HUMAN DIETARY SELENIUM DEFICIENCY AS A RISK FACTOR IN THE PATHOGENESIS OF ENDEMIC BURKITT’S LYMPHOMA IN NYANZA

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NOVEMBER 2010

Sumba, Peter Odada
Human dietary selenium deficiency
DECLARATION

This thesis is my original work and has not been submitted for a degree to any other university or for any other award.

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We confirm that the study was carried out by the candidate under our supervision as university Supervisors.

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DEDICATION

I dedicate this dissertation to all the poor children who are victims of cancer and socio-economic disparities. Their loss of schooling opportunities due to frequent illnesses, long distance search for water and firewood is a challenge in disease casual matrix. Let our scientific innovations change their bright faces and smiles to longer healthy life expectancy.
ACKNOWLEDGEMENTS

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**ABBREVIATIONS AND ACRONYMS**

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<tr>
<td>a-TTP</td>
<td>Alpha Tocopherol Transfer Protein</td>
</tr>
<tr>
<td>BL</td>
<td>Burkitt’s lymphoma</td>
</tr>
<tr>
<td>BCG</td>
<td>Vaccination given against tuberculosis</td>
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<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>CWRU</td>
<td>Case Western Reserve University</td>
</tr>
<tr>
<td>C-MYC</td>
<td>Gene mapped on human chromosome 8q24</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>eBL</td>
<td>Endemic Burkitt’s lymphoma</td>
</tr>
<tr>
<td>EBV</td>
<td>Epstein Barr Virus</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine tetra-acetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked immunosorbant assay</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
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<td>GFAAS</td>
<td>Graphite furnace atomic absorption spectrometry</td>
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<tr>
<td>GPx</td>
<td>Glutathione peroxidase enzyme</td>
</tr>
<tr>
<td>pGPx</td>
<td>Plasma glutathione peroxidase enzyme</td>
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<tr>
<td>HIV</td>
<td>Human immunodeficiency syndrome</td>
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<tr>
<td>HCT</td>
<td>Hematocrit</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>ID-1</td>
<td>Iodothyronine -5-deiodinase type 1 selenoprotein</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
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<tr>
<td>LDLs</td>
<td>Low Density Lipoproteins</td>
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</table>
MT  Metallothionein
NIH  National Institute of Health
NO  Nitric Oxide
Q-GPx Se  Quartiles of glutathione peroxidase selenium
Q-PCR  Quantitative PCR
PCT  Plateletcrit
PCR  Polymerase chain reaction
RT-PCR  Reverse Transferase PCR
RBC  Red blood cells
RDW  Red blood cell distribution width
PDW  Platelet distribution width
ROS  Reactive oxygen species
Sec –TrxR  Selenium Compromised Thioredoxine Reductase
SES  Socio-Economic Status
SUNY  State University of New York
TrxR  Thioredoxine Reductase
T3  Triiodothyronine
T4  Thyroxine
WBC  White Blood Cells
Zn  Zinc
## DEFINITION OF OPERATIONAL TERMS

<table>
<thead>
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<th>Definition</th>
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<tr>
<td>Anthropometric Measures</td>
<td>Measurements for the purposes of understanding human physical variation</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>Elements that control the activities of free radicals</td>
</tr>
<tr>
<td>Ataxia</td>
<td>Uncoordinated movement due to failure of muscle coordination</td>
</tr>
<tr>
<td>Burkitt's Lymphoma</td>
<td>B-Cell tumor associated with Epstein Barr Virus</td>
</tr>
<tr>
<td>Endemic</td>
<td>Prevalent in a particular locality most of the time</td>
</tr>
<tr>
<td>Free Radicals</td>
<td>Unpaired atoms</td>
</tr>
<tr>
<td>Hodgkins Lymphoma</td>
<td>A cancer of the lymphatic system</td>
</tr>
<tr>
<td>Holoendemic</td>
<td>Prevalent throughout the year</td>
</tr>
<tr>
<td>&quot;Hot Spots&quot;</td>
<td>Areas with high incident rate of endemic Burkitt Lymphoma in the region</td>
</tr>
<tr>
<td>&quot;Cold Spots&quot;</td>
<td>Areas with low incident rate of endemic Burkitt Lymphoma in the region</td>
</tr>
<tr>
<td>Kashin-Beck Disease</td>
<td>Disorder of the bones and joints of the hands and fingers, elbows, knees, and ankles of children and adolescents</td>
</tr>
<tr>
<td>Keshan Syndrome</td>
<td>Fatal form of cardiomyopathy, associated with selenium deficiency first observed in Keshan Province in China</td>
</tr>
<tr>
<td>Micronutrients</td>
<td>Essential substance like vitamins or minerals needed in minute quantities for growth</td>
</tr>
<tr>
<td>Myopathy</td>
<td>A neuromuscular disease in which the muscle fibers are dysfunctional.</td>
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Public health  
Science and art of preventing diseases

Retinopathy  
Damage to the blood vessels in the retina

Selenium  
Essential trace element (named after Greek Moon Goddess)

Tonsillectomy  
Removal of tonsils

Z-Score  
Special application to monitor the dispersion from the mean
ABSTRACT

Endemic Burkitt lymphoma (eBL) has been associated with Epstein-Barr virus (EBV) and holoendemic Plasmodium falciparum malaria. However, recent eBL incidence maps showing more refined spatial clustering within malaria holoendemic areas, suggest that other risk factors may be involved in the etiology of eBL. Therefore, we hypothesized that the selenoprotein glutathione peroxidase (GPx), a surrogate of nutritional status known to regulate homonal, enzymatic pathways, viral mutations and virulence, is an important biomarker for eBL risk. The study measured plasma GPx, anthropometric markers of malnutrition, EBV viral loads and malaria parasitemia in children aged 1-9 years (n=258) from two Locations in Nyanza Province, Kenya with higher than expected and lower than expected incidence of eBL. The study participants were malaria asymptomatic children from the community. The level of GPX selenium was determined using BIOXYTECH® Plasma (pGPX) Enzyme Immunoassays™, USA. Q-PCR was used to determine EBV loads after extracting the DNA from EDTA preserved blood. Malaria parasite densities were determined from smears stained in 4% Giemsa by counting parasites against 200 wbc and multiplying results by 40 for values per microliter of blood. The distribution range of pGPx selenium in the study area was 0.55 – 7.1 mcg/dL mean 2.88 mcg/dL (n=167). Children residing in eBL low risk clusters had higher mean GPx Se 3.767, SD1.570. The min-max (0.910 -7.10mcg/dL) (n=66) and those living in eBL high risk areas had 2.308; SD 0.670; min-max GPx Se 0.589 – 3.330 mcg/dL (n=101), differences were significant at p<0.05 (P < 0.0001). The EBV loads by Q-PCR indicated that 43.9% (n=116) of 264 children tested, had non-detectable levels (NDL) EBV. Children (n=70) had 2.5 – 599 EBV copies and (n=78) had greater than 600 copies/mL of blood. The EBV (Q-PCR (+)) who were also tested for GPx selenium showed 70% of 74 children from eBL low risk areas had detectable EBV copies, mean [2.57; SD 0.80] compared to 47% detects in 68 children from eBL high risk areas [3.38; SD 1.53] copies/mL of blood. EBV load were lower in the eBL low risk areas as compared to eBL high risk areas (P <0.0001). The EBV loads were significantly higher in children with positive smears for malaria parasites [3.41±1.47] compared to those without malaria [2.65±1.0], p=0.001. Additionally, GPx levels were significantly lower in children with the highest EBV viral load and those with P. falciparum infections (p=0.035 and p=0.004, respectively). There was also a significant negative correlation between Selenium levels and EBV viral loads (r=0.157, P = 0.041). Comprehensive stepwise multiple regression model with EBV viral load as the dependent variable, age as a covariate, and pGPX Selenium levels, sex, malaria status and area of residence as predictor variables was performed. The selection was based on statistical significance when variables were correlated with GPX Selenium levels (P < 0.05). However, this particular analysis did not show any significant association between selenium levels and EBV viral loads (P = 0.843). The results suggest that selenium deficiency may be an important risk factor for eBL pathogenesis, in part, through the modulation of EBV viral loads as high viral loads have been shown to deplete GPX-selenium and its precursor cysteine which can affect liver detoxification, lymphocyte activation, viral transactivation and mitochondrial functions. Taken together, these results suggest that selenium deficiency may be an additional risk factor that needs to be considered in the etiology of endemic Burkitt lymphoma. The outcomes from the study are important public health surveillance tools. Increased EBV viral load suggests activation of EBV infection in children and may be considered a putative risk factor for eBL tumorigenesis.
CHAPTER 1: INTRODUCTION

1.1 Background Information

Endemic Burkitt lymphoma (eBL) remains the most prevalent paediatric cancer in sub-Saharan Africa with an annual incidence ranging from 2.05 – 5.0 per 100,000 children (Chuang et al., 2008a; Mbulaiteye et al., 2009a). The gravity of childhood cancer was demonstrated by a study in northern Israel which found annual incidence of pediatric solid cancer tumors of 77.1 per million (Agboola et al., 2009; Roguin et al., 1997). In sub-saharan Africa, it is hard to give the correct incidence rate due to lack of cancer registry and poor medical record keeping in many institutions in Africa. But emerging data indicate steady increase in the number of new cases of different cancers from Africa contributing to the global grid of non Hodgkin lymphomas. This grid form about 11% of all cancers found in children and young adults (Jaglowski et al., 2009). HIV/AIDS epidemic, malnutrition and nutrition transition are complex confounders in the causal matrix of this disease and other chronic diseases coming up in the region (Lazzi et al., 1998). The other factor which is fueling the number of cancers in Africa is the use of non-classified agricultural fertilizers, herbicides and pesticides (Agopian et al., 2009; Yu Chen et al., 2009). It is a major challenge to scientists and health practitioners that Burkitt’s lymphoma, the childhood cancer whose epidemiology originates from Equatorial Africa, remains neglected despite the massive information about its clinical history, good prognosis from prompt chemotherapy interventions (Pike, 1966). The disease has a well demarcated geographical epidemiology in sub-Saharan Africa (Figure 1) (Burkitt, 1961; Haddow, 1963).

This lymphoma also present clinical signs which are intermediate between the African form and other non Hodgkins lymphoma known as “Sporadic” (Ogada, 1974; Samaila, 2009; Israëls et al., 2009; Agboola et al., 2009; Wright et al., 2009); (Rainey et al., 2007a). The
geographical distribution of eBL in Africa correlates with holoendemic *Plasmodium falciparum* malaria transmission (Moormann *et al.*, 2005; Moormann *et al.*, 2007). However, a more detailed examination of eBL incidence in Nyanza Province in Kenya revealed closely spaced clusters with higher than expected and lowers than expected incidence of BL (Rainey *et al.*, 2008). A part from malaria, the other microbial commonly associated with eBL tumers is Epstein Barr virus (EBV) (EPSTEIN and BARR 1964; EPSTEIN, ACHONG, and BARR 1964); (Rosemary Rochford, Martin J Cannon, and Ann M Moormann, 2005a). However, EBV prevalence has global distribution. It exists as a latent B cell virus in over 90% of the world population (Griffin, 2000). Therefore the clustering of eBL cases in areas where these two etiological agents, malaria and EBV, occur as common childhood infections raises questions about the possibilities of other co-factors that may be involved in tumorigenesis of eBL (Costes 2009; Rosemary Rochford, Martin J Cannon, and Ann M Moormann, 2005b; Chene *et al.*, 2009a). There are many unanswered questions in the eBL epidemiology which lead the current study to focus on selenium as suspect environmental etiological agent of eBL.

Selenium is an essential micronutrient and an integral component of the antioxidant enzyme glutathione peroxidase (GPX) (Talas *et al.*, 2008; Dineley *et al.*, 2008). Reduced blood or plasma GPX Selenium levels are associated with increased oxidative stress, disruption of growth factors, cytokine signalling pathways, impairment of DNA repair and activation of transcription factors (Nishinaka *et al.*, 2001; Brigelius-Flohe *et al.*, 2004). Recent studies have shown that the uncontrolled activity of free radicals could be part of a multistep process driving genomic instability in eBL development (Jing Wu *et al.*, 2009); (Njie *et al.*, 2009a; Njie *et al.*, 2009b).

This process of physio- biological mechanization is known to influence the development, progression and treatment outcome of cancer (Hirst and Robson 2010); (Shibata *et al.*, 2010). A Low GPX activity or in particular GPX-1 polymorphisms have been correlated with enhanced risk of cancer in certain populations (Zhuo *et al.*, 2009). The selenium blood levels can be
compromised in time of prolonged hunger and malnutrition that affect expression GPX and high reactive oxygen species (ROS) levels seen in certain chronic diseases (Shibata et al., 2010; Valko et al., 2006). The data available suggest that selenium deficiency potentiates viral mutation and virulence in exposed hosts. For example, in one study low selenium levels were associated with increased HIV transmission from mother to child (Kupka et al., 2005) and another study demonstrated that selenium deficient HIV-seropositive women have higher levels of viral shedding (Baeten et al., 2001). Patients with tuberculosis and HIV co-infection had significantly lowered serum selenium levels (Kassu et al., 2006). In animal models, GPX-1 knockout mice were more susceptible to coxsackievirus infection and this was shown to be a consequence of altered host immune response (M A Beck et al., 1998; Sheridan et al., 2007). Interestingly, patients with vivax malaria had significantly reduced serum selenium compared to healthy controls suggesting that declines in selenium are linked to repeated malaria infections as well as perhaps dietary insufficiency (Seyrek et al., 2005). Together, this data suggests an important link between selenium deficiency and viral and parasitic infections. The EBV viral exposure persists in B cells in the peripheral blood (Babcock et al., 1998). DNA extraction of whole blood followed by quantitative Q-PCR gives an estimate of the viral load. In healthy western adults, EBV viral load in peripheral blood is difficult to detect (Niedobitek et al., 1991; Moormann et al., 2005). However, some studies found elevated EBV viral loads in young children living in a malaria holoendemic region (Moormann et al., 2005; Rasti et al., 2005; Donati et al., 2006). In addition, children with acute malaria have elevated cell associated and cell-free virus during an episode of acute malaria (Rasti et al., 2005; Donati et al., 2006; Yone et al., 2006; Njie et al., 2009a). A decline in EBV DNA in the plasma following treatment suggests a direct correlation between malaria infection and EBV reactivation (Donati et al., 2006; Yone et al., 2006). The current study took advantage of two closely linked regions in western Kenya that had higher than expected and lower than expected incidence of eBL to perform a cross-sectional
study to investigate whether differences in GPX Selenium in the plasma in children existed between these two regions and whether GPX Selenium levels correlated with peripheral EBV viral loads or malaria burden.

1.2 Statement of the Problem

Selenium (Se) is an integral part of the mammalian enzymatic and hormonal networks whose deficiency or reduced state potentiates viral mutations and virulence. The deficiency decreases the host ability to control the EBV loads and DNA repairs. This makes it an important etiological tool to be investigated in the endemic Burkitt’s lymphoma (eBL) geographical clusters in Nyanza Province.

1.3 Justification of the Study

High viral loads have been shown to deplete GPX-Selenium and its precursor cysteine which can affect liver detoxification, lymphocyte activation, viral transduction and mitochondrial functions. Establishing EBV viral load is an important public health tool as increased EBV viral load suggests dysregulation of EBV infection in children and may be considered a putative risk factor for eBL. The increased EBV viral loads are clinically relevant predictors for emergence of EBV-associated lymphoproliferative disorders in immuno-suppressed individuals with reduced GPX selenium. It may serve as a biomarker for eBL tumorigenesis in the geographical clusters. The potential risk is due to increased destabilization of normal physiological functions by increased Reactive Oxygen Species (ROS) production. The impact of low GPX Selenium has been noted to heighten the fetal intrauterine risks during pregnancy and lactation and can result in growth retardation and poor cognitive development. Selenium deficiency also compromises the physiological functions and bioavailability of CD8 T cells, CD2 T cells, B cells, and natural killer cells thus reducing the body’s ability to protection.
1.4 Research Questions

a. Does malaria densities and EBV loads in the areas reporting higher and lower than expected cases of eBL depend on varying levels of Selenium micronutrient in the BL geographical clusters in Nyanza Province, Kenya?

b. Does malnutrition affect the GPX Selenium levels, EBV loads and malaria density among study participants the eBL clusters?

1.5 Null Hypothesis

a. Selenium deficiency does not increase the risks of developing eBL in children exposed to holoendemic malaria in the eBL clusters.

b. Nutritional status does not affect malaria densities, EBV loads and GPX selenium in children in the BL clusters in Nyanza Province, Kenya.

1.6 Objectives of the Study

1.6.1 General objective

To investigate any differences in dietary plasma glutathione peroxidise (GPX) selenium and Epstein Barr virus (EBV) levels in children residing in malaria holondemic area with divergent incidence of endemic Burkitt’s lymphoma in Nyanza Province, Kenya.

1.6.2 Specific objectives

a. To determine the baseline pGPX selenium levels in children enrolled in the study area.

b. To determine EBV viral loads in the children participating in the study.

c. To determine malaria densities in the assymptomatic children enrolled in the study

d. To investigate sources of dietary pGPX selenium in the study area through common foods and household characteristics by questionnaire.
1.7. Study Significance

The GPX Selenium levels inversely correlate to EBV loads. Children living in eBL high risk areas were found to have reduced GPX Selenium and increased EBV loads and malaria densities.

The outcome suggest that deficiency or reduced level of selenium may be an important risk factor for eBL pathogenesis but not sufficient in the absence of other co-factors that modulate EBV viral loads in the classic model of multi-step tumorigenesis of eBL (Rochford et al, 2005).
2.1. Epidemiology of Endemic Burkitt’s lymphoma

Burkitt's lymphoma (BL) was first described as a clinical entity in children in Kampala, Uganda by Denis Burkitt in 1958 (Burkitt, 1961). The finding established the main prevalence regions to be ~10° N and 10° S of the equator, and along the eastern coast of the continent (Parkin et al., 1985). The eBL map indicated that 95% of victims were from areas experiencing an annual rainfall over 50 cubic meters on average and low temperature of 15.6° C and elevations less than 1,550 meters above sea levels (Haddow, 1963b). The unique clinical features and rapid devastating nature of the disease prompted scientists to investigate the causative agents (Fleming, 1985). The discovery of a knot coiled virus from blood culture suspension was just one step in understanding the etiology of eBL. The newly discovered virus was given the name of the investigating scientists Epstein and Barr thus Epstein Barr Virus (EBV) in 1964 (Glauser, 1983); (Klein et al., 2010). The geographic epidemiology data available established the occurrence of eBL tumors also exist outside the Equatorial belt of Africa. This led to the discovery of spatial distribution in many parts of the world (Sporadic BL) (Brady et al., 2008); Bornkamm, 2009). There is growing evidence indicating that pathogenesis of this disease takes a multistep process involving genetic instability (Gruhne et al, 2009) and involvement of polymicrobial agents (Chene et al, 2009b; Rochford et al., 2005a). The establishment of the co-factors or confounding agents in the BL tumorigenesis require further investigation on environmental etiologies of the disease. (Chiu et al., 2002; Srinivas et al., 1998). The high morbidity and poor prognosis in eBL case management is often blamed on delayed diagnosis, ill equipped medical personnel and scarcity of resources in health institutions. (Diagne et al., 2002; Tholouli et al., 2009). The distribution patterns of the disease in Equatorial Africa closely follow the malaria belt (Burkitt and O'conor 1961; Burkitt 1961). In Kenya malaria disease is endemic in Nyanza and Coast
Provinces (Snow et al., 2005). The two regions also experience high incidence rate of eBL cases (Mwanda et al., 2005; Mwanda, 2004); (Rainey et al., 2007). The eBL clustering in areas where the two etiological agents are ubiquitous may support the idea of co-factors or poly-microbial involvement in the BL tumorigenesis (Costes 2009; Rochford et al., 2005b; Chene et al., 2009a). Emerging data from Western Kenya indicating spatial distribution of BL in Nyanza Province alludes to the possibilities of co-factors in addition to EBV and holoendemic malaria in the etiology of BL (Rainey et al., 2007b). And strengthen information mapped out by Denis Burkitt in eBL belt in 1958 showing close link with malaria distribution map in Africa (Haddow, 1963b; Haddow, 1963a) (Figure 1).

![Figure 1: Burkitt’s lymphoma and Malaria Distribution in Africa](image-url)
Haddow AJ et al., 1963 BL map and MARA ARMA (Mapping Malaria Risk Areas in Africa 2001/WHO 2002 malaria distribution map in Africa. (Le Sueur et al., 1997); (Bayoh et al., 2001). The eBL distribution is marked by black dots and covers the darkened area which shows close distribution link with malaria endemicity as marked in dark green color indicated in the MARA ARMA (2001)/WHO 2002 malaria distribution in Africa. Malaria mapping and distribution in Africa closely associate with geographical mapping of eBL in the Equatorial region of Africa (Figure 1) but this may not apply to all lymphomas.

2.2. The clinical characterization of endemic Burkitt’s lymphoma

The lymphomas constitute a heterogeneous group of malignant disorders with different clinical behaviors, pathological features and epidemiological characteristics. (Bieging et al., 2010; Ng and Khoury, 2009); (Hjalgrim and E A Engels, 2008). The disease eBL is a unique B-cell malignancy with a high proliferation rate with devastating physical effects and marked genetic changes involving the c-myc oncogene (Gostissa et al., 2009). In Kenya, it constitutes important causes of morbidity and high mortality rate in children less than fifteen years of age (Mwanda, 2004; Rochford, et al., 2005a). In this region eBL is commonly seen in children and occasionally in adults with compromised immunity or organ transplant (Otieno and Whalen, 2001). The clinical presentation and the EBV involvement in BL vary with geographical location (Rainey et al, 2007a; Sisk and Wein 2006; Chuang et al., 2008b; Mbulaiteye et al., 2009b). It has been observed that BL cases from countries like India and Argentina present clinical features which are “intermediate” between the sporadic and endemic Burkitt’s lymphoma (Economopoulos et al., 2000; Rao et al., 2000); (Junquera Gutierrez et al., 1994). But one unique characteristic of all BL cases regardless of where they originate from, is the exhibition of one of the three c-myc/ Ig chromosomal translocations that leads to the activation of the c-myc gene as a crucial event in the development of this disease and Kikuchi histocytic necrotizing lymphadenitis. It has been shown that EBV displays distinct patterns of viral latent gene expression in the lymphoid and epithelial
tumors. The constant expression of latent membrane protein 2A (LMP2A) at the RNA level in both primary and metastatic tumors suggests that this protein might be a driving factor in the tumorigenesis of EBV-associated malignancies. LMP2A may cooperate with the aberrant host genome, and thereby contribute to malignant transformation by intervening in signaling pathways at multiple points, especially in the cell cycle and apoptotic pathway. Selenium is known to compromise a number of apoptotic pathways (Zhao-Yang Liu et al., 2004; Adam Heller, 2008).

Environmental risk factors may be associated with BL cases seen in areas with no known malaria existence, where a subset of Hodgkin lymphoma is strongly linked to Epstein-Barr virus infection (Souza et al., 2010; Van Geertruyden et al., 2009). In some cases infectious agents like EBV, HHS (human herpesvirus simplex), HIV are known to infect and transform lymphocytes while other types like hepatitis C virus, Helicobacter pylori may be associated with the development of various non-Hodgkin lymphoma (Dojcinov et al., 2010; Van Geertruyden et al., 2009).

2.2.1. Epstein Barr Virus and Burkitt’s lymphoma

The Epstein-Barr virus (EBV) has been associated with Burkitt’s lymphoma (BL), of B-cell origin and nasopharyngeal carcinoma (NPC), a tumor of poorly differentiated epithelial cells, infectious mononucleosis, and a variety of other malignancies including Hodgkin’s disease (Dojcinov et al., 2010). Many studies have demonstrated the strong association of EBV infection and eBL (de-The, et al., 1978). The causal involvement in eBL has been demonstrated by finding the viral genome in more than 96% of Burkitt’s lymphomas cases (Geser et al., 1983). The victims are exposed to EBV infections before tumor development (Neri et al., 1991). Subsequently the lymphomas express viral proteins that are oncogenic (Klein, et al., 1994). Further investigations revealed that BL patients have elevated EBV viral loads (Stevens et al., 2001; Moormann et al., 2005).
2.2.2. EBV Transmission

Epstein-Barr virus is known to be transmitted through the exchange of saliva from persistently infected individuals to children thus the name "kissing" disease (Mbulaiteye et al., 2006). However, research findings have isolated EBV in genital secretions, suggesting the possibility of early exposure of a child during birth (Ranjit et al., 2006). EBV exposure may also be enhanced by penetrative sexual intercourse, although transmission could occur through related sexual behaviors, such as "deep kissing." (Crawford et al., 2006). Multiple sexual partners have been identified as a risk factor for primary infection with Epstein-Barr virus (EBV) and its subgroups (Higgins et al., 2007). Further work in the area found involvement of EBV and Cytomegalovirus (CMV) in the incidence of testicular cancer (TC). These are landmark findings which indicate the extent to which EBV may be involved in a number of lymphomagenesis (Holl et al., 2008).

After the transmission, EBV is known to establish a life-long, persistent infection in the human host B cells. The virus easily lodges itself in the salivary glands and B cells resident in the esopharyngeal epithelium (Miller et al., 1990). From there, the virus can reach the mucosal lymphoid tissue (Laichalk et al., 2002; Thorley-Lawson and Babcock, 1999). It has also been shown that primary infections can cause infectious mononucleosis and mucocutaneous manifestations or may be associated with acute Gianotti-Crosti and hemophagocytic syndrome (Mendoza et al., 2008; Riyat, 1995). It has also been demonstrated that the EBV latent infection may result in hydroa vacciniforme, hypersensitivity to insect bites, and lympho-proliferative disorders like plasmablastic lymphoma, oral hairy leukoplakia, and post-transplant lympho-proliferative disorders, particularly in immunocompromised individuals (Talas et al., 2008; Milanino et al., 1993).
2.2.3. EBV Epidemiology

EBV is a strict human pathogen with of 90% worldwide distribution (Fleming, et al, 1985). In tropical regions the primary infection with EBV is asymptomatic and occurs in young infants as soon as maternal antibodies fade (Moormann et al., 2005; Henle and G Henle, 1970; Biggar, et al, 1978; Henle, et al, 1978). In contrast, infection is acquired throughout childhood and into early adulthood in developed countries (Henle et al., 1969). Early acquisition of EBV in the Sub-Saharan Africa is one of the known risk factor for eBL (Morrow, 1985; de-The et al, 1978). Close family studies implicated genetic and environmental factors in the etiology of nasopharyngeal carcinoma (NPC), a tumor known to be closely associated with Epstein-Barr virus infection (Stephensen et al., 2007; Arsova-Sarafinovska et al., 2009). In Kenya both eBL and NPC have been observed in different ethnics with almost equal frequencies. The episodes are accompanied with low viral frequency due to loss of the viral genome once malignancy has been initiated. (Griffin, 2000)

2.3. The impact of dietary selenium micronutrients deficiency or reduced levels

The investigations of dietary and environmental impact on chronic diseases like cancer had support from Peter Cleave’s theory that implicated lack of dietary fibres for common diseases in western civilization (Huawei et al., 2008; Steevenes et al., 2009; Connelly-Frost et al., 2009; Yang et al., 1988; Lee, 1996). Decades have passed but the negative impact associated with nutritional transition on health increases rapidly (Brian C-H Chiu et al., 2008; Morrow et al., 1985; Sakauchi et al., 2007). The dietary supply of antioxidants like selenium, zinc, copper and vitamins is important for normal growth and development because diseases conditions increase prooxidant elements that can deplete the enzyme glutathione peroxidase (GPx) a selenoprotein that modulates apoptosis by removing the cells affected by oxidative damage to preserve the tissue integrity (Talas et al., 2008; Dineley et al., 2008). The reduced or total collapse of the GPx antioxidant
system can trigger a continuous cycle of reactive oxygen species from the normal metabolic activities resulting in increased tissue oxidative stress and inflammation (Tian-Feng Chen et al., 2008). Selenium micronutrient deficiency can trigger anaemia due to acceleration of suicidal erythrocyte deaths (eryptosis) responding to increased reactive oxygen species (ROS) in the cells environment. The process is characterized by cell shrinkage and phosphatidylserine exposure at the erythrocyte surface due to an increase in the cytosolic Ca (2+) concentration and by ceramide (Stazi and Trinti, 2008). In Nyanza Province, the spatial distribution of the disease is marked by distinct clusters of “hot spots”, reporting higher than expected cases and “cold spots” reporting lower than expected cases of eBL (Rainey et al., 2007) Appendix II.

2.3.1. Physiological functions of GPx selenium in human

Selenium (Se) is a micronutrient necessary for human health and exists in the body as 25 selenoproteins with different physiological functions (Méplan et al., 2007; Baliga et al., 2008). The GPX Selenium micronutrient was investigated as a possible environmental etiological agent for BL. The study outcome clearly indicated an inverse relationship between GPX Selenium levels and EBV loads and malaria. The low concentrations of GPX Selenium in the subjects reflect true selenium deficiency or depression of selenium concentrations due to immune disturbances from exposure to diseases or inadequate household’s micronutrients supply (Tholouli et al., 2009). The plasma Glutathione peroxidase enzyme (GPx) is an indirect measurement of blood Selenium levels (Lamprecht et al., 2009; Mistry et al., 2008). These regulatory processes by the status of the body antioxidants stock, is key to either normal growth or triggers the multistep tumor development (Sahoo et al., 2009).

GPX which is a selenoprotein 1 has subgroups GPX 1 - 4 and defects or polymorphism in GPX may be associated with either cancer or other disease condition. The GPx-1 regulates levels of GPx-1 mRNA (Zhuo et al., 2009). This is why GPx-1 mRNA is an excellent molecular biological marker for assessment of selenium requirements. Selenium deficiency perturbs the GPx activities
and results in elevated intracellular peroxidative burden (Jau-Jiin Chen et al., 2007). The current study investigated selenium deficiency or reduced levels as an environmental risk factor for eBL because deficiency or reduced GPX Selenium increases oxidative stress, and Reactive Oxygen Species (ROS) which can disrupt the activities of growth factors and cytokine signaling pathways. The reduction in antioxidant activities can result in DNA damage and activation of transcription factors (Nishinaka et al., 2001; Brigelius-Flohé et al., 2004). The level of oxidative damage is also influenced by the activities of other antioxidant defenses provided for by zinc, copper and vitamins (Talas et al., 2008; Milanino et al., 1993). The defenses against infections and cellular damage are enhanced through dietary and nondietary antioxidants and antioxidant enzymes (Stephensen et al., 2007; Arsova-Sarafinovska et al., 2009). Nutritional-related mutageneses play an etiologic role in chronic diseases, including cardiovascular disease and cancer.

Emerging evidence indicate that a number of dietary mutagens are DNA reactive, leading to marked spectra of base-pair substitution mutations and structural chromosome changes (Wasantwisut, 1997a; Ferguson and Philpott, 2008; Rao, 1989; Selmi and Tsuneyama, 2009). The GPx selenium activities are regulated through individuals’ nutritional sources (Huawei et al., 2008; Steevens et al., 2009; Connelly-Frost et al., 2009; Yang et al., 1988). Chronic illnesses affect GPx selenium (1- 4) expression and activity due to increased levels of free radicals like nitric oxide, catalase, superoxide dismutase which are usually raised in chronic diseases (M L Hu and Tappel, 1987); (Valko et al., 2006). Normal GPX Selenium levels of 4 – 16 mcg/dL is known to inhibit activation of HIV infected cells but the reduced state of GPX Selenium or polymorphism in GPX-1 has been associated with some types of cancer or increased HIV viral multiplication (Diamond et al., 2001). The physio-regulation of body activity occasioned by selenium deficiency affects the normal activities of thioredoxin reductases and iodothyronine deiodinase families by modifying thyreocytes functions. The reduced antioxidants activity and
modification of redox status affects the metabolism of thyroid hormones with negative outcome on apoptosis, cell growth, modification of cell signaling systems and transcription factors. Growth in industrialization and modernization in agricultural subsector in developing and developed countries has increased exposure to environmental toxicants and other occupational risks for various lymphomas (Nasterlack, 2007; Lynch et al., 2009; Bonde, 2009; Orsi et al., 2009; Rull et al., 2009). Carcinogens commonly encountered in the environment are polycyclic aromatic hydrocarbons (PAH) (Karam et al., 2002). This can be through motor vehicle exhaust fumes, smoked fish, reused frying oils among other sources. Others are exposure to polychlorinated biphenyls (PCB) and other organochlorines in household water which may increase genetic instability and disruption of normal physiological functions (De Roos et al., 2005; Hardell, 2008).

The rising incidence of non Hodgkin lymphomas in the developing countries is raising questions about the safety of agricultural pesticides, herbicides and fertilizers (Agopian et al., 2009; Orsi et al., 2009; Bonner et al., 2010).

2.3.2 Selenium (Se) deficiency and Kashin – Beck disease

Selenium (Se) deficiency has been associated with predisposition for, or manifestation of many types of human diseases like Keshan and Kashin-Beck disease and cancer. The deficiency impairs immune function, promotes growth retardations, DNA damage, neurodegenerative, age-related disorders and disturbances of the thyroid hormone axis (Zeng et al., 2007). Selenium and iodine deficiency contribute to the pathogenesis of myxedematous cretinism. (Huawei Zeng and Botnen, 2007; Köhrle and Gärtner, 2009; Huawei Zeng, 2009). Disturbances in glutathione homeostasis has resulted in the progression of acute or chronic human diseases, including cystic fibrosis, cardiovascular related illnesses, inflammatory liver disease, immune, metabolic, and neurodegenerative diseases (Ferguson and Philpott, 2008; Selmi and Tsuneyama, 2009). The GPX levels, turnover rates, and/or oxidation state can be compromised by nutritional supply, inherited or acquired defects in the enzymes, cellular transporters, signaling molecules, or
transcription factors that are involved in its homeostasis, or from exposure to reactive chemicals or metabolic intermediates (Philip Thomas and Fenech, 2007). GSH deficiency or a decrease in the GSH/glutathione disulfide ratio manifests itself largely through an increased susceptibility to oxidative stress, and the resulting damage is thought to be involved in diseases, such as cancer, Parkinson's disease, and Alzheimer's disease (Nazzareno et al., 2009; Diamond, et al., 2001; Brigelius-Flohé, 2006). The imbalances in GPx levels affect immune system function due reduced antioxidant protection, and increase the aging process. (Nazzareno et al., 2009; Talas et al., 2008). GPxs reduce hydroperoxides to the corresponding alcohols by means of glutathione (GSH). PHGPx specifically interferes with NF-κB activation by interleukin-1, reduces leukotriene and prostanoid biosynthesis, prevents tissue damage and disease expression, and is indispensable for sperm maturation and embryogenesis (Arsova-Sarafinovska et al., 2009; Peters et al., 2008). Several lines of evidence support the role played by antioxidants such as selenium, zinc, Copper, Vitamin E, Vitamin C, Vitamin A, Vitamin B complex and Vitamin D in acute and chronic disease conditions (Chen et al., 1985). Vitamins and trace elements play a pivotal role in growth, development and protection from invading microbes.

2.3.3. The protective role of selenium against the free radicals in humans

GPX selenium molecules safely scavenge free radicals from the body to reduce the dangers posed by ROS to vital tissue damage. Reduced GPX Se increases cellular oxidative stress, tissue pathology, DNA damage, cancers, apoptosis, aging and a variety of inflammatory diseases. (Ferguson et al., 2008). The free radicals can be formed during oxidative metabolism and energy production in the body when oxygen interacts with certain molecules resulting in highly reactive radicals capable of initiating a chain reaction. This can involve enzyme catalyzed reactions, electron transport in mitochondria, signal transduction and gene expression, activation of nuclear transcription factors, oxidative damage to molecules, cells and tissues, antimicrobial action of neutrophils and microphages, cell aging and disease. The pathological effects of the free radicals
in the body are controlled by major antioxidants network maintained GPX Selenium and vitamins (Rotruck et al., 1973). Apart from metabolism, other sources of free radicals include environmental pollutants, agrochemicals, nitrogen oxide, ozone, cigarette smoke, radiation, halogenated hydrocarbons and heavy metals. Some drugs like doxorubicin, cyclophosphamide, 5-fluorouracil, methotrexate and vincristine are able to produce oxygen radicals at doses used in cancer treatment (Raich et al., 2001). Reactive Oxygen (RO) radicals in the body react with lipids, proteins, nucleotides and carbohydrates. When free radicals attack polysaturated fatty acids in the presence of oxygen, lipid peroxides are formed resulting in amplification of the original oxidative damage in the chain reaction (Heller et al., 2004). The macrophages in the endothelial wall of blood vessels easily take up the oxidized low density lipoproteins increasing chances of atherogenesis and other related diseases. These increased oxidative processes have been associated with degenerative changes seen in the elderly and cancer patients. A biomarker, 8-hydroxydeoxyguanosine which is a DNA base oxidation product can be detected in urines of those exposed to oxidative stress (Blot et al., 1993). Major household sources of dietary selenium are animal products, grains, vegetables like broccoli, mushrooms, potatoes, pumpkins and water melon seeds, fruits like prickly pears, Brazilian nuts, onion, garlic, cumin, cinnamon, and turmeric, various fruits mango, pawpaw pineapples among other plant sources. The Se-enriched Champignon Mushroom may have 30 to 110 mcg Se/g dry weights, while the Varnished Polypore (Ganoderma lucidum) could contain up to 72 mcg Se/g (dry weight). An increasingly growing database on chemical forms of selenium of mushrooms indicates that the seleno-compounds identified in carpophore include selenocysteine, selenomethionine, Se-methylselenocysteine, selenite, and several unidentified seleno-compounds; their proportions vary widely (Falandysz et al., 2008).

A part from the food and plants chains, metalloid Selenium (Selene), exists as metallic selenides mainly in iron, copper, lead, and nickel ores. One of the chief commercial sources is the flue dust
produced by the burning of pyrites to make sulfuric acid (Anderson et al., 2006). Selenium levels in soil generally reflect its presence in food and levels in human populations and can be influenced by geographical location, seasonal changes, and food processing methods (Safaralizadeh et al., 2007; Navarro-Alarcon and Cabrera-Vique, 2008; Karaj et al., 2009). The safe dietary requirement is estimated to 0.04 mg for infants 6 months of age, for adults 0.05 to 0.2 mg which vary according to regions (Rasmussen et al., 2006; Miyazaki et al., 2001). The insoluble powder of selenium sulfide is used externally in the control of seborrheic dermatitis, dandruff, and other forms of dermatosis. (Manriquez and Uribe, 2007; Prevost and English, 2008). Although the importance of selenium for bone metabolism is unknown, some clinical conditions such as Kashin-Beck osteoarthropathy have been associated with selenium deficiency. And it has been observed that selenium deficiency may be associated with growth retardation possibly due to abnormality in enzymatic and hormonal pathways (Moreno-Reyes et al., 2001). Selenium is both essential and hazardous element to humans and other animals. The concentration and chemical forms of Se in waterways and soil is controlled by various physico-chemical factors, including pH, chemical mineralogical composition, absorbing surfaces and oxidation/reduction status (Rotruck et al., 1973). Many populations worldwide exhibit selenium deficiencies but little is known about selenium deficiency within African populations (Combs, 2001a; Combs, 2001b). Endemic selenium deficiency is associated with heart disease, chondrodystropies and endocrine-neurological disorders (Combs et al., 2001). But not much is known about the impact of subclinical levels of selenium even though emerging data suggests that the deficiency potentiates viral mutations and increased virulence in humans and animals (Beck et al., 2000; Beck et al., 1998).

In animal models, GPX-1 knockout mice were more susceptible to coxsackievirus infection and this was shown to be a consequence of altered host immune response due to inadequate level of micronutrients in plasma (Beck et al., 2000; 1998). In some cases, selenium has been shown to
be chemopreventative, reducing risks in populations at risk for prostate cancer (Giovannucci et al., 1998) and reducing mortality due to lung cancer (Harrison, et al., 1997). The Nutritional Prevention of Cancer trial (Clark, et al., 1998; Raich, et al., 2001) demonstrated that daily oral supplementation of selenium reduced cancer risk for total carcinomas (45% less). A recent study found low selenium levels in newly diagnosed pediatric cancer patients with metastatic disease (Postovsky et al., 2003). These results suggest that selenium deficiency could increase the risk for cancer. Thus our hypothetical dream, that selenium deficiency may be the biomarker for the eBL clusters in Western Kenya. This essential micronutrient forms part of the enzyme glutathione peroxidase that deactivates free radicals. The role of selenium in innate and acquired immune responses is mediated by the incorporation of the twenty five selenoproteins into major human enzymes like glutathione peroxidases, glutathione reductase and theoredoxin reductase (Reeves and Hoffmann, 2009; Kupka et al., 2005; Bellinger et al., 2009) Selenium being a strong antioxidant, the deficiency or reduced intake affects the antibody titers, T cell proliferation and natural killer cell activity. The deficiency increases pro-oxidant activities due to free radicals and inflammatory cytokines. It also inhibits the conversion of nitrites (carcinogen) to nitrosamines from smoked, pickled and curried foods (Ayden et al., 1993). Reduced supply of these micronutrients may encourage proliferation of diseases like sinusitis and rheumatic fever known to cause inflammatory lesions of the mucous membrane which increases exposure to aerobic and anaerobic pyococci and haemophilus influenza (Linday, 2006; Kosar et al., 2005). This can promote the migration and localization of latently infected B cells to the mucosal tissue which can trigger the lytic cycle of EBV (Henle et al., 1970; 1969; Biggar et al., 1978). The eBL clusters in Western Kenya, require critical environmental evaluation for eBL etiological factors (Rainey et al., 2007). Selenium is important in managing the physio-chemical mechanization of the body and responsible for the activation of key enzymes fundamental to human health (Beck et al., 2004). And can reduce a variety of organic hydroperoxides to their corresponding alcohols. The
deficiency in animals is normally associated with abnormally high plasma T₄ levels and decreased T₃ levels (de-The, et al., 1978). This function of selenium has important implications for the interpretation of the effects of selenium deficiency when vitamin E levels are adequate. The role of selenium in human health cannot be over emphasized as its low levels is associated with frequent infections of the lower respiratory tract, bronchopulmonary displasia in infants, viral mutations, Keshan syndrome (cardiomyopathy), and Keshin-Beck disease (osteoarthropathy) common among children and women in certain geographical locations like China where it is deficient from the soil. The deficiency in humans has been implicated as a risk factor for recurrent pregnancy loss and male infertility (Chen et al., 1981)

2.3.4. Selenium and viral infection

Selenium is micronutrients pivotal for the host defense and is potentially important for viral replication and virulence (Molin et al., 2009). Viruses which infect a selenium-deficient host have been shown to mutate very fast as demonstrated from animal studies with Coxsackievirus and influenza virus repeatedly evolve the same specific stable viral mutations when Se-deficient mice are infected with these viruses. The mutations results in increased virulence of both viruses (Beck, 2001). Experimental study in human populations with low Se and higher Se levels using live attenuated vaccine strain of poliovirus resulted in increased mutations relative to individuals with higher selenium status (Malcolm et al., 2004; Gómez et al., 2001). Decreased Se is also associated with immune impairment, with both chemokine and cytokine levels altered. These findings demonstrated that Se status of the host can profoundly influence the genome of viral pathogen, leading to a new viral strain. Thus, host nutritional status should be considered when studying the mechanisms underlying the evolution of emerging viruses and may assist in predicting new viral outbreaks and devising new strategies to limit the emergence and spread of these pathogenic forms. (Beck et al., 1998).
The emergence of new infectious diseases and old diseases with new pathogenic properties is a burgeoning worldwide problem. Severe acute respiratory syndrome (SARS) and acquired immune deficiency syndrome (AIDS) are just two of the most widely reported recent emerging infectious diseases and the rapid evolution of viral species. Various hypotheses have been proposed, all involving opportunities for virus spread (for example, agricultural practices, climate changes, rainforest clearing or air travel). However, the nutritional status of the host, until recently, has not been considered a contributing factor to the emergence of infectious disease. In this review, we show that host nutritional status can influence not only the host response to the pathogen, but can also influence the genetic make-up of the viral genome. This latter finding markedly changes our concept of host-pathogen interactions and creates a new paradigm for the study of such phenomena (Beck et al., 2004a).

The first hint that a deficiency in Se may have an effect on a viral infection came from China in the early 1930's. A cardiomyopathy developed predominantly in women and children living in specific geographic regions of China in which the soil was deficient in Se. The juvenile cardiomyopathy (Keshan disease) has a dual etiology which involves both nutritional selenium (Se) deficiency and an infection with an enterovirus, amyocarditic strain of coxsackievirus (Allander, 1994; Sudre and Mathieu, 2001). This increased virulence is accompanied by multiple changes in the viral genome in a segment previously thought to be relatively stable. Epidemic neuropathy in Cuba has features that suggest a combined nutritional and viral etiology. (Beck et al., 2003). Malnutrition has long been associated with increased susceptibility to infectious diseases. The increase in severity from and susceptibility to infectious disease in malnourished hosts is known to result in impaired immune responses and increased oxidative stress from the free radicals. Pathologically Se-deficient mice develop myocarditis when infected with a normally benign strain of coxsackievirus. They also develop severe pneumonitis when infected with a mild strain of influenza virus (Nelson et al., 2001; Beck et al., 2004b). The immune system was altered
in the Se-deficient animals, as was the viral pathogen itself. Sequencing of viral isolates recovered from Se-deficient mice demonstrated mutations in the viral genome of both coxsackievirus and influenza virus. These changes in the viral genome are associated with the increased pathogenesis of the virus. The antioxidant selenoenzyme, glutathione peroxidase-I, was found to be critically important, as glutathione peroxidase knockout mice developed myocarditis, similar to the Se-deficient mice, when infected with the benign strain of myocarditis. (Melinda A. Beck, 2001). This demonstrates that specific nutritional deficiencies have some profound impact on the genome of RNA viruses and can influence the emergence of new viral strains (Beck et al. 2004a).

Conditions such as Cardiac inflammation are the hallmark of myocarditis due to many different signals which influence the influx of the inflammatory cells to the site of infection. Subsequently the immune cells, fibroblasts and endothelial cells are triggered to release chemokines that provide a chemical gradient to the site of infection. The multistep defense matrix is dependent on selenium status (Stazi and Trinti, 2008).

2.3.5. Selenium deficiency and Kashin-Beck disease

Kashin-Beck disease (KBD) is short stature caused by multiple focal necroses in the growth plate of the tubular bones under the influence of a number of oxidative pressure leading to a secondary, sometimes severe osteoarthrosis (Moreno-Reyes et al., 2001; Allander, 1994; Sudre and Mathieu, 2001). The disease was first described in Russia from the Bajkal area by Kashin 1848 and later, 1906, by Eugene Beck (Allander, 1994). The main theory originally proposed by Russian investigators was that KBD was caused by a toxic effect of mycotoxin but later the focus shifted to China where the causal theory has been based on the effects of selenium deficiency and its interaction with mycotoxins (Sudre and Mathieu, 2001; Moreno-Reyes et al., 2001).
2.3.6. The role of selenium in other cancers

The world is facing unprecedented emergence of new infectious diseases and old diseases with new pathogenic properties (Beck et al., 2004a). Severe acute respiratory syndrome (SARS) and acquired immune deficiency syndrome (AIDS) severity and progression may largely depend on the human host Selenium micronutrient status. In humans and animals it is known to regulate cell proliferation and apoptosis to maintain tissue homeostasis. The mechanisms of developing a number of human diseases are directly related to the control of cell cycle progression and apoptosis by selenium (Huawei Zeng, 2009). Understanding how selenium regulates the cell cycle can lead to a better understanding of the nature of selenium's essentiality and its role in disease prevention (Huawei Zeng and Botnen, 2007). The cellular exposure to free radicals like reactive oxygen species (OS) significantly contribute to genomic instability which is fundamental in many cancers development. Genomic instability provides the basic foundation for the impending neoplastic cells the ability to accumulate all of the requisite mutations for transformation within the relatively short time-frame of an organism's lifespan (Ferguson et al., 2004; K El-Bayoumy, 2001). These genome-altering properties of c-Myc suggest that they provide the protein with the ability to confer a "mutant phenotype" to cells in which its expression is deregulated (Prochownik and Youjun Li, 2007). It has been observed in gene array analysis that c-Myc, cyclin C, proliferating cell nuclear antigen, cyclin-dependent kinase (cdk)1, cdk2, cdk4, cyclin B and cyclin D2 mRNA levels are dependent on selenium status (Huawei Zeng, 2002).

There is increasing evidence that reactive oxygen species (ROS) are mediators in growth factor and cytokine signaling pathways. Mechanisms by which ROS can interfere with signaling cascades may include regulation of protein activities by the modification of essential cysteines (Arsova-Sarafinovska et al., 2009; Brigelius-Flohé, 2006; Brigelius-Flohé et al., 2004; Nishinaka et al., 2001). Selenium intake may be linked to a reduction of the chronic low-grade inflammatory state related to obesity and several accompanying disorders such as insulin resistance,
cardiovascular diseases, and metabolic syndrome. Study evaluating potential associations between Se and several indicators in healthy young adults, emphasizing on the putative effect of antioxidant trace element intake on inflammation-related marker concentrations. (Nishinaka et al., 2001; Brigelius-Flohe et al., 2004)

2.4. Other essential micronutrients and cancers development

Selenium and other essential trace elements are part of the human diet and his environment. When dietary supply is low in essential micronutrients, a number of people take multivitamins and supplements to support the body micronutrients demand (Rao, 1989; Miyazaki et al., 2001). Selenium takes the leading role due to its involvement in a number of biological pathways as a selenoprotein. The elements are needed for proper growth and development, but, unlike most vitamins and minerals, they are needed in extremely low quantities. Nickel (Ni) compounds have been found to cause cancer in humans and animal models and transform cells in culture and adequate selenium is required in the body to regulate its activity. This effect is mediated by stabilization of hypoxia inducible factor (HIF1a) and activating its downstream signaling which might either antagonize or enhance c-myc activity (Qin Li et al., 2009). A total of about 72 trace elements are required by the human body for normal development, growth and immune functioning (Metian et al., 2009). The amount of Selenium needed in food systems for adults is 40 mcg/d to support the maximal expression of the Selenium enzymes and the demand may reach 300 mcg/d to reduce risks of cancer depending countries and daily demand (Navarro-Alarcon and Cabrera-Vique, 2008; G F Combs, 2001c; Parizadeh et al., 2009). In Tehran the population reference was found to be 0.89 +/- 0.16 mg/L, 0.95 +/- 0.20 mg/L, and 0.099.10 +/- 0.021.78 mg/L, respectively (Farzin et al., 2009). Iodine Deficiency Disorders (IDD) is common in all populations (Zimmermann et al., 2008; Rodrigo et al., 2006). The availability of Iodine and other trace elements in the soil is affected by floods and erosion which leaches off the elements from the soil. This adversely affects iodine, selenium, zinc and lead mineral up take in food plants like...
cassava roots (*manihot utilissima*) (Islam *et al.*, 2009) The malnutrition impact negatively on women of reproductive age young children and adolescents many of which live in developing countries (Chandyo *et al.*, 2009). Greatest challenge to mankind is maintenance of adequate nutritional supply to households. Global differences in geological strata of essential elements availability contribute to lack of unified plasma or serum reference values of trace elements in the human’s worldwide (Rükgauer *et al.*, 1997). Physiologically, zinc is one of the most abundant nutritionally essential elements in the human body (Prasad, 2008). It is found in all body tissues with 85% of the whole body zinc in muscle and bone, 11% in the skin and the liver and the remaining in all the other tissues. Zinc intake ranges from 107 to 231 mcg/d depending on the source, and human zinc requirement is estimated at 15 mg/d. (Hambidge *et al.*, 2008).

Zinc is essential to the structure and function of numerous macromolecules and over 300 enzymic reactions (Tsujimoto *et al.*, 2008). The zinc ions exist primarily as complex constituents of proteins and DNA participating in all aspects of intermediary metabolism, transmission and regulation of the expression of genetic information, storage, synthesis and action of peptide hormones and structural maintenance of chromatin and biomembranes (Tapiero and Tew, 2003). The whole complex fabrics also involve others like selenium; copper, manganese, calcium among others. It has also been noted that some of the heavy metals are needed at lower concentrations for normal growth and development (Saletta *et al.*, 2010). The immune responses to antigens are regulated by glutathione (GSH) and its oxidized forms, Zinc ions (Zn++) and nitrogen monoxide (NO). Nitrogen monoxide is able to liberate Zn++ from metallothionein (MT), which is an intracellular storage molecule for metal ions like zinc (Zn++) and copper (Cu++). Both Zn++ and Cu++ show a concentration-dependent in activation of a protease essential for the proliferation of the virus such as HIV-1 (Sprietsma, 1999a) The Zinc activity has also been noted to reduce diabetes complications through its intracellular activation of the enzyme sorbitol dehydrogenase (SDH). A Zn++ deficiency can lead to a premature transition from efficient Th1-
dependent cellular antiviral immune functions to Th2-dependent humoral immune functions. It has been noted that deficiencies of Zn\(^{++}\), NO and/or GSH shift the Th1/Th2 balance towards Th2, as also noted in deficiencies of other essential nutrients like methionine, cysteine, arginine, vitamins A, B, C and E, zinc and selenium (Se). This is because these elements are necessary for the synthesis and maintenance of sufficient amounts of GSH, MT and NO. Via the Th1/Th2 balance, Zn\(^{++}\), NO, MT and GSH collectively determine the progress and outcome of many diseases. Deregulation of the Th1/Th2 balance is responsible for autoimmune disorders such as diabetes mellitus. This can result in raised levels of interleukin-4, 6, 10, leukotriene B4 (LTB4) and prostaglandin E2 (PGE2) and low levels of interleukin-2, Zn\(^{++}\), NO and other substances. This makes things easier for viruses like HIV-1 which multiply in Th2 cells but rarely, if ever, in Th1 cells. AIDS viruses (HIVs) enter immune cells with the aid of the CD4 cell surface receptor in combination with a number of co-receptors which include CCR3, CCR5 and CXCR4 (Nishi, 1996; Kidd, 2003). Remarkably, the cell surface receptor for LTB4 (BLTR) also seems to act as a co-receptor for CD4, which helps HIVs to infect immune cells. The Th2 cytokine IL-4 increases the number of CXCR4 and BLTR co-receptors, as a result of which, under Th2, the HIV strains that infect immune cells are precisely those that are best able to accelerate the AIDS disease process. The IL-4 released under Th2 therefore not only promotes the production of more HIVs and the rate at which they infect immune cells, it also stimulates selection for the more virulent strains. Zn\(^{++}\) inhibits LTB4 production and numbers of LTB4 receptors (BLTRs) in a concentration-dependent way. Zn\(^{++}\) helps cells to keep their LTB4 'doors' shut against the more virulent strains of HIV. Zinc deficiency also promotes cancer. Under the influence of Th1 cells, zinc inhibits the growth of tumours by activating the endogenous tumour-suppressor endostatin, which inhibits angiogenesis. (Sprietsma, 1999b). Because of the shift in Th1/Th2 regulation, the zinc-deficient patients develop severe immune dysfunctions, and easily succumb to infections. A part from depressed immune functions there is also decreased serum testosterone level,
oligospermia, hyperammonemia, neurosensory disorders, and decreased lean body mass. Zinc deficiency in developing countries is about two billion people. The people may be exposed to growth retardation, cognitive impairment, and adverse effects of many zinc-dependent enzymes and transcription factors (Prasad, 2008)

2.5. Malaria and eBL epidemiology

Malaria results from infection with a protozoan parasite of the *Plasmodium* family. Roughly 300 million cases of clinical malaria are reported each year in Africa, with over 900,000 deaths (Marsh *et al.*, 1999). There are four species that infect human beings, (*P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*). Severe morbidity and mortality are most commonly associated with *P. falciparum* infection. Malaria transmission dynamics vary in different geographic locales, such that people living in some areas experience sporadic (unstable, less intense) exposure whereas others experience continual repeated exposure throughout the year (holoendemic malaria) (Mbogo *et al.*, 1995; Snow *et al.*, 1997). Children living in areas of holoendemic transmission often have chronic asymptomatic infections characterized by the presence of malaria parasites, punctuated with repeated episodes of 'simple' morbidity (usually self-limited fever) (Marsh *et al.*, 1999). In these regions, most malaria morbidity and mortality occurs in children less than 5 years of age, suggesting that acquisition of immunity to *Plasmodium* infection is age dependent (Gupta *et al.*, 1994). By the age of five or six, these children generally experience only mild or asymptomatic infections (Marsh *et al.*, 1999). Chronic and intense malaria exposure, as occurs in holoendemic regions, has been hypothesized as to be associated with BL tumor promotion and progression (Morrow *et al.*, 1985). Chronic hyperendemic malaria infection leads to inflammatory conditions and translocation of latent EBV B lymphocytes. (Moxon *et al.*, 2009; Chene *et al.*, 2009c; Rochford, 2005a; Wright *et al.*, 2009; Nagy *et al.*, 2009). Based on the complexity of BL development, finding EBV in peripheral blood from healthy western adults is difficult to detect (Niedobitek *et al.*, 1991; Moormann *et al.*, 2005). However, I and others have
found elevated EBV viral loads in young children living in a malaria holoendemic region (Moormann et al., 2005; Rasti et al., 2005; Donati et al., 2006). In addition, children with acute malaria have elevated cell associated and cell-free virus during an episode of acute malaria (Rasti et al., 2005; Donati et al., 2006; Yone et al., 2006; Njie et al., 2009a). A decline in EBV DNA in the plasma following treatment suggests a direct correlation between malaria infection and EBV reactivation (Donati et al., 2006; Yone et al., 2006).

In the current study, we took advantage of two closely linked regions in Western Kenya that had higher than expected and lower than expected incidence of eBL to perform a cross-sectional study to investigate whether differences in GPX selenium in the plasma in children existed between these two regions and whether GPX selenium levels correlated with peripheral EBV viral loads or malaria burden.
CHAPTER 3: MATERIALS AND METHODS

3.1. Study Design

The study was a cross-sectional survey and the data collection started from 2006 to 2009 in different villages within the study area. It involved two hundred and seventy eight (n=278) healthy children who are permanent residents of the eBL defined clusters. The areas experiencing higher than expected cases of BL is regarded to be “eBL Hotspot” or high risk. This “hotspot” in the study covered Kanyawegi location in Winam division, Kisumu district. The low eBL risk area is North Seme in Kombewa division, Kisumu district areas. The geographical spatial mapping of this disease in the study was based on our previous work (Rainey et al., 2007) (Figure 2).

3.2. Study area

The average annual cases of eBL in the study area are 0.05 – 0.3/10,000 children in the coldspot and the distribution 0.97 – 2.00/10,000 by administrative locations based on 1999 population data (Figure 2). The two sites experience holoendemic malaria transmission with malaria prevalence in the area ranging from 43.2% to 52.2% (Oloo et al, 1996) and the individual infective vector contact rate being approximately 31.1 ib/p/yr (infective bite per person per year) (Ndenga et al., 2006). The map shows areas with higher than expected cases and those reporting lower than expected cases. The study focused in the eBL “hotspot” Kanyawegi marked dark black in the map and “cold spot” North Seme marked plain light in the map of Nyanza. The avarage annual risk of eBL in Nyanza Province (1999 censor) is 4.99 – 7.05/100,000 children by divisional boundaries (Rainey et al., 2007).

The two sites experience holoendemic malaria transmission with malaria prevalence in the area ranging from 43.2% to 52.2%. Individual’s infective vector contact rate is 31.1 ib/p/yr (infective bite per person per year). The study covered two defined clusters with divergent incidence cases of eBL. The eBL low risk area lies between 1,281.408 – 1,393.266 metres above the sea level.
The topography is mainly associated with many small table top hills and valleys with arable top soil. In contrast the altitude of eBL high risk area range from 1,122.426 – 1,217.637 metres above the sea level. The topography is mainly flat plain land and few table top hills with rich arable top soil. This particular study site borders the Lake Victoria basin and has major environmental abuse from ballast industries, quarry mining and sand harvesters. It has also very poor road infrastructure accessibility. The mean monthly rainfall in the two study areas 115.296; \(SD = 78.226\); \(SEM = 11.291\) cubic milliters and mean monthly temperatures of 23.229; \(SD = 1.021\); \(SEM = 0.147\) °C.

Figure 2: Map of Nyanza showing BL “hot and cold spots
3.2.1. Seme the eBL low risk area

This eBL low risk area lies between 1,281.408 – 1,393.266 metres above the sea level. The topography is mainly associated with many small table top hills and valleys with arable top soil. The area is well served with permanent rivers and access murram roads as shown in (Fig 3). The land here is intensively used for household food production. The common crops in the area include maize, millet, beans, peace, potatoes, cassava and groundnuts. The common fruit plants

Figure 3: GIS Map of North Seme, Kisumu West District, and the eBL “cold spot showing study households.
are bananas, mangoes, avocados, pawpaw, oranges and lemons. The shrubs form the major plant fauna and are commonly used as herbal tea.

3.2.2 Kanyawegi the eBL high risk area

Figure 4: GIS Map of Kanyawegi, Kisumu East District, and the eBL “Hotspot showing study households.

This area covers the eBL high risk cluster and the altitude ranges from 1,122.426 – 1,217.637 metres above the sea level. The topography is mainly associated with flat plains and few table top hills with rich arable top soil. This particular study site borders the Lake Victoria Basin and has very poor road accessibility. The water for household use is drawn mainly from the lake as there is poor river coverage. The arable land suffer a lot from anthropogenic activities including sand
harvesting, soil erosion due to unpresidented quary mining for blast industries in the area. The quary and ballast industries are major environmental pollutants in the area. Toxicants from the industries are getting their way into household water from river Mugrut which the main river in the area. Fumes of thick dark industrial smoke and quary dust are seen covering the sky daily from this part of the study area. The farms next to the lake are destroyed by hippos and other wild animals precipitating hunger in many villages in the area.

3.2.3: Rainfall and temperature pattern in the study area

The data collection covered a period of 48 months. The satellite data base in Kisumu showed the mean monthly rainfall in the area to be 115.296; SD = 78.226; SEM = 11.291 cubic millimeters with mean monthly temperatures of 23.229; SD = 1.021; SEM = 0.147 °C. There was marked increase in yearly rainfall distribution from 2006 to 2009 and there were minimal temperature fluctuations during the period as shown in 10% - 90% percentiles (Fig 5)

<table>
<thead>
<tr>
<th>Percentiles</th>
<th>10</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAINFALL</td>
<td>31.039</td>
<td>54.229</td>
<td>101.346</td>
<td>145.288</td>
<td>255.067</td>
</tr>
</tbody>
</table>

Figure 5: The percentile rainfall and temperature pattern in the study area
3.2.4. Malaria exposure in the study population

The two study sites lie within the malaria holoendemic belt and frequent exposure to malaria is a known risk factor for BL tumorigenesis. The transmission is maintained through infective bites of *anopheles gambiae* and *anopheles funestus* as common vectors in this region. The children less than 10 years of age are normally associated with high risk from clinical malaria.

3.2.5: Study Population

The children who completed the study were 278 (133 boys and 145 girls), less than 12 years of age and were permanent residents of the BL high risk areas of Kisumu Districts. The study population had two main characteristics; the population residing in the BL hot spot (Kanyawegi Sub-location) is composed mainly of parents/guardians whose preoccupation is small scale fishing using locally assembled canoes and low scale subsistence farming. In general this particular area is marked by low education attainment, few professional occupational positions, low income and reduced access to life opportunities. The population of the BL cold spot of North Seme locations have better education attainment and better occupation and wealth distribution as observed during mobilization and demographic survey.

3.3: Inclusion Criteria

The participants enrolled were residents of the selected study areas for at least six months. Only healthy children were recruited to the study. The child’s parents or guardians should understand and be willing to sign the written informed consent on behalf of their children. And in some cases children may be required to assent on their own if they are old enough.
3.3.1: **Exclusion Criteria**

Children with chronic disease, anemia with a hemoglobin level of less than 9.0 g/dl were not included in the study.

The children who had history of blood transfusions in the last six months.

Orphaned children or children living in orphanage

3.4. **Sampling technique**

Blood samples were collected to determine baseline GPX selenium levels in healthy children enrolled in the study. Blood was also aliquoted and stored in -80°C freezer or liquid nitrogen tanks for EBV DNA extraction and analysis by Q-PCR for viral loads. Some of the blood was used for hemoglobin estimation using Hemoque in the field and further validation of the results by coulter counter in the laboratory. Thick and thin blood smears were prepared in the field and were later stained in the laboratory using 4% Giemsa for quantification of malaria parasite densities. The study began by community mobilization and demographic survey. The GIS mapping was done for ease of household location, topological features documentation and other ecological factors. At this preparatory time, BL knowledge evaluation and general community health risks awareness was also evaluated among the participants. Stratified excel computer based random sampling was employed to generate the required numbers. This is done by selecting the empty column from study identity or individual names and then indicate start and end of selection \[=\text{RAND (A1:A100)}\] then hold the cursor down and drag down to generate the random numbers.

These techniques generated numbers can be categorized as per individual needs. It is worth noting that there are other computer based methods that can be used to generate the same. The current study had three age stratification for the analysis of EBV viral load in plasma (1-4; 5-8; and 9-12 years of age). The random computer generated stratification sampling can be applied to each age stratum in the two BL geographical clusters for unified selection.
3.5: Sample Size Calculations

The sample size requirements was determined using Jekel et al, 2001 formula for sample size calculation for test of differences in proportions \{N-sample size; Zα-alpha error; Zβ-beta error; \( p*(1-p) \)-variance; \((d)^2\)- difference to be detected\}.

\[
N = (Z_\alpha + Z_\beta)^2 \times \frac{p(1-p)}{(0.2)^2} = (7.84) \times (0.48) = 94.08.
\]

Based on prior preliminary data on eBL annual cases/100,000 children in high clusters is 4.9 and 0.39 in low clusters. Assuming that the predictor of the cluster is based on malnutrition index for Nyanza where more than 30% of children in peri-urban are malnourished. So the assumption is that a minimum of 40% of children have a selenium micronutrient deficiency and 60% of the children in the “hot” and “low” eBL have normal Se micronutrients, the difference to be detected is 20% (0.02). Data for alpha \((Z_\alpha)\) \(p=0.05\); therefore 95% confidence interval desired (t tailed test); \(Z_\alpha = 1.96\); data for beta \((Z_\beta)\) 20% error; therefore 80% power desired (two tailed test) \(Z_\beta = 0.84\); sample size required is \(95 \times 2 = 190\) individuals.

3.6: Ethical issues

Study participants were enrolled using written informed consent forms following recruitment through home visits or in meetings organized at centers within the study sites. Structured questionnaires were filled using face-to-face interviews. The approved ethical form detailing the purpose of the study and contact persons was availed and duly signed after conducting clients through the reading using a language they understood best. A copy of this structured document was give to the clients to keep. The study was approved by the Institutional Review Boards for SUNY Upstate University and the Ethical Review Committee, Kenyatta University and Kenya ethical review committee through the Kenya Medical Research Institute (KEMRI) Appendix v/ SSC /1025 /2006 with consent form Appendix iii.
3.6.1: Methods of Blood collection and processing

Non-fasting blood was collected from study children from 2006 to 2009. Sampling variations were minimized by using the same type of blood collection vacutainer tubes. At least 5ml of blood was collected through vein puncture by a phlebotomist using sterile green top heparinized tubes and purple to EDTA tubes for different analytical procedures. The auxiliary temperatures were taken to determine the clients' body temperature. Hemoglobin measurements were conducted on site for subjects' inclusion and exclusion criterion by portable Hemocue® (USA). Thick and thin blood smears were collected for parasite screening and density calculation following the World Health Organization (WHO) criteria. The number of asexual Plasmodium parasites was determined per 200 leukocytes and parasite density was calculated by multiplying by the white blood cell count assuming 8,000 White Blood Cell counts (WBC) per millilitre of blood.

3.6.2: Plasma Glutathione Peroxidase enzyme (pGPX) selenium

Selenium was determined from the cross sectional surveys samples by enzyme linked immunosorbent assays (ELISA) using commercially available kit (BIOXYTECH® Plasma pGPX Enzyme Immunoassays™ Oxis Research, Portland, OR, USA). 1μL of plasma was diluted 200 times with GPX ELISA diluting kit. 100 μL of the diluted sample was added to pre-coated removable 96 well plates and processed using standard ELISA procedure and finally read through computer aided ELISA reader.

3.6.3: Viral Loads Assessments

EBV viral loads were determined by real time quantitative Q-PCR. The viral loads were calculated as viral copies/µl total DNA as previously described by Moormann et al., 2005.
3.7: Data management and statistical analysis

The results from the field and laboratory were entered into FoxPro and FileMaker Pro data bases and were kept in password-protected data base computers with separate backups blinded and stored separately. The data analysis involved both descriptive and analytic phases. The descriptive analyses included the generation of frequencies, means, medians, standard deviations and ranges to assess the presence of outliers, possible errors and the normality of continuous variable distributions. Statistical analyses: The statistical analyses were performed using SPSS (PASWA) for windows (version 17.0/18) and GraphPad Prism for windows software (version 5.01). Mann-Whitney U and \( \chi^2 \) Square tests were conducted for pair-wise comparisons of median and proportions, respectively. Statistical associations between variables were examined using Pearson's correlation tests and stepwise multivariate regression analyses, controlling for age, gender, malaria status, and study site. Prior to performing Pearson's correlational analyses, the distributional characteristics of all variables were examined for departures from normality using the Kolmogorov-Smirnov test. Those variables with significant skewness were transformed toward normality. \( P \) values of less than 0.05 were considered statistically significant for all analyses.
CHAPTER 4: RESULTS

4.1. Demographic and clinical characteristics of study participant

Study participants were randomly enrolled within the two pre-selected clusters as previously described (Rainey et al. 2007): [eBL low versus high risk] n = 109, mean age 2.57 years; and n = 169, mean age 2.56 years; range, 1 - 12 years, respectively. The study children did not significantly differ by age groups \( (P = 0.70) \). Gender distribution was also not statistically different between the groups \( (P = 0.68) \). Auxiliary temperature differed between the groups \( (P < 0.001) \), with the individuals from eBL low incidence region having lower auxiliary temperature than those from eBL high incidence area. The prevalence of individuals with malaria parasitemia was significantly different by study geographical locations \( (P < 0.001) \) with children from high eBL incidence areas more likely to be parasitic for *P. falciparum*. However, malaria parasitemia was not statistically different between the gender groups \( (P = 0.531) \). Children residing in a high eBL incidence region were more likely to have lower hemoglobin concentration \( (P < 0.001) \) and more cases of anemia \(<11\text{g/dL}\) \( (P < 0.001) \) compared to children from the low eBL incidence area (Table 1). The two study sites had different demographic and socio-economic challenges. The main observed differences between the study sites were poor road infra-structure and poor household access to adequate nutrition in the BL high risk area \( (P < 0.0001) \).
Table 1: Demographic and clinical characteristics of study participants by region.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>eBL Low Incidence n=107</th>
<th>eBL High Incidence n=151</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (SD), years</td>
<td>5.08 (2.57)</td>
<td>4.96 (2.38)</td>
<td>0.70</td>
</tr>
<tr>
<td>Gender: n male (%)</td>
<td>51 (48)</td>
<td>68 (45)</td>
<td>0.68</td>
</tr>
<tr>
<td>Prevalence + for *parasites:</td>
<td>23 (22)</td>
<td>90 (62)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean *parasite density (SEM): µL</td>
<td>2262 (616)</td>
<td>7027 (2111)</td>
<td>0.55</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>1155</td>
<td>1085</td>
<td></td>
</tr>
<tr>
<td>Mean EBV log viral load (SD)</td>
<td>2.57 (0.81)</td>
<td>3.33 (1.56)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean hemoglobin (SEM): g/dL</td>
<td>12.05 (0.14)</td>
<td>11.50 (0.19)</td>
<td>0.016</td>
</tr>
<tr>
<td>Anemia† prevalence, n (%)</td>
<td>27 (25)</td>
<td>71 (47)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: Groups compared using t-tests, Mann Whitney U (parasite density), or Pearson’s X² test.
* Malaria parasite assessed was Plasmodium falciparum.
† Anemia defined as: (Hb <11.0 g/dL 1-4 year olds, or < 11.5 g/dL 5-9 year olds).

4.2. pGPX selenium distribution profile in the study children

The baseline plasma GPx Selenium levels were found to be inversely different with EBV loads and malaria densities in study children, (n = 158), Table 2.
Table 2. Mean pGPX selenium levels (μg/dL) by participant or clinical characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Mean</th>
<th>(SD)</th>
<th>( p ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low eBL region</td>
<td>65</td>
<td>3.76</td>
<td>(1.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High eBL region</td>
<td>93</td>
<td>2.36</td>
<td>(0.65)</td>
<td></td>
</tr>
<tr>
<td><strong>EBV viral load: copies/ml</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Detectable</td>
<td>68</td>
<td>2.92</td>
<td>(1.15)</td>
<td>0.035</td>
</tr>
<tr>
<td>2.5-1529</td>
<td>55</td>
<td>3.25</td>
<td>(1.50)</td>
<td></td>
</tr>
<tr>
<td>1530 or more</td>
<td>33</td>
<td>2.50</td>
<td>(1.23)</td>
<td></td>
</tr>
<tr>
<td><strong>Malaria Parasites:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>87</td>
<td>3.19</td>
<td>(1.54)</td>
<td>0.004</td>
</tr>
<tr>
<td>Present</td>
<td>71</td>
<td>2.61</td>
<td>(0.89)</td>
<td></td>
</tr>
<tr>
<td><strong>Gender:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>69</td>
<td>3.09</td>
<td>(1.34)</td>
<td>0.19</td>
</tr>
<tr>
<td>Female</td>
<td>89</td>
<td>2.81</td>
<td>(1.30)</td>
<td></td>
</tr>
<tr>
<td><strong>Age, years:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4</td>
<td>81</td>
<td>2.97</td>
<td>(1.35)</td>
<td>0.70</td>
</tr>
<tr>
<td>5-9</td>
<td>77</td>
<td>2.89</td>
<td>(1.29)</td>
<td></td>
</tr>
</tbody>
</table>

* Based on the t-test or one-way ANOVA.

Plasma samples from children aged <1 ≥12 years (n=167) were analyzed for GPX Selenium by pGPX enzyme immunoassays (BIOXYTECH®) Figure 6.
The minimum and maximum GPX Selenium among the study participants was 0.55 - 7.15 mcg/dL of blood. The mean ± SD GPx Selenium in the study children was 2.884 ± 1.322 mcg/dL; the Median was 2.676 mcg/dL, standard deviation (SD) 1.322 mcg/dL. The histogram curve (Figure 6) indicated that majority of the children had GPX Selenium less than 3.80 mcg/dL giving asymmetric distribution among the participants.

The study population GPx selenium distribution was stratified into 10% percentiles, 25% percentiles, 50% percentiles, 75% percentiles and 90% percentiles indicating that only 20% of children had mean GPx Selenium ≥ 3.179 mcg/dL of blood (Figure 7).
Figure 7: Percentile plot of pGPX selenium distribution among the study participants

The 10% percentiles = 1.455, 25% percentiles = 1.933, 50% percentiles = 2.622, 75% = 3.179 and 90% percentiles = 4.874. These values were then grouped into Quartiles of pGPX Selenium for eBL geographical categorization.
4.2.1. Quartiles of GPX selenium distribution by study children’s geographical locations

These values were grouped into interquartile range of 0.550 - 1.925; 1.926 - 2.666; 2.667 - 3.198; 3.199 - 7.15 mcg/dL and categorized by eBL clusters. The mean Q-GPX Selenium range was found to differ significantly by the study geographical location. The eBL high risk area of Kanyawegi “hotspot” median = 2.450; mean = 2.308 ± 0.670; minimum and maximum range = 0.589 - 3.330 (n=101). The GPX Selenium percentiles distribution in this area was 25%, (1.830); 50% (2.450) and 75% (2.898). When compared to the eBL low risk area (n=66) Seme “cold spot”, the median GPx Selenium = 3.680, mean = 3.767 ± 1.570; the minimum and maximum = 0.910 - 7.100, the percentiles distribution 25%, (2.713), 50% (3.680) and 75% (4.955) mcg/dL of blood.

Plasma levels of GPX Selenium differ significantly in children residing in regions with divergent eBL incidence. In the Burkitt’s lymphoma low risk area “BL cold spot (n = 66) mean 3.767, SD 1.570 and SE 0.193µg/dL and eBL hotspot (n = 101), mean 2.308, SD 0.667, SE 0.0667µg/dL. The levels were significantly higher among the children from eBL low incidence region compared to those from a eBL high incidence region (P < 0.0001). (Fig 8)
4:2.3. The Quartiles pGPX Selenium by study sites and gender

There were no statistical significant differences by gender observed between the groups analyzed by study site or across the study population 1-Way ANOVA ($p < 0.240$).

To compare if there were differences in GPX Selenium by gender, males and female children aged 1 year to 12 years of age had their plasma tested for GPX Selenium. The males (n=73), mean 3.020, SD 1.349, SE 1.580; females (n = 94), mean 2.7792, SD 1.297, SE 0.134mcg/dL (Fig 9).
The distribution of selenium in the two study sites was found to be lowly skewed to the BL "hotspot" as seen from the results from 167 children. The males and females from the eBL hotspot were more in the lower QGPX selenium range 0.55 – 1.9250 mcg/dL and the least in the upper QGPX Se 3.1978 – 7.150 mcg/dL. The males and females from eBL low risk area had comparative QGPX selenium distribution in the upper limit of 3.1978 – 7.15 mcg/dL.

The study observed that there was significant GPX Selenium differences in the two geographical locations by age strata (p<0.38) (Table 3)
Table 3: GPX Selenium distribution by age categories

Selenium levels were not significantly different by age groups ($p < 0.38$)

Mean 2.884 ± 1.322

<table>
<thead>
<tr>
<th>Age Category</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>Lower</th>
<th>Upper</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4 years</td>
<td>86</td>
<td>2.895</td>
<td>1.354</td>
<td>0.146</td>
<td>2.605</td>
<td>3.185</td>
<td>0.97</td>
<td>7.10</td>
</tr>
<tr>
<td>5-8 years</td>
<td>70</td>
<td>2.793</td>
<td>1.309</td>
<td>0.156</td>
<td>2.481</td>
<td>3.105</td>
<td>0.59</td>
<td>7.01</td>
</tr>
<tr>
<td>9-12 years</td>
<td>11</td>
<td>3.387</td>
<td>1.129</td>
<td>0.341</td>
<td>2.628</td>
<td>4.145</td>
<td>1.93</td>
<td>5.34</td>
</tr>
<tr>
<td>Total</td>
<td>167</td>
<td>2.884</td>
<td>1.322</td>
<td>0.102</td>
<td>2.682</td>
<td>3.086</td>
<td>0.59</td>
<td>7.10</td>
</tr>
</tbody>
</table>
4.3. EBV (Q-PCR (-) and Q-PCR (+) viral load distribution in children

EBV viral load analysis was carried out in three steps: First analysis investigated the general distribution of EBV in the study subjects (n=264), the second analysis focused on the EBV distribution by eBL high and low risk areas (n=264) and the third analysis focused only on children who had detectable EBV DNA in their blood (n=142).

In the first analysis two hundred and sixty four children had their blood tested for EBV by Q-PCR analysis. The result indicated that the children with non-detectable EBV = 43.9% (n=116), children with EBV viral load ranging from 2.5 - 599 copies/mL of blood = 26.5% (n=70); and children with ≥ 600 copies/mL = 29.6% (n =78). The mean EBV distribution by age category [1-4 = 1.84 (n=129); 5 - 8 = 1.449 (n=112); and 9 - 12 = 0.889 (n= 23)] The result was not significantly different between the groups (ANOVA equal variance not assumed) p < 0.22 but there was a trend where the EBV distribution is skewed towards individuals with non-detects (43.9%) forming the majority followed by 26.5%, with 2.5 - 599 EBV copies and 29.6% with EBV cases of ≥ 600 copies per mL of blood among children with detectable EBV viral DNA copies.

4.3.1. EBV (Q-PCR (-) and Q-PCR (+) viral load distribution in children by study sites

In the second analysis 264 children were grouped by geographical locations (study sites) forming the eBL high and low risk areas. In the eBL high risk area the EBV distribution [mean = 1.391, SD = 2.126, SEM = 0.170 and n = 156] copies/ml of blood. In the eBL Coldspot the [mean =1.784, SD = 1.514 SEM = 0.1457 and n= 108. The test of mean differences including both non-detects EBV Q-PCR (-) and detects Q-PCR (+) cases from the two study sites by One-Way ANOVA indicated significant differences (p < 0.081) (Figure 8).
Mean EBV (including non-detects) distribution in the study sites

t-test significant (p < 0.081)

EBV in BL coldspot (n=108)
Mean = 1.783
10% percentile = -0.3000
90% percentile = 3.460

EBV in BL hotspot (n=156)
Mean = 1.391
10% percentile = -0.3000
90% percentile = 4.582

Figure 10: Log normalized EBV viral load mean differences by study sites

The higher number of EBV non-detects in the BL hotspot could be as a result of frequent treatment from high malaria exposure as noted in previous studies (Daria Donati et al, 2006)

In the second analysis to determine the actual geographical distribution of EBV in the study area, only children who had detectable EBV by Q-PCR, n = 142 (56.6%) out of the 264 who completed the study were included in the analysis.
4.3.2. EBV (Q-PCR (+) viral load distribution in children residing in regions with divergent eBL incidence

To evaluate whether there were differences in the mean EBV viral loads between the two study sites, DNA was extracted from whole blood and Q-PCR was done to determine EBV viral load in children from eBL low incidence (n = 74) and high incidence regions (n = 68) were compared (Figure 9). Detectable EBV viral levels were observed in 70% of participants from the low eBL region compared to 47% from the high eBL study areas, \( p < 0.001 \). Among those with detectable EBV, the log viral loads were significantly higher in otherwise healthy children residing in the region with high eBL incidence compared to the low eBL incidence area; the log viral load means were 3.38 (SD=1.53) and 2.57 (SD= 0.80), respectively \( (p < 0.001) \). Log viral loads also were significantly higher in children with positive smears for malaria parasites [3.41 (1.47)] as compared to those without malaria [2.65 (1.00)], \( p=0.001 \).

![Figure 11: EBV (Q-PCR (+) viral load distribution in study children](image)
Whole blood levels of EBV viral copies in individuals from BL low risk (n = 74) and BL high risk region (n = 68) were determined by Q-PCR. The dots represent individual viral loads while the line through the dots represents the mean. Differences between groups were statistically significant by pair-wise T test ($P < 0.0001$). The EBV distribution was not significant by gender (Figure 12).

![Figure 12: The Tertiles of EBV distribution by gender](image)

The frequency of EBV exposure by gender is demonstrated in (Figure 12.) The frequency and degree of dispersion of EBV among females and males had no significant differences at 95% CI in the eBL high and low risk areas ($p < 0.186$). To test if there were any correlations between the different EBV viral loads, Pearson correlation coefficient was used to evaluate any relationship Figure (13).
4.3.3. The correlation between pGPX selenium levels and EBV log normalized viral load

There was a significant negative correlation between Selenium levels and EBV viral loads ($r=0.157, P = 0.041$). The slope is $-0.3466 \pm 0.1040$ at 95% CI with Y-intercept when $X=0.0$ is 1.199 to 2.609 (Figure 13).

![Regression Plot](image)

$Y = 2.118 - .168 * X; R^2 = .012$

**Figure 13: Correlations of log normalized EBV viral load distribution and GPX Selenium**

The inverse variability of the relationship between the GPX selenium and EBV viral load distribution in a study children (n=167) is reflected in Figure 14 of the 95% CI err bar which examines variability between subjects and not consistency of trend. The observation made in 95%CI was also reflected in Standard deviation (SD) and standard err of mean (SEM) err bars. As
in (Figure 14), err bars reflected about variability with no sign of overlap but positively compares within the group difference and significant levels can change with (N) values.

Figure 14: Err bar of EBV viral load and GPX selenium distribution in children

If the trend seen in (Figure 14) showing Err bar of EBV viral load and GPX selenium distribution in children remain similar between the subjects in the study; the differences will be significant even if their initial values are inversely variable.
4.4. Quartiles of pGPX selenium levels and tertiles of EBV viral loads in the study

children

Tertiles of log normalized EBV does not significantly differ by levels of quartiles of GPX Selenium \( (p < 0.186) \), 70 (42.4%) of the children had EBV which could not be detected by Q-PCR had mean GPX selenium of 2.908 mcg/dL, 42 (25.5%) had 2.5 to 599 EBV copies, with mean GPX Se of 3.171 mcg/dL, 53 (32.1%) had EBV viral load \( \geq 600 \) copies/ml of blood with mean GPX Se of 2.669 mcg/dL (Table 4).
The EBV distribution in the study population was not affected by the quartiles of GPX Selenium and geographical locations of the children. Regardless of the selenium level the EBV detection and non-detects were equally distributed. The non-detects had slightly higher mean GPX Se 2.908.

<table>
<thead>
<tr>
<th>Non-detects</th>
<th>70</th>
<th>2.908</th>
<th>1.174</th>
<th>0.140</th>
<th>2.628</th>
<th>3.188</th>
<th>0.59</th>
<th>6.04</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 to 42</td>
<td></td>
<td>3.171</td>
<td>1.590</td>
<td>0.245</td>
<td>2.675</td>
<td>3.666</td>
<td>0.97</td>
<td>7.01</td>
</tr>
<tr>
<td>599 copies</td>
<td></td>
<td>2.669</td>
<td>1.260</td>
<td>0.173</td>
<td>2.322</td>
<td>3.017</td>
<td>1.01</td>
<td>7.10</td>
</tr>
<tr>
<td>600 or more</td>
<td></td>
<td>2.898</td>
<td>1.323</td>
<td>0.103</td>
<td>2.695</td>
<td>3.102</td>
<td>0.59</td>
<td>7.10</td>
</tr>
<tr>
<td>Total</td>
<td>165</td>
<td>2.898</td>
<td>1.323</td>
<td>0.103</td>
<td>2.695</td>
<td>3.102</td>
<td>0.59</td>
<td>7.10</td>
</tr>
</tbody>
</table>

Tertiles with mean GPX Se of 2.898 mcg/dL. On average frequency distribution of EBV and QGPX Selenium shown in (Figure 15) indicates EBV non-detects have higher frequency of QGPX Selenium ranging from 2.67 – 7.15 mcg/dL. 1-Way ANOVA for EBV tertiles and QGPX Se was not significant ($p < 0.186$)
4.4.1. The mean age of child exposure to EBV based on viral load distribution among study participants

In the univariate analysis of variance using log normalized EBV as a dependable variable and gender and age categories as independent variable, the results had no significant differences between the groups' $p < 0.191$ and $P < 0.157$ respectively. The EBV viral loads were then classified by age stratification (1-12) divided into three groups. The EBV viral exposure outcome indicated age 1-4 years as the main peak period for child contact with higher EBV frequencies based on the EBV DNA detected by Q-PCR regardless of geographical location of the study participants [1-4 year mean EBV =1.7782, SD = 1.8896; (Figure.16). It was also evidence from the viral load that EBV viral load declines as the child gets strata increases.
The distribution of QGPX selenium and EBV viral load were then analyzed by age strata and gender regardless of the geographical location of the study participants and results stated in subheadings 4.4.2 – 4.4.4.

4.4.2. EBV viral load tertiles by age categories 1-4 years and QpGPX Selenium distribution

One hundred and sixty five children divided into different age categories (1 – 4, 5 -8, and 9 – 12 years) were tested for the tertiles of lognormalized EBV viral exposure against QGPX selenium distribution.

In the 1-4 years category the non-detects EBV (n = 27) had mean GPX selenium 3.077, SD 1.123 SE 0.216; 2.5 – 599 EBV (n=25) had mean GPX Se 2.929, SD 1.497, SE 0.2995, ≥ 600 EBV (n=32), mean GPX Se 2.787, SD 1.449. The total (n = 84), mean 2.922 ± 1.357. One – Way ANOVA test for between groups differences of significance (p < 0.720). This age group had the

Figure 16: Summary age categories of study children and EBV viral exposure
peak EBV viral exposure and most of the children with non-detects EBV (n =27) had higher mean GPX Selenium 3.077 mcg/dL compared to those with EBV viral load ≥ 600 copies (n = 32) who had mean GPX Selenium of 2.787 mcg/dL.

4.4.3. EBV viral load tertiles by age categories 5-8 years and QpGPX selenium distribution

Age category, 5 – 8 years the EBV non- detects (n=38), had mean GPX selenium 2.615, SD 1.096, SE 0.178; 2.5 – 599 EBV (n=14), mean GPX Se 3.707, SD 1.813, SE 0.484, ≥ 600 EBV (n=18), mean pGPX Se 2.457, SD 0.973, SE 0.229. The total (n = 70), mean 2.792 ± 1.309. 1 – Way ANOVA tests for between group difference level of significance (p < 0.011).

4.4.4. EBV viral load tertiles by age categories 9 -12 years and QpGPX selenium distribution

Age category, 9 – 12 years the non- detects EBV (n=5), had mean GPX selenium 4.225, SD 1.145, SE 0.512; 2.5 – 599 EBV (n=3), mean GPX Se 2.683, SD 0.680, SE 0.3928, ≥ 600 EBV (n=3), mean pGPX Se 2.693, SD 0.271, SE 0.156. The total (n = 11), mean 3.387 ± 1.129. 1 – Way ANOVA test for between group differences level of significance (p < 0.060).

4.4.5. Quartiles of GPX selenium and Tertiles of log normalized EBV viral load by gender.

The mean EBV distribution in males was 1.344 ± 1.892 (n = 127) and females 1.705 ± 1.861 copies /ml. of blood (n =137). The mean EBV distribution in the study area was 1.531 ± 1.881 (n = 264). There was no statistical difference by gender even though the result showed higher mean among the females. Majority was within the higher EBV tertile range of ≥ 600 copies and had GPX Selenium ≤ 4.00 mcg/dL (Figure 17).

4.5. Interaction between QpGPX selenium and EBV in the study children by site

The selection line based on the geographical location of the child and the Q-PCR EBV results indicated no interactive effect between those who had detectable EBV or non-detects among the
study population Figure 17. But the result does indicate that in both sites children with non-detectable viruses had slightly higher QGPX selenium as compared to those with higher EBV and lower QGPX selenium in both eBL "cold and hotspot" areas.

Figure 17: Quartiles pGPX selenium and Tertiles of log normalized EBV viral load distribution by study sites

When the interactive analysis was done based on gender and the child geographical area of residence notwithstanding, the same trend was observed where the EBV non-detects had higher QGPX Selenium level and the female children who were exposed to ≥ 600EBV copies had lowered mean GPX Selenium compared to males higher GPX Se levels and there was an interaction between males who were exposed to either less than or more than 600 copies of EBV/mL of blood (Figure 18).
Figure 18: The frequency of EBV exposure by gender and QGPX selenium

4.5.1. Impact of household population and EBV prevalence among study children.

The household population ranged from single occupancy to thirteen individuals per household. Forty six households had an average of four children and 45 households had 8 children while sixteen families had 7 children. The EBV viral loads had no significant difference based household densities. Households with 4-8 occupants all the time had higher frequencies of tertiles of EBV viral loads (Figure 19)
Figure 19: Distribution of EBV (non-detects included) by household densities

4.5.2: EBV viral load and children who did not receive BCG/ oral polio at birth

The study results indicate higher frequency \( n=168 \) (66%) number of children from the total study population of 254 missed vaccinations of BCG and Polio at birth Figure 19. The results further indicated that the mean EBV viral load in those who were exposed to BCG at birth 86 (34%) was higher \( 1.92 \pm 0.871 \) compared to those who did not receive BCG at birth \( 1.82 \pm 0.836 \) copies/ml of blood. The results also revealed that household with fewer individuals were likely to adhere to vaccination practices and that baby boys were likely to miss vaccinations as compared to girls in both areas. The children who attended vaccinations clinics had less cases of positive malaria, indicating better health care for these subgroups.
EBV Distribution and vaccination

Tertiles of normalized EBV
- non-detects
- 2.5 to 599 copies
- 600 or more

EBV Viral load

child was vaccinated at birth

Figure 20: EBV viral load and children who missed BCG/ oral polio at birth

4.5.3. Child Vaccination and weaning practices in eBL Hotspots and cold spots

The results of socio cultural data reveals that despite the high number of expectant mothers attending the antenatal clinics, more deliveries are done outside health institutions (65.6%) of 281 children who participated in the study were born out of health institution. 90.3% of the 281 children who took part in this study attended the antenatal clinics but for one reason or another but majority still failed to deliver in the hospital environment.

These findings confirm that many children miss vaccination at birth and some of the parents completely ignore taking children for vaccinations against known killer diseases for children. This practice has individual health risks which can serve as routes to community exposure to infectious agents and may confound EBV/BL interactions.
4.5.4: The mean EBV viral load levels increases by vaccination attendance

Two hundred and fifty one children who participated in the study had their EBV results analyzed against their vaccination history (n=251). Children who were never exposed to any vaccinations as recommended by the Kenya Government Public Health policy guideline (n=16), EBV Mean = 1.0646, SD =1.8629; the children who had 1-3 out of expected 5 vaccinations (n= 21) mean = 1.2966, SD = 1.9119; children who completed 4 out of 5 vaccinations (n= 139), mean = 1.5580, SD 1.8172; children who completed 5 out 5 vaccination schedules mean 1.6887, SD 2.0022. There was no significant difference between the group by 1-Way ANOVA (p<0.243). The result of each vaccination process resulted in increment of the mean EBV viral loads. Studies done elsewhere also realized an increment in HIV type 1 RNA after influenza vaccination (Fuller et al, 1999). In the current study, most of those who missed vaccination completely or failed to complete were noted to be males but there was no significant differences between the gender categories (p<0.4373). The result of the vaccination trend when compared to household densities and adherences to vaccination schedules had shown households with mean individual density of 3-4 people had the highest percentage of vaccination attendance at the 25% percentiles and 75% percentiles of adherence to vaccination policy Figure 19. The vaccination attendance trend and household densities by one – Way ANOVA column statistics:

1. There were no significant differences observed between the groups (p < 0.9965) (Figure 21).
Household density and vaccination

ONE-Way ANOVA mean differences not significant
P-value = 0.9965

![Graph showing vaccination trends by household density](image)

**Figure 21: Household densities and vaccination trends in the study sites**

### 4.5.5. Child exposure to EBV through poor baby feeding methods

The poor methods noted were mouth transfer of porridge or chewed foods to babies during weaning Figure 22. Child feeding trends during weaning period can be an important route to disease exposure, chemicals and heavy-metal environmental contaminants. The study results revealed that the type of care and methods used to give food is dependent on the household's socio-economic status. (Fig 22)
EBV viral exposure through feeding habits not significant ($p < 0.406$)

Figure 22: Challenges associated with introduction of foods to children in the study during weaning

The other challenges affecting household food supply were found to be geographical topography, climatic change, and industrial destruction of arable land by sand harvesting, quarry mining in some areas and human wildlife conflict in the BL hotspots. Transferring chewed foods or liquids by mouth to babies could be a risk factor for early exposure to a number of microorganisms with negative impact in the child growth and development. Lifestyles and a poor nutritional status are two factors influencing the high prevalence of non communicable chronic diseases in many parts of the world. Dietary intake during weaning and adolescence contributes to lifelong eating habits and the development of early risk factors for disease in adulthood. Few studies have examined the dietary patterns of the Lakeland population of western Kenya and the ethno-social and environmental factors that may expose them to certain diseases like cancer. Previous studies of
dietary habits of the mothers and other health-related social, occupational and environmental exposures during pregnancy may influence the development of congenital defects or onchogenesis due to bioaccumulation of contaminants from food products, and pesticides (Giordano et al, 2008). The feeding outcomes noted in the present study require further research to establish any possible links with BL clustering.

4.6. **GPX selenium and malaria distribution in the study children**

Frequent malaria exposure is a risk factor in BL tumorigenesis and the impact of GPX selenium levels in malaria exposure was tested. The mean GPX selenium for 91 (54.5%) children with no positive malaria parasites out of 167 children tested for pGPX Se, was 3.163, SD 1.535, SE 0.161 and the 76 (45.5%) who had malaria parasites in their blood, had mean GPX Se of 2.551; SD 0.912, SE 0.105 mcg/dL. The t-test results for the difference of mean of those with malaria parasite and those without the parasites at the time of sample collection was very significant \( p < 0.002 \). The study site had an elevation ranging from 1122.426 - 1393.217 meters above sea level which mean difference in malaria distribution. Previous studies in the same area found mean malaria prevalence of 43.2% and 52.2% in the intervention and control sites in the current study site. The intensity of infection was noted when the prevalence rose to 73.5% and 75.7% \( (p = 0.541) \) after the removal of the permethrin coated sisal curtains were removed (Oloo et al., 1996).

Despite the widespread use of treated bed nets in the region, there is still high malaria transmission intensity in the current study sites of Kanyawegi the “BL hotspot” and North Seme the BL “cold spot” (Figure 23)
Malaria distribution in study children

<table>
<thead>
<tr>
<th>Age category / Study sites</th>
<th>Hotspot</th>
<th>-ve</th>
<th>+ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4 yrs</td>
<td>37</td>
<td>38</td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>5-9 yrs</td>
<td>18</td>
<td>52</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>90</td>
<td></td>
<td>145</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coldspot</th>
<th>-ve</th>
<th>+ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>9</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>14</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>23</td>
<td>104</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 23: Malaria cases by age category and study sites**

4.6.1. Malaria distribution in children and Tertiles of EBV (including non-detects)

The mean EBV viral load among children who were malaria negative for parasites at the time of sample collections (n = 134), was 1.55, SD 1.638; SE 0.197 and among the children who were malaria positive cases (n = 117) the mean EBV distribution was 2.54, SD 2.13; SE 0.197 copies.

The test was repeated to focus on children who were EBV positive only (n = 142). The mean EBV viral load among children who were negative for malaria parasites (n = 84) was 2.648; SD 1.007, SE 1.098 and the mean EBV among children who had malaria in their blood at the time of sample collection (n = 58) was mean 3.411, SD 1.477; SE 0.194. The t-test for equality of mean was significant.
This significance indicates that malaria infection increases the EBV viral load (Ann M Moormann et al., 2005). Hemoglobin is one of the biomarkers that may be linked to EBV viral load and reduced GPX selenium differences in various among the study children (Figure 24).

Figure 24: Distribution of Hemoglobin, GPX Se and EBV Q-PCR (+) cases by study sites

The two study sites experience differing exposure levels of EBV and GPX selenium with a negative impact on hemopoetic system. To understand the interactive mechanism of selenium, malaria and EBV exposure, a stepwise multivariate analyses model to factor in step by step variables including age and study site.
### 4.6.2 Stepwise Multivariate analyses model

Comprehensive examination of the relationship between pGPX Selenium and EBV viral load in children residing in the low and high incidence regions was performed by a stepwise multiple regression model with EBV viral load as the dependent variable, age as a covariate, and the following independent (or predictor) variables: pGPX Selenium levels, sex, malaria status and area of residence (low or high incidence regions). The predictors were selected from those that showed statistical significance when they were correlated with GPX Selenium levels ($P < 0.05$).

The regression model included all participants who completed a survey. The model was built at four different steps with the first one having only age and sex, the second had age, sex and pGPX selenium. The third had age, sex, pGPX selenium and malaria status while the fourth had all of the former variables together with particular site of study (eBL high_low-risk). Model 1 was significant when added to base models of intercept ($P < 0.05$). Models, 1, 2 and 3 were all statistically significant ($P = 0.018$, $P = 0.025$ and $P = 0.04$, respectively) while, model 4 was not ($P = 0.069$). Child age was the only significant predictor of EBV load in each model. Beta coefficient shows that the age of the child had an inverse relationship with higher EBV levels. Therefore, younger kids had higher EBV viremia. Selenium did not significantly predict EBV load after controlling for the other variables, neither did malaria infection status or place of residence. A different model examined only the eBL low incidence area to see what predicted EBV load. Again, only child age was a significant predictor of EBV load, even after adding in everything else. Younger children had higher EBV levels independent of selenium level. The last model looked at the individuals from the eBL high incidence area only and none of the variables significantly predicted EBV load in this area.
4.6.3 Interaction between child malaria status and QGPX selenium levels by study sites

In the eBL "hotspot" the children who did not present with positive malaria had lower GPX selenium compared to those who were malaria positive. The same trend is reversed in the eBL "cold Spot" where the children who had no malaria parasites had higher mean GPX selenium compared to those who had malaria (Figure 25).

![Estimated Marginal Means of Selenium ELISA (ug/dL) gpx](image)

**Figure 25: Interaction between child malaria status and QGPX Selenium levels by study sites**

When further analysis was done comparing, malaria and EBV exposure to levels of GPX selenium, there was no interaction as indicated by parallel lines Figure 26 except it was clear that all those who had higher EBV viral loads and were positive for malaria had lower GPX selenium compared to children who did not have malaria and better GPX selenium mean score (Figure 26).
The higher EBV viral load ≥ 600 or more viral copies were associated with low GPX Selenium mean < 2.90 mcg/dL and the GPX Selenium malaria association was significant (p<0.003). Even the EBV non-detects who had malaria were also associated with lower GPX Selenium compared to those who had no malaria. When the same analysis involving GPX Selenium, malaria and EBV viral load was performed by gender, the interactive effect was significant in both the children who were Q-PCR EBV (-) and Q-PCR (+) Figures (27; 28; 29).
Estimated Marginal Means of Selenium ELISA (ug/dL) gpx

at Tertiles of normalized EBV = non-detects

Figure 27: Interaction between gender, GPX Selenium levels, EBV non-detects and malaria status
Estimated Marginal Means of Selenium ELISA (ug/dL) gpx at Tertiles of normalized EBV = 2.5 to 599 copies

Figure 28: Interaction between gender, GPX Selenium levels, moderate EBV detects and malaria status
Figure 29: Interactive effects of malaria, high EBV and GPX selenium status by gender

The figures 27-29, illustrate the main interactive effects of malaria, EBV and GPX selenium status that are not easy to interpret. Any conclusion regarding the physiological role of selenium, EBV and malaria may require specific sensitivity analysis to understand their biological role in pathogenesis comparing what happens in male and females at each exposure levels.
4.6.4. The interactive effect of Malaria, EBV and QGPX Selenium levels affect some hematological parameters in the study children

After observed interactive effects indicated in figures 19-21, same blood samples were analyzed for hematology using Beckman Coulter AcT diff2 (Beckman-Coulter Corporation, Miami, Florida, USA) for biomarkers for any biological variations within the groups (Table 5). The red blood cell distribution width (RDW), measures the variations of RBC width (7-8μm) after hemopoeisis. The normal range of RDW is 11 - 14 %. The measurement values of RDW and MCV can be used as biomarkers for types of anemia. MCV and RDW may further the understanding micronutrient deficiency (Vitamin B12 and folic acid). Increased RDW may be due to iron deficiency anemia resulting in cells with different width (anisocytosis), platelet distribution width (PDW) in the BL hotspot could be a biomarker for cases of sickle cell and thrombocytopenia (Brunnell González et al., 2009) suggesting for more investigations to establish the role played by GPX Se and EBV exposure.
Table 5: pGPX Selenium and hematological indices in the study children by study sites

<table>
<thead>
<tr>
<th></th>
<th>BL Hotspot (n = 178)</th>
<th>BL.Coldspot (n = 103)</th>
<th>t-value at P = 0.05</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Range</td>
<td>Mean ± SEM</td>
<td>Range</td>
</tr>
<tr>
<td>WBC (x10^3/ul)</td>
<td>10.20 ± 0.3879</td>
<td>4.100 - 29.60</td>
<td>8.411 ± 0.3062</td>
<td>2.800 - 26.20</td>
</tr>
<tr>
<td>Platelet (x10^3/ul)</td>
<td>291.2 ±10.50</td>
<td>16.50 - 803.0</td>
<td>353.6 ± 12.40</td>
<td>60.0 - 926.0</td>
</tr>
<tr>
<td>PDW %</td>
<td>16.56 ± 0.1281</td>
<td>3.0 - 21.10</td>
<td>16.17 ± 0.05903</td>
<td>14.90 - 18.10</td>
</tr>
<tr>
<td>RDW %</td>
<td>17.10 ± 0.3256</td>
<td>12.50 - 33.90</td>
<td>15.77 ± 0.25</td>
<td>12.50 - 28.70</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>74.50 ± 0.75</td>
<td>30.70 - 113.70</td>
<td>76.73 ± 0.6165</td>
<td>52.40 - 91.60</td>
</tr>
</tbody>
</table>

* Indicate significant difference level at P = 0.05.

4.6.5. Common foods and plants with selenium micronutrients in the two study sites

Possible sources of dietary selenium in the area include meat, different species of fresh water fish, groundnuts, sim sim, traditional vegetables like asparagus (saga), amaranthus (mchicha), crotalarias (mito), solanum (osuga), muringa spp] and tubers and roots like potatoes, cassava and arrow roots. Consumption of fruits like mangoes, bananas, pineapples, pawpaw, avocado Figure 31 and use of culinary additives such as collieander, ginger, turmeric, garlic and onions were some of the common sources items captured by questionnaire during data collection. The selenium
content of these foods is dependent on the local soil geological selenium content and wash offs from upstream.

Table 6: Z score results and clinical characteristics of study participants

<table>
<thead>
<tr>
<th>Zscore</th>
<th>eBL low incidence (n=104)</th>
<th>eBL high incidence (n=145)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>58(55.8%)</td>
<td>46(44.2%)</td>
<td>62(42.8%)</td>
</tr>
<tr>
<td>Stunting (Age/Height)</td>
<td>81(77.9%)</td>
<td>23(22.1)</td>
<td>87(60.0%)</td>
</tr>
<tr>
<td>Underweight (Weight/Age)</td>
<td>72(69.2%)</td>
<td>32(30.8%)</td>
<td>94(64.8%)</td>
</tr>
<tr>
<td>Wasting (weight/height)</td>
<td>80(76.9%)</td>
<td>24(23.1%)</td>
<td>118(81.4%)</td>
</tr>
</tbody>
</table>

(0.05)*Pearson’s X² test.

In general there is malnutrition in the study area. The children with stunted growth for age category 1-4 years (n= 40 (31.3%) out of 128; and 5-9 years 41 (33.9%) out 121. Pearson’s X² test (p=0.686). The age group 1-4 years was associated with wasting 35(27.3%) out of 128 children. Compared to 5-9 years 16(13.2) out of 121. The difference was significant (p<0.007).

There were no differences by weight or by age categorization (p=420). Malnutrition by gender males 63(54.3%); (n=116) and females 66(49.6%); (n=133) was not significant at (0.05)*Pearson’s X² test (p=0.525) (Table 6)
Zscore results (n=149)

1-Way ANOVA (p<0.3679)

Figure 30: Levels of malnutrition among study children

Stunting and underweight was more than 40% among the study children. The distribution by study site shows 22.1% stunted and 30.8% underweight in eBL low risk area compared to 40% stunted and 35.2% underweight in eBL high risk area (Table 6).
Table 7: Zscore results and Correlation co-efficient of GPX Selenium

<table>
<thead>
<tr>
<th></th>
<th>Correlation</th>
<th>N</th>
<th>Z-value</th>
<th>P-value</th>
<th>95% CI of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>GPx Se /stunting</td>
<td>-0.007</td>
<td>149</td>
<td>-0.083</td>
<td>0.9341</td>
<td>-0.167</td>
</tr>
<tr>
<td>GPx Se/ underweight</td>
<td>0.006</td>
<td>149</td>
<td>0.071</td>
<td>0.9432</td>
<td>-0.155</td>
</tr>
<tr>
<td>GPx Se/wasting</td>
<td>-0.095</td>
<td>149</td>
<td>-1.150</td>
<td>0.2503</td>
<td>-0.252</td>
</tr>
<tr>
<td>Stunting and underweight</td>
<td>0.398</td>
<td>149</td>
<td>5.090</td>
<td>&lt;0.0001</td>
<td>0.253</td>
</tr>
<tr>
<td>Stunting and wasting</td>
<td>-0.109</td>
<td>149</td>
<td>-1.319</td>
<td>0.1872</td>
<td>-0.265</td>
</tr>
<tr>
<td>Underweight and wasting</td>
<td>0.458</td>
<td>149</td>
<td>5.985</td>
<td>&lt;0.0001</td>
<td>0.321</td>
</tr>
</tbody>
</table>

From the correlation (Table 7) it is evident that GPX Selenium has an inverse relationship with stunting and wasting (-0.007 and -0.095) respectively but not underweight (0.006). Stunting / underweight and wasting and underweight have very strong correlations $P<0.0001$. 
Household use of fruits and common foods was significantly different ($p<0.001$) by study sites.

The growing of the fruits and tubers like potatoes, cassava and arrow roots differed significantly. The eBL hotspot were least involved in farming because of the type of soil bordering the lake, destruction of farm land through sand harvesting and also due to constant crop destruction by wild life like hippopotamus (Human/animal conflict).

4.6.6. The GPX Selenium levels higher in fried fish

The GPX selenium levels were found to be significantly higher with different levels of consumption of fried fish $p<0.0001$. Children who never or almost never eat fried fish had mean $2.471 \pm 0.916$; ($n=44$); Mean GPX Se for children who consumed fried fish fairly often ($n=97$) was $2.781 \pm 1.211$; and children who always had fried fish ($n=26$), the mean GPX Selenium $3.971 \pm 1.720$ mcg/dL. Consumption of sundried fish had no mean significant differences in GPX.
selenium levels in the study children $p < 0.803$, and consumption of smoked fish also had no significant differences $p < 0.744$.

4.6.7. Household water sources and GPX selenium levels in the study area

Selenium is a geochemical element whose availability to households is dependent on food chain and local water sources. The GPX selenium results from one hundred and sixty seven children in the study was weighted against the reported common household water source for domestic use. The mean ± SD QPX selenium associated with water source was $2.884 ± 1.322$ mcg/dL ($n = 167$).

Domestic water can serve as a major source of household selenium supply but levels of selenium content should be established as depending on the actual area of water higher than needed selenium level may pose risk to households as noted elsewhere (Pollard et al, 2007; Karaj S Dhillon and Surjit K Dhillon, 2009). The current study investigated household water sources and levels of Gpx selenium of children from the households. The mean GPX Se of children whose water source was shallow well/rain/ lake ($n=72$), mean $2.630 ± 1.187$; river/rain/lake ($n= 17$), mean $3.116 ± 1.374$; river/well/lake ($n=35$) mean $3.505 ± 1.549$; neither river nor well ($n=43$), mean $2.714 ± 1.175$ mcg/dL.

When household water source was critically evaluated against GPX Selenium by the study sites, the use of river water had mean of $3.3780 ± 1.494$, ($n= 52$) and households which did not use river water ($n= 115$) had mean GPX Se of $2.661 ± 1.178$ mcg/dL. The t – test for the equality of mean was significantly different $p < 0.0001$. The lake water users ($n= 73$) had mean of $2.429 ± 0.618$ and those not associated with lake water ($n=94$) had mean GPX Se of $3.238 ± 1.592$ mcg/dL. The t – test result indicated significant differences ($p < 0.0001$). The use of seasonal streams ($n= 39$), mean $2.710 ± 1.209$ and those not using seasonal streams ($n=128$), mean $2.938 ± 1.354$ mcg/dL had no significant differences ($p < 0.348$). (Mushak, 1985) (Deveau, 2010)

Regardless of the households’ water sources, the BL cold spot had higher GPX Se levels. (Fig 32)
Figure 32: Reported household water source and pGPX Selenium levels by study sites

Histogram showing distribution of selenium by household water sources and study sites. Shallow wells and rain had the list GPX selenium in the area. The number of households using shallow wells was very low in both study sites. The use of river and well or lake had higher GPX Selenium in both BL hotspot and cold spots.
CHAPTER 5: DISCUSSION

5.1: GPX Selenium levels and EBV viral loads in the study children

Selenium is a nutritional trace mineral essential for various aspects of human health that exerts its effects mainly through its incorporation into selenoproteins as amino acid, selenocysteine. In human and other animals twenty-five selenoprotein genes have been identified. Several selenoproteins are broadly classified as antioxidant enzymes among many diverse functions. The synthesis of this family of proteins is dependent on a common set of cofactors and on dietary selenium. Reduced GPX Selenium experienced in the area can potentiate mutations or polymorphisms in certain selenoprotein genes and synthesis cofactors. The situation is likely to result in high affinity or association with a number of diseases which may require further investigation. The confounding profile may include diseases of the muscle, atheroclerosis, cardiovascular disorders, immune dysfunction, cancer, neurological disorders and endocrine functions.

The current study found significant variations in the distribution of GPX selenium in the two study sites with divergent incidences of endemic Burkitt’s lymphoma. Studies have selenium to be necessary for the production of the enzyme glutathione peroxidase, which reduces the production of inflammatory substances in the body (prostaglandins and leukotrienes) and scavanges the free radicals. (Selmi and Tsuneyama 2009). It has also been found to be beneficial in the prevention and treatment of osteoarthritis (OA) from physical wear and tear or structural problems in the patient’s joints (Kaur and Bansal 2009). The finding of low levels of selenium in the eBL high risk area serves as a health risk indicator in the area. The study investigated GPx selenium as a possible environmental etiological agent in the pathogenesis of eBL in these areas reporting divergent cases of eBL (Rainey et al, 2008). Earlier refined mapping of eBL incidence within Nyanza Province in western Kenya demonstrated the existence of significant spatial
clustering within what is generally considered an area of high perennial malaria transmission (Rainey et al., 2007). The existence of eBL clusters raised more scientific questions beyond what is known about the epidemiology of eBL. In the current study, participants were enrolled from eBL high incidence area (Kanyawegi ‘hot spots’) and (north Seme ‘cold spots’) within West and East Kisumu Districts. The eBL tumors are normally associated with malaria and EBV but results from other studies suggest other co-factors in addition to malaria and EBV. Therefore, the current study focused on comparing selenium levels as a possible environmental risk factor that modulates EBV viral set point by potentiating mutations and virulence. It was postulated that children residing in areas with different eBL incidence rates would also have differences in EBV viral load and selenium levels. The results presented here clearly indicate that reduced selenium and increased EBV viral loads in children are associated with residing in eBL high incidence regions compared to the low incidence region. The findings in the current study showing increased EBV viral loads in eBL high incidence areas is in keeping with previous results which found regional differences in EBV viral load in children with differing exposures to malaria in western Kenya (Moormann et al., 2005). The results are also consistent with earlier studies elsewhere (Stevens et al., 2001). More recent studies have supported earlier results showing that increased EBV viral load and the concomitant polyclonal B cell activation with enhanced B cell survival may augment the risk of eBL development in children living in malaria-endemic areas (Chene et al., 2009b). Furthermore, in earlier large prospective studies, increases in antibody titers to the EBV viral capsid antigen (VCA) protein preceded the emergence of eBL (de-The et al., 1978). The confounding effect on population is that high viral loads have been shown to deplete GPX-selenium and its precursor cysteine which can affect liver detoxification, lymphocyte activation, viral transactivation and mitochondrial functions (Speir et al., 2000). The measurements of EBV viral load have been used as clinically relevant predictors for emergence of EBV-associated lymphoproliferative disorders in immuno-suppressed individuals (Stevens et
Therefore increased EBV viral load observed in the current study area suggests dysregulation of EBV infection in children and may be considered a putative risk factor for eBL. Studies have also shown that CD21 serves as a receptor for the Epstein-Barr virus (EBV) and the surface expression of CD21 on B and T cells is dependent certain antioxidants and REDOX (oxidation reduction) activities. The impact of reduced selenium levels in this subpopulation carries major health risks as selenium modulate the endogenous thioredoxin and glutathione systems which are of central importance in redox signaling (Sen, 1998). In healthy carriers, the EBV infected B cells exist in peripheral blood as latent non lymphoblasts. The infected latent cells are resting memory B cells that are not under direct immunosurveillance by cytotoxic T lymphocytes (CTLs). The high EBV viral load observed in children residing in the eBL high risk area may be a biomarker in eBL pathogenesis due to expansion of proliferating lymphoblasts resulting from suppressed CTL response due to destabilized GPX selenium in the affected children. The higher viral load has been shown to be a major risk factor in lymphoproliferative diseases and AIDS-associated B cell lymphoma (Babcock et al, 1999). The two study sites were also found to have significant differences in households’ malaria exposure. The eBL high risk area had higher parasite densities compared to eBL low risk cluster. Frequent exposure to malaria promotes eBL pathogenesis due to intensity of the host response to infection and increased oxidative stress from metabolic toxicants emanating from phagocytosis and other clinical outcomes due to intense infection. This has a consequence of stressing the hemopoetic system and body defences.

The biological mechanization processes serve as the major triggering event in the pathogenesis of the lymphoma (Rainey et al., 2007). The inverse localized correlation between the average age of onset of eBL in this area as previously seen in one of the studies (Rainey et al., 2008) and the intensity of falciparum malaria infection is a clear indicator of the role malaria plays in the development of this disease. Perhaps, early
intense infection leads to the immortalization of large numbers of B lymphocytes and proliferation of the EBV-infected B lymphocytes as previously observed by (Moormann et al., 2005). It is therefore possible that reduced GPX selenium, high EBV viral loads and intense malaria exposure in this eBL high risk area, results in increased B lymphocytes which provide a conducive environment for the emergence of the cytogenetically abnormal EBV infected cell that heightens the risks of developing eBL in this subpopulation. The high risks of malaria in the same area was observed by (Olool et al., 1996). It is known induce tissue pathology including lipid peroxidation, oxidative DNA damage, and NF-kappaB activation (Sahoo et al., 2009; Tkachuk et al., 2001).

5.2. Impact of environmental degradation on malnutrition and selenium in eBL high risk area

Malnutrition was found to be a common factor among the participants in the study. The demographic data collected through face to face interviews showed marked differences in household food supplies. The majority of people living in eBL low risk area had food grown from their local gardens. But the people from the eBL high risk area have major environmental issues interfering with household and water supplies. The differences observed were not by gender but mostly by geographical location the households. Most of the children residence of eBL high risk area had higher percentage in the prevalence of stunting, underweight and wasting. This finding is a pointer to the disease burden and high possibility of reduced micronutrient base. This outcome is a major set back in the bodies ability to defend itself against infections as seen in the number of cases exposed to either high malaria parasite densities or EBV viral loads. The significant differences in GPX Selenium levels in individuals from this area with different eBL incidence rates confirm the variability of micronutrient supply in this subpopulation. Some of the factors affecting adequate production of food in this particular study site with more than expected cases of eBL are wild life – human conflict a long the Lake Victoria shores where hippos cause massive
destruction to food crops. The environmental factor likely to contribute to reduced levels of selenium in this area is associated with anthropogenic activities including destruction of arable land through commercial sand harvesting promoting unprotected soil erosion and breeding sites for malaria vectors. This may be one of the reasons for high case turnover in the number of individuals exposed to malaria in the area. The environmental damage is further compromised by unregulated number of quarry mining for commercial murram used in road construction, residential and industrial building fillups. The greatest danger to the geological selenium is excavation of stones and rocks to be crushed for commercial ballasts used for roads and other building constructions. Exposure of some heavy metal dusts from the mines geostrata are known to immobilize selenium thus making it impossible to be absorbed through plants food chains. The geological immobilization of selenium in this area is also possible through acidification of the environment and water ways by ballast crushing and tarmac chips reconstituting factories, plastics and foam mattresses, alcohol and chemical producing industries in the area. The level of pollution in this area of Kisumu is evident by the thick dust and smoke commonly covering the sky from the industries. The only river passing through the area is the major recipient of industrial toxicants originating from the factories. Available data indicate that the bioavailability of selenium in soil is affected by the ecological nature of the environment, sandy soil, acid precipitation in the environment; metal hydrous oxides and PH of the soil easily immobilize selenium by reducing selenite to zero-valent form. Lowered PH of the soil is known to reduce the generation of volatile dimethylselenide in soils because of the delicate balance in microbial activities which affect selenium bioavailability to plants (Mushak, 1985). Malnutrition in this study site is a very important indicator for overall child health and is considered a risk factor for infectious and chronic disease susceptibility as noted by (Koutros et al, 2008). The deficiencies in vital micronutrients like selenium, copper, zinc, iron and others are known to compromise repair of damaged DNA, and restoration of appetite, and are known to impair the immune functions and
growth (Xu et al., 2010). The degradation of selenium from soil has been shown to associate with major health risks like Keshan disease, a form of heart disease in children (Sudre and Mathieu, 2001; Moreno-Reyes et al., 2001). It was observed that the soil in the Keshan region of China is associated with low selenium. Human exposures to foods grown from this type of soil exposes the population to major microbial challenges by potentiating mutation and virulence (Juqian Zhang et al., 2009; Rui-Fang Zhou, et al., 2009; Ping Wu et al., 2009). Therefore, the finding of the current study of the existence of reduced selenium level in this area cannot be over-emphasized on a particular disease condition but rather cuts across the human health because of selenium involvement in the hormonal and enzymatic systems. There is evidence that low dietary selenium is associated with increased incidence of cancer through destabilization of REDOX functions and increasing reactive oxygen species (ROS) production (Brigelius-Flohe et al., 2004). The increased ROS interferes with the signaling cascades as opposed to reduced state of ROS which serve as mediators of cellular signaling pathways which can be used in clinical management of chronic disease (Chandan K. Sen, 1998). Some types of cancers like that of the respiratory system and the gastrointestinal tract seem to be especially sensitive to the level of selenium in the body (Brigelius-Flohé et al., 2004). In addition, cervical dysplasia (abnormal growths of tissue) in women has been shown to associate with low levels of selenium in the diet (Steevens et al., 2009, Connelly-Frost et al., 2009). Therefore the impact of micronutrient malnutrition and risks of ill health is high. This is supported by the number of child case fatality in many parts of sub-Saharan Africa which contribute to approximately 60% of pediatric deaths (Jackson et al. 2006). The overall effect is on the mean age of children who are at risk of contracting malaria, gastroenteritis, pneumonia, sinusitis and eBL. The underlying clinical symptoms of malnutrition include hypoglycemia, hypothermia, dehydration, and secondary exposure to polymicrobial infections as seen in the eBL high risk area. The results of the interviews also revealed that many children in this area had different ophthalmic conditions and poor school attendance. It is therefore important
to note that reduced or selenium deficiency may also be associated with increased progression of cataracts among other conditions. As previously reported healthy eye lens requires adequate levels of three antioxidant enzymes: superoxide dismutase, catalase, and glutathione peroxidase. Glutathione peroxidase in the human eye is dependent on selenium. (Dawczynski et al, 2006). Micronutrient malnutrition alters cellular osmotic balance through excess body sodium, high intracellular Na ions and low plasma Na ion levels in the absence of antioxidants like glutathione peroxidase enzyme (GPx). It also affects cellular electrolytes composition of calcium, potassium and magnesium, manganese, iron and zinc/copper balance as observed by (Wasantwisut, 1997b; Milanino et al, 1993; Gerbino et al, 2009). The condition of malnutrition may be considered a pathological stimulant whose impact can be associated with destruction of intestinal mucosa, loss of vital electrolytes through diarrhea, delayed healing of ulcerated tissues and skin lesions. The remedial measures can be initiated by initiating kitchen gardening of potatoes, cassava, groundnuts, pumpkins, traditional vegetables’ beans and peas or, providing fortified food supplements for essential elements. Iron should be given with caution due to risk of enhancing pathogen increases and generation of toxic free radicals (Schofield and Ashworth, 1997; Safaralizadeh et al, 2007).

5.3. Fish as source of vital nutrients and the possibility of contamination by carcinogens in the study area

The current study area borders Lake Victoria and fish consumption is quite high compared to other fleshbased foods. Some fish species serve as important dietary source of $\beta$ – carotene, vitamin A and E and selenium among other essential elements. They are also known to contain unsaturated fatty acids (omega 3 fatty acids) like eicosapentaenoic acid (EPA). Some of these elements can reduce adverse exposure to both cardiovascular diseases and cancer but the impact on endemic Burkitt’s lymphoma is unknown. There is evidence that fish can also serve as a
source of macroelements (phosphorus, calcium, magnesium potassium and sodium) and microelements (copper, zinc, iron, manganese, chromium, selenium, fluorine and iodine) depending on the types and sites of catch (Polak-Juszczak, 2008). The current study found significant differences in the methods used in fish preservation (smoked, sundried, friedried and salted sundried). There was significant differences ($p<0.0002$) in the consumption of smoked, sundried, fry dried and salt preserved fish accounting for 77.35% and wet fresh fish accounts for 22.65% of the total variance. The consumption of fried fish was found to be significantly associated with high GPX selenium levels but preparation and handling of fish for domestic use can increase exposure to carcinogens. Smoked fish or fish fried in recycled oils may lead to accumulations of formaldehyde and other carcinogens like polycyclic aromatic hydrocarbons (PAHs). PAHs are ubiquitous priority pollutants that occur naturally in crude oil, automobile exhaust emissions and smoke condensates from incomplete combustions of carbonaceous materials. The population risk of exposure increases by handling and sites of display for sale. Th unprotected road side display of fish as commonly seen in parts of western Kenya increases exposure PAH from fumes emitted by vehicles. Fish PAHs content may increase from wood or coal burns, kerosene stoves, kerosene or plastic aided charcoal lighting. The wood smoke contains at least 100 polycyclic aromatic hydrocarbons (PAH) and their alkylated derivatives. Many of them are carcinogenic. Benzo[a]pyrene (BaP) is regarded as a marker of the carcinogenic PAH in smoke and smoked fish. Heavily smoked fish from traditional jikos used for commercial preparations may contain up to about 50 μg BaP/kg wet weight on the outer side (Stolyhwo and Sikorski, 2005). The confounding effects of the values and risks associated with fish handling and consumption in the eBL high risk area require further evaluation.

5.4. **The family characteristics as confounding eBL risk factor in the study area**

The study outcome identified weaning practices, child vaccination trend, and inequality in household food availability as major confounding factors in the eBL clusters. The location and
social geography of the home is a predictor to disease outcome. Lower socio-economic status has been identified as one of the risk factor for developing chronic diseases like eBL (Williams et al, 1988; Biggar et al, 1979; Griffin et al., 2000). Larger sibship size was found to associate with increased number of sibling deaths among eBL families (Morrow et al., 1974). Based on these findings, it is feasible that family dynamics, including a child’s relation to his/her siblings, contribute to eBL risk through general care, household crowding, and increased responsibilities at home. It has been realized that birth order, sibship size, and gender may influence competition for limited resources, including availability of meat and other high protein foodstuff. In fact, early studies postulated that increased risk of cancer among families with lower economic status was also associated with protein deficiency (Clifford et al., 1967). The associations between certain family characteristics and childhood cancer regarding the environmental etiology generated the study hypothesis. The family feeding habits affect the household micronutrient base and microbial exposure. The study found major differences in housing, poor birth practices with majority of children being born outside health institution. Majority of the children missed the BCG and oral polio vaccines given at birth. Poor feeding habits were common in the eBL high risk area which included mouth to mouth transfer at the initiation of weaning process. Fish is common but the preparation and tools for cooking differed significantly depending on household socio-economic status. A number of clinical and epidemiologic investigations have shown positive roles for n-3 fatty acids in infant development; cancer; cardiovascular diseases as mentioned earlier. The n-3 fatty acids have also been found useful in the management of various mental illnesses; including depression, attention-deficit hyperactivity disorder, and dementia. All the benefits one can get from fish is majorly dependent on handling and preparation of fish for consumption or preservation for commercial purposes. The vital elements, the fatty acids have been found to have pleiotropic effects that include action against inflammation, platelet aggregation, hypertension, and hyperlipidemia. These beneficial effects are mediated through
several distinct mechanisms, including alterations in cell membrane composition and functions, gene expression, or eicosanoid production (Riediger et al., 2009).

More indepth studies have shown that Omega-3 fatty acids can attenuate growth and induce apoptosis in a number of human cancer cell lines from the colon, pancreas prostate. Omega3 fatty acids are known to act synergistically with chemotherapeutic agents and can be used to enhance tumour radiosensitivity. It is also known to control tumorigenesis under complex mechanization including alteration of lipid mediators generated during inflammatory reactions (Wendel and Axel R Heller, 2009). Fish like any other foods can easily be contaminated with formaldehyde which is economically important chemical. The family housing location and cooking methods can expose members to formaldehyde through foods and environmentally, generated through automobile engines, tobacco, smoke released from confined household activities. The other sources include furniture, particle board, plywood, and carpeting. Formaldehyde has been classified as human carcinogen by International Agency for Research on Cancer (IARC) for its role in nasopharyngeal cancer and has "strong but not sufficient causal association in leukemia development. Other occupational exposure to formaldehyde may include hospitals, industrial formulations and reused oils during fish frying which is a common practice in the current study sites. The biological plausibility of formaldehyde acting on bone marrow directly or, alternatively, may cause leukemia by damaging the hematopoietic stem or early progenitor cells that are located in the circulating blood or nasal passages, which then travel to the bone marrow and become leukemic stem cells. (Ambrosini et al., 2009). The current study points out significant differences in family characteristics, EBV viral loads, GPX selenium levels and malnutrition as fundamental in the divergent incidence rate of eBL in the two clusters. These are major predictor of disease burden in the area and require intense epidemiologic surveillance.
CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

6.1.1 GPX Selenium levels were found to differ significantly in the two study sites. There was higher mean GPX selenium level in children living in EBL low risk area as compared to those living in EBL high risk areas. There was an inverse correlation between selenium levels and EBV loads and malaria densities.

6.1.2 The EBV viral load differed significantly in the two sides. The children in the EBL high risk areas had up to 50% of non detectable levels of EBV copies. As for those who had detectable EBV levels, the viral load in them was very high as compared to the children living in the EBL low risk areas.

6.1.3 Malaria densities in children living in EBL high risk areas was high.

6.1.4 Malnutrition was also common in children living in EBL high risk areas

6.1.5 Summary of conclusions

The study outcome clearly indicated that reduced selenium and increased EBV viral loads in children are associated with residing in eBL high risk regions compared to the low incidence region. However, in multivariate modelling, EBV viremia and selenium levels were significantly associated with age and not place of residence. This suggests that synergy of multiple risk factors (ie malaria and EBV co-infections in conjunction with low selenium levels) may be necessary to set the stage for eBL tumorigenesis but each covariate is not sufficient to independently result in eBL. The higher number of Q-PCR EBV (-) (EBV non-detects) individuals from the eBL high risk area, were possibly due to the frequent use of malaria drugs which suppresses the EBV DNA in blood. The EBV viral loads did not differ by gender but by age of child. There was an inverse relationship between pGPx Selenium levels and EBV viral loads in the study children. The study also found a household population density to have some influence in child vaccination adherence.
as seen in cases where household with more than six occupants' likely fault on completion of child vaccination schedules. The main sources of GPX Selenium in the area were common food crops and fruits. River water and fried fish were found to be some the major sources of selenium in the study area. The use of river water was common in the BL cold spots as the eBL hotspot were likely to use the Lake Water. The GPS longitudinal distribution of malaria by households in the area indicated reduction of malaria positive cases $RR = 0.64$ per 100 meters increase (95% CI: 0.55, 0.76 $P < 0.0001$).

The increase in RDW (the coefficient of variation of mean RBC volume) is a biomarker for malnutrition status (vitamin $B_{12}$, folic acid, and iron) as in higher number of children with in the BL hotspot which indirectly points to the underlying nutritional differences in the study areas. The study found social demographic practices to affect child vaccination trend. 90.3% mothers out of 281 attended the antenatal clinics but only 35.4% delivered in a health institution, leaving out 65.6% mothers delivering outside health institution. This translates to only 35.8% of 281 children receiving BCG/ Polio vaccine at birth. Out of 65.6 children delivered outside, 59.2% were later taken for vaccination at different stages in life but 6.4% of 281 children completely missed any vaccination even at latter date. The majority of the victims came from the eBL hotspot.
6.2. Operational Recommendations

1. Future research in this area should locate resource for analysis of essential elements

2. Future activities should involve geo-chemical analysis

6.3. Recommendations for future research in the area

1. Future research on the impact of nutrition and emergence of cancer

2. Mapping of selenoproteins and other essential elements in the BL clusters as a bio-predictor for epidemiological surveillance.
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Appendix I: Picture showing fish preserved by different methods displayed for sale by the road side in Kisumu city, Kenya

Adapted from odada Kasumba research pictures 2009

Smoked and fry dried fish may be a source of carcinogen, polycyclic aromatic hydrocarbons (PAH) and formaldehydes associated with recycled oils and smoke.
Appendix II: Map of Annual Distribution of endemic Burkitt's lymphoma in Nyanza Province

Map of Nyanza Province, Kenya. Shown are the 6 Districts within Nyanza Province.

1999 Division Boundaries.
Average annual cases / 100,000 children
- 0.39 - 1.50
- 1.59 - 3.09
- 3.10 - 4.99
- 4.99 - 7.05
- No reported cases

(Adapted from Rainey et al., 2007)
Appendix III: Study Consent Forms

Peter Odada Sumba KEMRI Consent for Human Investigational Studies

**Study Title:** Nutritional Deficiency as a risk factor for endemic Burkitt’s lymphoma in Western Kenya.

**Consent form:** Endemic Burkitt’s lymphoma cross section study

**Purpose:** This is endemic Burkitt’s lymphoma (eBL) environmental epidemiology study looking at how nutritional deficiency (selenium and Vitamin E) and environmental ecology relates to this childhood cancer. The micronutrient deficiencies are known to be associated with pro-oxidant activities thus reducing the child's ability to neutralize the effects of free radicals and high viral load. We suspect that lack/reduced state of the micronutrients and antioxidants result in the multiplication of eBL infected lymphocytes. Mr. Peter Odada Sumba from the Kenya Medical Research Institute (KEMRI) and his colleagues at Kenyatta University and Suny Upstate Medical School, USA, are inviting you to enroll your child to participate in this research study. We are doing this study because we want to understand the environmental etiological factors associated with clustering of eBL in Nyanza when all the children have the risk of exposure to malaria and Epstein Barr virus. The goal of this research study is to understand how individual’s ecological environment influences disease development. Information gained from this study will help researchers design environmental measures to protect vulnerable children against this childhood cancer and other infections. This information will also be helpful for antioxidant supplementation to global population at risk of the disease.

**Procedure:** In order to understand the environmental influence on malaria/EBV interaction and subsequent development of eBL, we need to know about the child’s environmental habitat by having information about your child, living arrangement, feeding habits and some cultural practices. We shall also be requesting a small blood sample (2 mls) from your child once every six months for the next 3 years. It is important if you agree to be in this study that you will not migrate from the area for the next three years. Blood will be drawn from a vein in your child's arm using a needle attached to a blood collection syringe or tube. About 2 teaspoons of blood (2 mls) will be requested each time. Blood will be transported to the KEMRI/SUNY laboratory at Kisian in Kisumu city. Tests done in the laboratory will tell us the nutritional status of your child and malaria and EBV infections. To monitor your child’s growth, we will also measure their height and weight.

If you do not agree to have your blood/saliva stored for viral load testing, we will destroy the sample after we have tested it for nutritional status and malaria parasite. If, at any time you wish to withdraw your agreement, please contact Peter Odada Sumba at KEMRI Kisumu Tel. 254-57-2022989 or 254-733-746854/254-720-766550 and we will destroy the samples. If you do not wish to have the blood stored, you may still participate in this study. You /child will still be examined for nutritional deficiency and participate in environmental questionnaire. If the child has symptoms such as diarrhea, fever or anemia (lack of blood) and is infected with malaria, the clinical officer or nurse assisting the investigators in this project will provide you with free medicine to treat malaria infection as recommended by ministry of health malaria treatment policy.
Long-term storage and future studies: I agree for KEMRI to store my blood/saliva for future studies of etiological factors that may influence eBL development. I understand that if any test results are found that are important for my health, KEMRI will try to report this to me, if possible. I understand that I have the right to withdraw my agreement for future research anytime and for any reason. I may also ask that my blood not be used for certain types of testing. To do this, I may tell Peter Odada Sumba the KEMRI principal investigator, of my request and he will inform the other investigators at Kenyatta University and SUNY Upstate Medical School. I understand that the KEMRI SSC and ERC will approve any future testing not described here.

If you agree, circle “YES”. If you do not agree, circle “NO”

YES   NO

Signature* ___________________________ Date ___________________________

Witnessed by ___________________________ Date ___________________________

*A person can sign, or verbally state his/her consent in the presence of a witness who will then sign or put a thumb impression

Risks and Benefits: There are minimal risks in donating saliva or having your child’s blood drawn. The blood drawings include a little bleeding, pain, bruising and rarely infection. All of these are uncommon events that may occur in very few people. This research group has not noted any of these risks in the group previously studied. The benefit of having the child’s ecological environment tested is to establish the essential background information likely to be in the food chain. The benefit of testing of saliva is to establish the EBV viral load of the mother/caretaker likely to be transmitted to the child. The benefits of child’s blood drawn are free testing and treatment for malaria illness and establishing the viral loads. Blood will be checked for malaria by blood smear. If the blood smear is positive and your child has malaria symptoms, such as fever and headache, the Clinical Officer assisting the principal investigator will offer you the drugs currently recommended by the Kenya Ministry of Health for the treatment of uncomplicated malaria.

The current medication recommended by the Kenya National Health Guidelines for the treatment of uncomplicated malaria is Artemether-Lumefantrine (CoArtem®). Artemether-Lumefantrine has been shown to be very effective for treating uncomplicated malaria even if the parasites are resistant to other antimalarial drugs. The side effects of this drug may include dizziness and fatigue, loss of appetite, nausea, vomiting, abdominal pain, myalgia, racing heart beat, trouble sleeping headache, rash and aching joints. These side effects are extremely rare. This drug should not be taken by women who are pregnant. The benefits of taking the medication are the treatment of symptomatic malaria, which can cause severe sickness and even death.

Confidentiality: The results of the studies using saliva and blood will be assigned a study number to preserve confidentiality. A database linking personal identifiers to the study number will be kept by the principal investigator and relevant key personnel. The other people assisting the investigators will be blinded to the true identity of the study participants.

Summary of your rights as a participant in a research study: Your participation in this research study is voluntary. Refusing to participate will not alter your usual health care or involve any penalty or loss of benefits to which you are otherwise entitled. If you decide to join the study, you may withdraw at any time and for any reason. If information generated from this study is published or presented, your / or child’s identity will not be revealed. If you or child
experiences physical injury or illness as a result of participating in this research study, contact
The Director of the Center for Global Health and Research CGHR) at KEMRI in Kisumu at PO
Box 1578, Kisumu 40100 Tel. 254-57-2022924 or Peter Odada Sumba at 254-57-2022989/ 254-
733746854/254-720-766550

**Contact Information:** Further information with respect to illness or injury resulting from a
research procedure as well as a research subjects’ rights is available from KEMRI/National
Ethical Review Committee (ERC), PO Box 54840, Nairobi at 254- 2-02722541 or The Director
of KEMRI, PO Box 54840, Nairobi at 254- 2-02722541. If you have any questions about this
study, you may also speak to The Director of CGHR, KEMRI in Kisumu at 254-57-2022924.
Peter Odada Sumba can be contacted at CGHR, KEMRI, PO Box 1578, Kisumu at 254-57-
2022989 / 254-733-746854 / 254-720-766550

**Signature:** Signing below indicates that you have been informed about the research study in
which you voluntarily agree to participate; that you have asked any questions about the study; and
that the information given to you has permitted you to make a fully informed and free decision
about your participation in the study. By signing this consent form, you do not waive any legal
rights, and the investigators are not relieved of any liability they may have. You can withdraw
from this study at any time. A copy of this consent form will be provided to you.

**First sample collection:**

Signature __________________________________________ Date __________
Witnessed by __________________________________________ Date __________
Date __________

Signature of Principal Investigator (Affirming subject eligibility for the Study and that informed
consent has been obtained.)

**Sample collection # __ :** --------------------------------------------

Signature __________________________________________ Date __________
Witnessed by __________________________________________ Date __________
APPENDIX IV: Questionnaire for Nutritional Deficiency as a Risk Factor for Endemic Burkitt's lymphoma in Western Kenya

A.1. Study number
A.2. Household number
A.3. Date of interview
A.4. Interviewer
A.5 Child's name--Date of birth--
   Sex-----
A. Your relationship to the child? 
A.6. District--A.9. Location
A.7. Sub location--A.11. Village
A.8. Name of the head of the homestead
A.9. Number of household at the homestead
A.10. Name of assistant chief
A.11. Study site ('Hot spot')--‘cold spot’
A.12. Samples collected -- Saliva, Blood
A.13. Temperature--Hemoglobin--Hb/dl

HOUSEHOLD INFORMATION

B.1. How many people are there in your household, that is, people whose main residence this is and who share at least one meal a day, or share living accommodation with you?
B.2. Which of the following best describe your house
   1. Traditional hut (mud floor/mud wall/grass thatched roof)
   2. Semi permanent (mud floor/mud/cement wall/iron roofed)
   3. Permanent (cemented floor/bricks/stone wall/iron/tile roof)
   4. Other types of housing--

B.3. Do you have a separate cooking house (kitchen) or you cook in the same house?
   1. Yes
   2. No
   If answer to QB3 is No then move to QB5

B.4. Do you use the separate cooking place (kitchen) for any of the following?
   1. Keeping chicken;
   2. Keeping calves, goats, sheep, etc;
   3. Children’s study room;
   4. Children’s sleeping room;
   5. Store for food/ agricultural produce;
   6. Store for agrochemicals and farm equipments;
   7. None of the above
   8. Others----------------------------------

B.5. Do you share your main dwelling house with any of the following?
   1. Chicken;
   2. Calves, goats, sheep, etc;
   3. Younger children;
   4. Store for food/ agricultural produce;
   5. Store for agrochemicals and farm equipments;
   6. None of the above
   7. Others----------------------------------

B.6. How many windows are there in the main living house?  ---------

    None

B.7. How many windows does the kitchen have?  ---------

    None

B.8. Observe for other ventilations like eaves sizes------------------

B.9. What are the common plants in/around the compound?  ----------------

B.10. What are the common sources of domestic water?
     1. River,
     2. Lake,
     3. Common well
     4. Pond
     5. Seepage
     6. Rain catchments
     7. Stream
     8. Piped water

B.11. What are the common plant flora around the domestic water source?  ----------------

B.12. How long have you lived in your current residence?

B.13. In the last one year have you/family stayed out of this residence for more than six months?
      Yes
      No
      If yes where were you staying ---------------------------------------

B.14. Before you settled /or farmed on this particular parcel of land, can you recall if any of the following ever took place on this piece of land or any adjacent land near your current home?
      1. Juggary
      2. Tobacco farming,
3. Tobacco drying huts,  
4. Sugar cane farming,  
5. Quarry,  
6. Used to be a mineral mine site,  
7. Kerosene/Petrol pump  
8. Waste damping site,  
9. Carving/black smith  
10. None of the above  
11. Others-----------------------------------

SECTION C

C.1. How many children have you given birth to, including any who are not living here and any who may have died since birth?  
NB. INCLUDE ANY STILLBORN  
1. Specify number  
2. Declined to provide actual number  
C.2. Have you any stepchildren of any age living with you (including any children from your partners' previous relationship or deceased relative)?  
1. Yes  
2. No  
C.3. Has the child---name----- been living with you always?  
1. Yes  
2. No elsewhere (name----------------------------------)  
3. No deceased  
C.4. Where was the child name------------------- born?  
1. Hospital  
2. Home  
3. Can’t remember  
C.5. How old was your youngest child when child name----- ------------------was born?  
1. One year  
2. Two years  
3. Three years  
4. Four years and above  
5. I don’t know  
C.6. Since the birth of child name----- who has been assisting you in caring for him/her?  
1. Mother in law  
2. Father in law  
3. Sister in law  
4. Maternal relative  
5. Husband  
6. House help  
7. I cared for her/him a lone.  
8. My husband’s grandmother  
C.7. Did you change helper/care taker for child name-------during the time when you were breast feeding/weaning?
1. Yes
2. No
3. Can't remember

C.8. If yes, how many times did you change the care takers?
   1. None
   2. One time
   3. Two times
   4. Three times
   4. More than three times

C. 9. From which district/village was the helper who stayed with child name----- longest originating from?
   1. District -----------------------
   2. Village -----------------------
   3. Don't know ---------------------

C.10. When you were expecting child -----name-----were you attending any antenatal clinic?
   1. Yes
   2. No

C.11. When you were expecting child name------ did you receive Tetanus toxoid injection usually administered as an injection on the thigh (2 injections or 1 booster)?
   1. Yes
   2. No

C.12. Can you tell me if the child was ever offered the following immunization? (Prompt and ask for the card wherever possible)
   1. After birth BCG below right shoulder/ oral POLIO
   2. 1st clinic visit (1-2 months) DPT on the thigh / oral POLIO / (BCG if not given at birth)
   3. 2nd visit (2-3 months) DPT on thigh/ oral POLIO
   4. 3rd visit (3-6 months) DPT on thigh /POLIO oral
   5. 4th visit (9 months) MEASLES
   6. HEPATITIS
   7. Others------------------------
   8. No immunizations

C.13. Has the child ever had any accidental injuries at home or in school?
   1. Yes
   2. No (If no move to C.19.)

C.14. When did the most recent accidental injury occur....
   1. within the last 12 months
2. within the last 5 years
3. within the last 10 years
4. Longer than ten years

C.15. What was the nature of the injury/s? 

C.16. As a result of that most recent accident, how soon was the child offered treatment?
1. within a day,
2. after one day,
3. after two days,
4. after three days and over,
5. No treatment provided

C.17. Where was the child treated?
1. Home based therapy,
2. Hospital,
3. Traditional healer,
4. Herbalist
5. Medicines from local shops
6. Private clinic
7. Drugs bought from pharmacy
8. Others

C.18. Has the child name --- been diagnosed in hospital/clinic or by a health worker to be malnourished? Yes------- No----------------

C.19. Over the last twelve months would you say this child’s health has on the whole been good, fairly good, or not good?
1. Good
2. Fairly good
3. Not good

C.20. Do any of your children under 16 years have any long standing illness, disability or infirmity? By long standing I mean anything that has affected them over a long period of time
1. Yes (any child) 2. No (None of them)

C.21. During the last 12 months, (that’s from yesterday back to –), have any of your children under 16 been to hospital as an inpatient overnight or longer?
1. Yes
2. No
SECTION D

D.1. Thinking of when child named was born, for the first feed was he/she breastfed or bottle-fed?

Note: breastfed includes expressed breast milk in a bottle – bottle-fed include formula only, fresh cow/goat milk, and commercial milk packets.

1. Breastfed
2. Bottle-fed (powdered formula only)
3. Bottle fed (fresh cow milk)
4. Bottle fed (fresh goat milk)
5. Bottle fed (commercial packed)
6. Can’t remember

D.2. At 2 weeks was your child. (Run prompt)
1. Only breastfed, 2. Only bottle-fed,
3. Both 4. don’t know/Cant remember

D.3. And at 4 weeks was your child (Run prompt)
1. Only breastfed, 2. Only bottle-fed,
3. Both 4. don’t know/Cant remember

D.4. And at 6 weeks was your child
1. Only breastfed, 2. Only bottle-fed, 3. Both
4. Don’t know/Cant remember

D.5. At 4 months old was your child...
1. only breastfed (no solids), 2. only bottle-fed (no solids),
3. Breastfed and bottle-fed only (no solids)
4. Breast, bottle-fed and solids, 5. Breast and solids,
6. Bottle-fed and solids, 7. don’t know/Cant Remember

D.6. Finally, at 6 months old was your child…… (Run prompt)
1. Only breastfed (no solids)
2. only bottle-fed (no solids)
3. Breastfed and bottle-fed only (no solids)
4. Breast, bottle-fed and solids
5. Breast and solids,
6. Bottle-fed and solids
7. don’t know/Cant Remember

D.7. I understand times can be hard to provide the young child with standard meals, may I know if at one time you mouth chewed the food for the baby when you were introducing him/her to solid foods?
1. Yes
2. No

D.8. How did you introduce the child to porridge?
   1. Bottle fed
   2. Cup fed
   3. Hand held feeding
   4. Mouth transfer feeding
   5. Others

D.9. How many times have you seen the child playing with soil or actually eating soil?
1. I never saw him/her eat soil
2. Once or twice
3. Occasionally during the time the baby was learning to crawl,
4. Many times when the baby started walking
5. Can’t remember

D.10. Are there times you noticed the child’s mouth had some traces of soil though you never saw her/him eat soil?
1. I never saw him/her eat soil
2. I saw him/her once or twice
3. Occasionally during the time the baby was learning to crawl,
4. Many times when the baby started walking
5. Can’t remember

D.11. Have you at any one time seen or heard that the child name---- playing with plants like euphorbia triculi
1. I never saw him/her play with plants like euphorbia
2. I saw him/her once or twice
3. Occasionally during the time of fetching firewood
4. Many times when the children were playing in school/home
5. Can’t remember

D.12. Have any of your children under 16 ever been to a dentist’s surgery, either for treatment or for some other reason?
1. Yes
2. No

D.13. Last time this child went to the dentist, was it because............... (Run prompt)
1. He/she was having trouble with their teeth
2. He/she went for a check-up
3. You had a note from the school
4. He/she had false teeth
5. Had fever at the time of teething,
6. Others, none of above

D.14. When was the last time this child went to the dentist surgeon----------------------
1. less than one month ago
2. 1 month – less than 3 months
3. 3 months – less than 6 months
4. 6 months – less than 9 months
D.15. When your child has trouble with their teeth in the past, have they ever gone anywhere other than the dentist/hospital?
1. Yes 2. No [next section]

D.16. If the response to D.15 is yes, where did they go?
4. Private hospital, 5. Dental technician
6. Traditional healer 7. Others? Specify

D.17. I would like to ask you if the child ever had false teeth
1. Yes 2. No

D.18. Has the child name ------- been taken to hospital/health worker for removal of false teeth or uvula
1. Yes 2. No

SECTION E

E.1. Which of these animals do you keep in your compound?
1. Cattle 2. Donkeys
5. Pigs 6. Chicken/ducks/turkey

E.2. What are the common crops or fruits you grow in your farm? ----------------------------

E.3. What are the common culinary practices do you use when preparing meals for the family?
1. Frying 2. Roasting
5. Grilling 6. Stewing

E.4. Which of the following fuel source do you commonly use in your household?
4. Electricity 5. Others ----------------------

E.5. How often does your family eat a meal of sun dried fish/meat?
1. Always 2. Very often
3. Fairly often 4. Some of the time
5. Almost never 6. Never

E.6. How often does your family eat a meal of smoked fish/meat?
1. Always 2. Very often
3. Fairly often 4. Some of the time
5. Almost never 6. Never

E.7. How often does your family eat a meal of fry dried fish/meat?
1. Always 2. Very often
3. Fairly often 4. Some of the time
5. Almost never 6. Never

E.8. How often does your family eat a meal of pickled (Salted) dried fish/meat?
1. Always 2. Very often
3. Fairly often 4. Some of the time
5. Almost never 6. Never

E.9. How often do you use tomatoes and/or onion and/or dania (Coriander leaves) when preparing the family meal?
1. Once in a while when I have money to buy them,
2. Occasionally when I have visitors,
3. I use them commonly during the rainy season when I have some in the garden,
4. I always use tomatoes and onions when preparing meals,
5. Rarely because some of my family members do not like them
6. I do not use them at all.

E.10. Which are the common vegetables you commonly use in your household?  
E.12. Do you have any allergy to foods? ; or have you at any one time been advised not to consume certain foods while expecting any of your children 
1. Yes  
2. No 
3. Don’t remember  
4. Never 
If yes can you name some 

SECTION F 
F.1. What is your denomination? 
10. Other Christians 11. Free Presbyterian 12. Legion Maria 
13. Non-Christian 
F.2. And how often do you attend your place of worship? 
1. More than once a week 
2. At least once a week 
3. At least once a fortnight 
4. At least once a month 
5. At least once every few months 
6. At least once a year 
7. Less often 
8. Never 
9. Unable to attend 

F.3. Do you usually use mosquito net at night while sleeping? 
1. Yes 2. No 
G.4. Does the child name --- use mosquito net? 
1. Yes 2. No 
G.5. Are the nets impregnated with insecticides. 
1. Yes 2. No 
G.6. Have you or any other persons including health workers sprayed your house with a residual spray for mosquitoes? 
1. Yes 2. No
TO: MR. PETER S. ODADA (Principal Investigator)

THROUGH: DR. J. VULULE, THE DIRECTOR, CGHR, KISUMU

RE: SSC PROTOCOL No. 1025 (REQUEST FOR STUDY RENEWAL): NUTRITIONAL DEFICIENCY AS RISK FACTOR FOR ENDEMIC BURKETT’S LYMPHOMA IN WESTERN KENYA

Dear Sir,

Reference is made to your letter email 14th April 2009. We acknowledge receipt of the revised continuing review report.

The Committee notes that:

3. The data on birth and child vaccination records have been updated for clarity.

A paper titled "Baseline Selenium Levels and Endemic Burkitt's Lymphoma in Western Kenya" has been presented at the Kampala AORTIC Conference in 2008. No new information is currently available that may affect the current status of the study.

The Committee is satisfied that the issues raised at the 165th meeting of 14th April 2009 have been adequately addressed and granted approval for continuation for a further term of twelve months effective this 16th day of April 2009. Please note that authorization to conduct this study will automatically expire on 18th March 2010. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat on Thursday, 4th February 2010.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

Yours sincerely,

C. WASUNNA,
FOR: SECRETARY,
KENYAT/NATIONAL ETHICS REVIEW COMMITTEE
May 16, 2007

To
Rosemary Rochford, Ph.D.
Dept. of Microbiology and Immunology
SUNY Upstate Medical University
750 East Adams St.
Syracuse, NY 13210

Re: R03 Application: Micronutrient Malnutrition and EBV persistence in children

Dear Dr. Rosemary,

I am delighted to be the Foreign Principal Investigator on the proposal that we are submitting to examine the role of micronutrient malnutrition and the effects on EBV persistence. This study is a continuation of our collaboration that we initiated when you first sent your student Adam MacNeil here to examine exposure to *Euphorbia tirucalli* and have continued through the last 5 years. The question of why do children get Burkitt’s lymphoma is a pressing one in Kenya and the role of nutrition in increasing this risk has not been explored. I look forward to our continued collaborations and will be the director of this project in Kenya when we begin these studies.

Sincerely yours,

P. Odada Sumba, MPH, HND, MLT
Senior Research Scientist
Centre for Vector Biology and Control Research
Kenya Medical Research Institute, Kisumu, Kenya
P.O.Box 1578 Kisumu 40100
254 720 766550 /254 733746854
odadakasumba@yahoo.com or podada@kisian.mimcom.net

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In Search of Better Health
Dear Sir,

RE: SSC Protocol No. 1025 (3rd Revised) – Nutritional deficiency as a risk factor for endemic Burkitt’s Lymphoma in Western Kenya

This is to inform you that during the 133rd Meeting of KEMRI/National Ethical Review Committee held on 23rd May 2006, the above protocol was discussed.

The Committee noted that you would wish to determine the role of selenium, vitamin E and socio-cultural practices in the development of endemic Burkitt’s Lymphoma in children living in malaria holo-endemic regions of Nyanza Province.

The study is hereby granted approval for one year. You are however, responsible for reporting to the ERC any changes to the protocol or in the Informed Consent Document. This includes changes to research design or procedures that could introduce new or more than minimum risk to human subjects.

You may begin your study.

C. L. Wasunna,
For: Secretary,

KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE

In Search of Better Health
KENYATTA UNIVERSITY
OFFICE OF THE REGISTRAR (ACADEMIC)

P. O BOX 43844
Nairobi, Kenya
Tel: 810901-19
Ext: 57183/4
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admissions@kju.ac.ke

DATE: 13 May 2008

OUR REF: 184/15301/2005

SUMBA PETER ODADA
CB KENYA MEDICAL RESEARCH INSTITUTE
CENTRE FOR VECTOR BIOLOGY AND
CONTROL RESEARCH
PO. BOX 1578 - 40100
KINUMU

Dear Mr. Sumba,

RE: DEPARTMENTAL TRANSFER

Your letter dated 14/2/2008 on the above subject refers.

Please note that your request to transfer from the Department of Public Health to the Department of Pathology was considered and approved.

You are requested to liaise with the Chairman, Department of Pathology for further advice on your studies.

Thank you.

Dr. (Mrs.) J.A. Samhauni
FOR: REGISTRAR (ACADEMIC)

Cc:
- Dean, School of Health
- Dean, Graduate School
- Chairman, Department of Pathology
- Chairman, Department of Public Health

JAS/gam
September 26, 2007

Peter Odada Sumba, MPH
Case Western Reserve Univ
PO Box 1578
Kisumu 40100
KENYA

Dear Peter Odada Sumba:

Congratulations on being selected as a travel award winner for the ASTMH 56th Annual Meeting. This year's meeting will be held at the Philadelphia Marriott Downtown in Philadelphia, Pennsylvania on November 4-8, 2007.

Please note...as a travel award winner, you are required to attend the entire meeting from Sunday, November 4 through Thursday, November 8. In addition, please attend the opening plenary session on Sunday evening, November 4. All travel award winners will be recognized and will take a group picture at the end of the session.

Your travel award includes the following:

1) **Airfare.** Round-trip coach airfare to Philadelphia, Pennsylvania USA. You will arrive in Philadelphia on November 3 or November 4 and depart on November 8.

2) **Cash Award.** Your travel award includes an $800.00 cash award. **This $800.00 is to be used for your hotel, ground transportation and other related travel costs while attending the conference.**

3) **Registration.** As a travel award recipient, you receive a complimentary meeting registration.

The ASTMH Annual Meeting is the premier source for education in the tropical medicine field. Annual Meeting features include oral presentations in scientific sessions devoted to specific topics in tropical medicine, scientific poster presentations, topical symposia and invited lecturers and exhibits showcasing valuable products and services for the tropical medicine and hygiene field.

ASTMH is pleased to support your travel to the 56th Annual Meeting. We are confident that your participation in the meeting will be a worthwhile educational experience.

Sincerely,

Judy DeAcetis
Administrative Director
APPENDIX X: POSTER FOR AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE (ASTMH 56TH ANNUAL MEETING NOVEMBER 2007), PHILADELPHIA, USA.

Seelenium Levels, Malaria and Endemic Burkitt's Lymphoma in Western Kenya

**ABSTRACT**

Endemic Burkitt's lymphoma (EBL) is one of the most prevalent cancers in Kenya. The etiology of this lymphoma is unclear. Increased knowledge early age infection with Plasmodium falciparum (PF) and Helicobacter pylori has been proposed. There's much evidence on the children being at the endemic area. One potential factor could be selenium deficiency. Selenium is an essential micronutrient and an integral component of the anti-oxidative enzymes glutathione peroxidase (GPx). Many populations worldwide exhibit selenium deficiency. In India, however, there is a strong association between selenium deficiency and Burkitt's lymphoma (BL). This paper reports on a study conducted in Western Kenya that compared selenium level and its relationship to children living in EBL endemic areas. The study used nutritional and anthropometric measures to examine children with and without selenium deficiency and concluded that children with BL had higher selenium levels than those without. The study also suggested that children living in areas with higher selenium levels were at lower risk of developing BL.

**STUDY DESIGN & POPULATION**

The study assessed 75 children aged 1-7 years in high and low risk areas of EBL in Kenya. The study was conducted in two stages: the first stage assessed the effect of selenium deficiency on children in the high-risk area, and the second stage assessed the effect of selenium deficiency on children in the low-risk area.

**CURRENT STUDY SITE BY ADMINISTRATIVE LOCATIONS**

**INTRODUCTION TO SELECTED AND METHODOLOGY**

- One student's model in each
- Human genome project, molecular biology, and gene therapy
- Ethical values: human rights range from 0-200 (1-4.5)
- Selenium (Se) is a mineral element and integral component of glutathione peroxidase (GPx), glutathione reduction and glutathione reductase. It is also argued that the use of selenium as an antioxidant is associated with an increased risk of cancer.

**DATA ANALYSIS**

- P-value: 0.05
- Pearson correlation coefficient: 0.70
- Chi-square test: 0.01

**RESULTS**

We found that children living in high-risk areas of EBL had a lower selenium level (0.45 mg/dL) than those in the low-risk areas (0.65 mg/dL). These differences were statistically significant (p < 0.05). In the high-risk area, the median selenium level was 0.45 mg/dL, while in the low-risk area, it was 0.65 mg/dL. These levels were compared using a t-test for equality of means and the difference was highly significant (p < 0.001).

**ACKNOWLEDGMENTS**

2007 System Medical University
Dontology Medical University
Eckersley University
Dental College
Kenya Medical Research Institute
Johoffe Medical
Oro Medical
Cheshire Hospital
Creswell Medical Center
Ann M. Montane University
Kakuma School
Jane Financial
Kenya Medical Council
Alien G. Group

**CONCLUSIONS**

The difference in selenium levels noted in the high and low EBL areas with the lower research area as an essential oxidant and integral component of glutathione peroxidase (GPx), glutathione reductase, and glutathione reductase. It is also a strong antioxidant in its capacity as an antioxidant in a number of antioxidant systems. An important role of antioxidant function is well established. However, the effect of differences in specific antioxidant enzyme levels like selenium (Se) in relation to the incidence of certain cancers.
APPENDIX XI: AORTIC CONFERENCE (50TH ANNIVERSARY OF BURKITT’S LYMPHOMA) FEBRUARY. 2008 KAMPALA, UGANDA,

SELENIUM LEVELS AND ENDEMIC BURKITT’S LYMPHOMA IN WESTERN KENYA.

Peter Odada Sumba¹, E.W.Kabiru², Opiyo Michael¹, Dickens Kowuour¹, Chelimo Kiprotich¹, Alloys S.S. Orago², Paula Rosenbaum³, Ann M.Moormann⁴, Rosemary Rochford³

¹Kenya Medical Research Institute, Kisumu, Kenya; ²Kenyatta University, Nairobi, Kenya; ³SUNY Upstate Medical School, Syracuse, New York, USA; ⁴Case Western Reserve University, Cleveland, Ohio, USA.

Endemic Burkitt’s lymphoma (eBL) is the most prevalent pediatric cancer in Equatorial Africa. The etiology of this lymphoma is multi-factorial involving early-age infection with Epstein Barr Virus (EBV) and frequent exposure to Plasmodium falciparum malaria. Since these co-infections are common in African children living in malaria endemic areas, other eBL risk factors are being investigated. One potential co-factor could be selenium deficiency. Selenium is an essential micronutrient and is an integral component of the antioxidant enzyme glutathione peroxidase (GPX). Many populations worldwide exhibit selenium deficiencies but little is known about selenium deficiency within African populations. Moreover, there is emerging data to suggest the selenium deficiency potentiates viral infections. We hypothesized that deficiencies in selenium could contribute to increased risk for eBL by decreasing host ability to control EBV infection. To test this hypothesis, a cross sectional survey was conducted in children living in a region of Western Kenya that has high incident rates of eBL. Blood was collected from 79 children ages 1 to 11 years, to determine selenium levels using pl¹GPx enzyme immunoassay for selenium GPX. In addition, EBV viral loads were measured by RTQ-PCR. The mean selenium levels were 2.4 mcg/dl +/- 0.7 (range 0.63-3.38 mcg/dl). This level is lower than those reported for developed countries where levels range from 6-8 mcg/dl (New Zealand and Finland) to 14-25 mcg/dl (USA). To test whether the selenium levels correlated with EBV viral load, we measured EBV viral load in the same study participants. Although several children had elevated EBV viral loads, we did not find a correlation of selenium levels with EBV viral load. Our data demonstrates that there is evidence of selenium deficiency in children living this region in Kenya but more studies are needed to determine what impact selenium deficiency has on immune function.

Key Words: Selenium; malaria; Epstein Barr virus; endemic Burkitt’s lymphoma
Baseline Selenium Levels and Endemic Burkitts Lymphoma in Western Kenya

Sumba P.O1, Ephantus Kabiru2, Paula Rosenbaum3, Ann M. Moormann4, Alloys S. Orago2, Rosemary Rochford3

1Kenya Medical Research Institute, Kisumu, Kenya, 2Kenyatta University, Nairobi, Kenya, 3SUNY Upstate Medical University, Syracuse, NY, USA, 4Case Western Reserve University, Cleveland, OH, USA

ABSTRACT

Endemic Burkitt’s lymphoma (eBL) is a common pediatric cancer in Kenya. The etiology of this cancer is multi-factorial involving early-age infection with Epstein Barr virus (EBV) and frequent exposure to Plasmodium falciparum malaria. Other environmental risk factors being investigated are Thevetia peruviana a plant associated with electrolyte disturbances and selenium. Selenium is an integral component of the antioxidant enzyme glutathione peroxidase (GPx). Little is known about selenium deficiency within African populations though emerging data suggests that selenium deficiency potentiates viral mutations. We hypothesized that deficiencies in selenium could increase risks for eBL by decreasing host ability to control EBV infection. To test this hypothesis, 145 children aged 1 to 11 years were sampled in a cross sectional survey in two areas with higher and lower than expected incident rates of eBL and tested for selenium using Plasma GPx enzyme immunoassay. We found that children living in a low BL risk area had a mean selenium level of 3.74 ug/dl (n=78) while children living in a high BL risk area had a mean selenium level of 2.44 ug/dl (n=67). These values were compared using a t-test for equality of means and the differences were highly significant (p<0.0001). Our data demonstrates that there is evidence that children living in a high BL risk regions have significantly less selenium GPx than children living in a low BL risk region but more studies are needed to determine what impact selenium deficiency has on immune function.

Keywords: endemic Burkitt’s lymphoma (eBL), selenium deficiency,