol levels,  $\alpha$ -tocopherol levels and CD4+ cell count levels in both the HIV positive and negative respondents. Of the named five indicators, only one, the CD4+ cell count levels, was found to have a statistically significant difference in terms of low count between the two study groups, 24.4% low count among the HIV positive group and 3.9% low count among the HIV negative group (P value < 0.001). The significant difference in CD4 levels among the two study arms can easily be attributed to the low immunity among the HIV positive individuals, where generally, a low immunity is equated to low levels of CD4+ cell count. In general, the CD4 (%CD4+ or absolute count) progressively decreases as HIV disease advances. According to WHO guidelines, the pathogenesis of HIV infection is largely attributable to the decrease in the number of T cells (a specific type of lymphocyte) that bear the CD4 receptor (CD4+). Progressive depletion of CD4+ T cells is associated with progression of HIV disease and an increased likelihood of opportunistic infections and other clinical events associated with HIV, including wasting and death [12]. Thus, as anticipated, the PLWHA had significantly lower more CD4+ cell count than their HIV negative counterparts.

It was also noted from this study that more than 60% of the population was deficient in all the other indicators, with no statistical significant difference in terms of deficiency between the HIV positive and negative respondents. These were; serum zinc, 69.7%; vitamin A, 69% and vitamin E, 67.7%. These deficiencies were contrary to the anticipated results. At the conceptualization of the study, it was anticipated that deficiency of the biochemical and immunological indicators would definitely occur among the HIV positive study population and not necessarily among the HIV negative study population. But as seen from the results of this study, it can be deduced that deficiency in zinc, vitamin A and vitamin E, seems to be a common occurrence among the wider population in Busia County, Kenya. Most of the population was deficient in these indicators whether one had HIV or not. Being HIV negative did not necessarily translate into one not being deficient in the said indicators. Similar results have been observed in studies done in Addis Ababa, Ethiopia by Fufa [13] and Ndagije [14] in Rwanda.

### **Morbidity patterns of the respondents**

During clinical evaluation, twenty-three (23) signs and symptoms were reviewed for both the HIV positive and negative respondents. Of these, only three (3) symptoms were found to have a statistical significant difference between the two study groups. These were lymphadenopathy, (12.8% HIV positive and 3.1% HIV negative, P value = 0.005), Upper Respiratory Tract Infections, (34.6% HIV positive and 19.5% HIV negative, P



value = 0.034), and Skin rash, (25.6% HIV positive and 7.8% HIV negative, P value = 0.003). These three symptoms are mainly associated with an immuno-compromised immune system resulting from infection with HIV virus [11].

Another indicator that showed a statistical significant difference between the HIV positive and the HIV negative respondents was the Performance Activity Scale in stage 1 (48.7% HIV positive and 77.9% HIV negative, P value = <0.001). This indicator, was a measure of how the respondents were actively involved in their day-to-day activities without experiencing either weight loss and/or being bedridden due to sickness. The significant difference resulted in stage 2 where more of the HIV negative respondents did not experience either 10% or more total body weight loss within one month nor were they bedridden due to sickness for less or more than 50% of the time within one month as compared to their HIV positive respondents. This result was generally anticipated due to the heavy disease burden experienced by the HIV positive respondents. They were mores sick, making them seek more rest and hence be less able to go about their daily activities compared to their HIV negative counterparts.

Two other clinical indicators showed a statistical significant difference between the HIV positive and negative respondents. These were the use of medication and other nutritional supplements (53.9% HIV positive and 9.1% HIV negative, P value = <0.001) and the use of Septrin (29.5% HIV positive and 2.6% HIV negative, P value = <0.001). Generally, use of any type of medication and other nutritional supplements among the participants revealed a significant association with those who were HIV sero-positive. The medications used were Septrin, Anti-TB drugs and Antibiotics while the nutritional supplements included food supplements and traditional herbs. It can be deduced from the findings that that the HIV positive respondents seemed to really value their health and thus used various methods to treat their opportunistic infections and also boost their immune system in order to prevent further ill-health. Of the medications used, use of septrin was highly associated with HIV positive sero-status (OR= 15.68; 95% CI: 3.55 – 69.32; P<0.001). Septrin was the commonly used drug among the HIV positive respondents, whether it was by prescription of a qualified physician or self prescribed by the individuals, being in line with the Ministry of Health (MOH) guidelines of care of HIV patients. It can also be noted here that use of micronutrient supplements was found exclusively among HIV positive individuals, although the exact nature of the micronutrients was not established. Results from the Kenya Aids Indicator Survey [8], show similar findings of septrin and micronutrient use among the HIV positive subjects.

### **Dietary practices and food intake pattern of the respondents**

The analysis of food frequency showed that close to two-third of the participants (60.6%) consumed two meals per day as compared with only 26.5% who consumed three meals. This can be interpreted that the socio-economic status of the majority of the

study participants was not high enough to enable them afford more than two meals per day. In a similar study [15] in Uganda, only 21.8% of the participants consumed three or more meals per day and this was greatly attributed to changes in prices of food being a major contributing factor. The frequency of meals has been shown per se to serve as a proxy indicator of consumption of macronutrients [16], [17]. Following that line of reason, it can be assumed in this study that the participants who consumed three meals per day would have higher amounts of the micronutrients in question as compared to the participants who consumed only two meals per day. It can also be argued that since the majority of the participants (60.6%) consumed two meals per day which equates to inadequate consumption of nutrients, it is therefore no surprise that more than 60% of the study participants were deficient in the three micronutrients in question in this study.

Analysis of the nutritional content of the foods that were never consumed by majority of the study population revealed that they are the very foods that were rich in the micronutrients in question in this study. For example, Avocado fruit is high in zinc and rich in Vitamin E. Pumpkin is rich in Zinc, Vitamin A and Vitamin E. Green Peas are rich in both Zinc and Vitamin A. Both Jackfruit and Watermelon are rich in Vitamin A. Spinach is high in all Zinc, Vitamin A and Vitamin E while Carrots are high in Vitamin A. If most of the study population did not consume the above named different categories of food, then it explains the high deficiency of most of the study population (up to 60%) in the micronutrients Zinc, Vitamin A and Vitamin E.

A comparison of consumption of the foods in the different food categories between the HIV positive and the negative study participants showed no significant difference among the two groups. Chicken, which was consumed more among the HIV negative and Pumpkin leaves which were consumed more by the HIV positives, were the only foods that showed a significant difference among the two groups at a P value of 0.042 and 0.023 respectively. This could possibly explain why the levels of serum zinc, retinol (vitamin A) and  $\alpha$ -tocopherol (vitamin E) were not significantly different in terms of deficiency between the HIV positive and negative respondents. It can be argued here that since micronutrients are derived from the food we consume and if the respondents had equal access to and consumed similar variety of foods regardless of one's HIV sero-status, then the respondents ended up having similar levels of micronutrients regardless of their individual HIV status.

## **Conclusion**

Deficiency in the micronutrients Zinc, Retinol (Vitamin A) and  $\alpha$ -Tocopherol (Vitamin E), seemed to be a common occurrence among the study population of Busia County. Most of the study population (more than 60%) was deficient in these indicators whether one had an HIV infection or not. Thus, being HIV positive did not necessarily translate into one being deficient in Zinc, Retino or  $\alpha$ -Tocopherol. More research is needed to

establish the reason behind the widespread deficiency of the micronutrients zinc, retinol and  $\alpha$ -tocopherol in the population of Busia County despite the availability of a wide variety of food

The pattern of morbidity was also similar as shown by the occurrence of signs and symptoms related to HIV/AIDS among the study participants. Being HIV positive did not make one experience more illnesses, neither did it make one less active in their day-to-day activities as shown by comparison of the Performance Indicator scale among the two study groups. But being HIV positive predisposed one to more frequent use of Medication as compared to being HIV negative.

Dietary practices were also similar among the HIV positive and HIV negative study subjects. Most of the HIV positives consumed two meals per day and ate a wide variety of foods from the different food groups examined in the study just as the HIV negatives did. there is need to promote and encourage the consumption of foods rich in Zinc, Vitamin A and Vitamin D, which will not only help the PLWHA to prolong their lives more, but will also boost the immunity of the whole population at large

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