

**BURDEN OF MALNUTRITION IN 7-10 YEAR OLD CHILDREN BORN IN A
PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HIV INFECTION
PROGRAMME IN ZIMBABWE**

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Dedication

This thesis is dedicated to my grandfather; Elias Toperesu Mateveke who believed in the potential of his daughters and granddaughters changing the lives of the women in my family. In addition, I also dedicate this work to my children Elizabeth Chido Kuona and Alison Thandiwe Kuona. The greatest gift bestowed on humans is hope for a brighter future.

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Thesis Summary

Background:

Africa carries the dual burden of malnutrition co-existing with HIV infection. Under-nutrition underlies almost half of the under-five childhood deaths while HIV/AIDS is among the top 10 causes of childhood mortality. Though under-nutrition is an essential problem in under-five children, it has also remained an important issue in school-aged children from low income settings and has many adverse consequences that can be immediate or long term. Under-nutrition is often associated with macronutrient and micronutrient deficiencies. Iron deficiency, a multisystem disorder leading to anaemia, is the commonest micronutrient deficiency globally. Less commonly described micronutrients such as selenium deficiency burden have not been clearly defined in children from low income setting particularly ones with high HIV infection burden. In addition, there is very limited information concerning important macronutrient deficiencies such as omega 3 fatty acids which coupled with the multiple micronutrient deficiencies associated with under-nutrition plus HIV infection contribute to poor growth and neurodevelopmental outcomes of children.

The objectives of this thesis were to measure the prevalence of malnutrition, anaemia, iron deficiency, selenium deficiency and describe the omega 3 fatty acid status in HIV unexposed and HIV exposed (infected and uninfected) children 7 to 10 years old who were born in a national mother-to-child transmission of HIV infection prevention programme (The BHAMC study) from a peri-urban setting in Zimbabwe, a low income country with a high burden of HIV infection.

Methodology: This cross-sectional study was a sub-study in the BHAMC study which recruited mother-baby pairs between 2002 and 2004 and was designed to assess impact of sexually transmitted infections on mother-to-child transmission of HIV infection. The study was carried out at 3 peri-urban primary care clinics, just outside Harare the capital city of Zimbabwe, offering maternal and child health services from August 2011 to June 2012.

Main outcome measures- The main outcome measures were the nutritional status(stunting, thinness, underweight, overweight), haemoglobin, serum Ferritin, soluble transferrin receptor, serum selenium and omega 3 fatty acid levels [Eicosapentaenoic acid (EPA), Docosapentaenoic acid (DPA) and docosahexaenoic acid(DHA)]. The nutritional status was defined using the World Health Organization (WHO) criteria for classifying nutritional status of children between 5 and 18 years.

Results: A total of 318 participants 7 to 10 years old were recruited of whom 21 (7%) were HIV infected, 116 (36%) HIV negative exposed and 180(57%) HIV negative unexposed. Majority of the children were in the 8 and 9 year old age groups. There were more female participants (57%). Prevalence of stunting, thinness, underweight and overweight was 12%, 4%, 8% and 5% respectively. Stunting was associated with HIV infection and exposure. Selenium deficiency (<0.89µmol/L) was present in 129 (48%) children. Selenium deficiency was associated with monthly household income below US\$250 and was not related to the participant's HIV status. The prevalence of anaemia (Hb<11.5g/dL), iron deficiency (Ferritin<15µg/L) and iron deficiency anaemia (Hb<11.5g/dL and either F<15µg/L or sTfR>8.3µg/L) were 15%, 4% and 2% respectively. When a higher cut-off for ferritin (<30µg/L) was applied, prevalence of ID and IDA increased to 32% and 5% respectively. Anaemia was more likely to be present in HIV infected children (p-

value<0.001) with an odds ratio of 4.9 (CI 1.9- 12.4). The 7 year old age group had the lowest EPA levels. There was no difference in EPA, DPA and DHA levels by HIV status, gender and nutritional status.

Conclusions and recommendations:

Chronic under-nutrition and selenium deficiency were common in these 7-10 year old children, therefore nutrition programmes targeted for children above 5 years are recommended in our setting and they should be continuous with the already present nutrition programmes for under-fives. More research is required to ascertain selenium status of all children from our setting including rural and urban children above and below 5 years as this may guide the need for supplementation of this micronutrient in our setting.

Prevalence of anaemia and ID were of mild public health significance. Anaemia association with HIV infection calls for strengthening of mother-to-child transmission of HIV prevention programmes in our setting as preventing HIV infection in children could help reduce anaemia burden in children above 5 years. ID can be addressed by teaching consumption of iron rich foods and fortification of basic foods.

This study did not find any differences in omega 3 fatty acid status by HIV status, gender and nutritional status from a peri-urban setting with high burden of HIV and under-nutrition. Further and larger studies are recommended that include urban and rural children to ascertain the relationship of omega 3 fatty acid levels with HIV status plus nutritional status.

List of Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
ART	Anti-retroviral Therapy
ARV	Antiretroviral
AZT	Zidovudine
BHAMC	Better Health for the African Mother and Child
BMI	Body Mass Index
CD4	Cluster of Differentiation 4
CSPRO	Census and Survey Processing System
DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Eicosapentaenoic Acid
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
LCPUFA	Long Chain Polyunsaturated Fatty Acid
MAM	Moderate Acute Malnutrition
MUAC	Mid-Upper-Arm Circumference
OIs	Opportunistic Infections
PCR	Polymerase Chain Reaction
PMTCT	Prevention of mother-to-child transmission of HIV
RNA	Ribonucleic Acid

RUSF	Ready-to-use-supplementary Food
RUTF	Ready-to-use-therapeutic Food
SAM	Severe Acute Malnutrition
SPSS	Statistical Package for Social Science
sTfR	Soluble Transferrin Receptor
UNDP	United Nations Development Programme
USA	United States of America
UZ-UCSF	University of Zimbabwe-University of California, San Francisco
WHO	World Health Organization
ZDHS	Zimbabwe Demographic Health Survey
3TC	Lamivudine

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List of Papers Included in the Thesis

1. **Kuona P**, Mashavave G, Kandawasvika GQ, Dzangare J, Masanganise M, Chandiwana P, M Munjoma, K Nathoo and B Stray-Pedersen. (2014) **Serum Selenium Levels and Nutritional Status of School Children from an HIV Prevention Programme in Zimbabwe.** *J Trop Dis* 2: 134. doi:10.4172/2329-891X.1000134
2. **P Kuona**, G Mashavave, G Q Kandawasvika, M P Mapingure, M Masanganise, P Chandiwana, M Munjoma, K Nathoo and B Stray-Pedersen. **Anaemia and Iron Deficiency in Peri-Urban School Children Born in a National HIV Prevention Programme in Zimbabwe: A Cross-Sectional Study.** *Central African Journal of Medicine* 2014; 60(5/8): 22-28
3. **Kuona, P.**, Mashavave, G., Dzangare, J., Munjoma, M., Nathoo, K. and Stray-Pedersen, B. (2015) **Whole Blood Omega 3 Fatty Acid Levels of HIV Exposed and HIV Unexposed 7 - 10 Years Old Children from a Low Income Country with High Burden of Under-Nutrition.** *Food and Nutrition Sciences*, 6, 485-493. <http://dx.doi.org/10.4236/fns.2015.65050>

1. INTRODUCTION AND BACKGROUND

1.1 Introduction

1.1.1 Global Prevalence of Malnutrition in Children

Malnutrition encompasses both under-nutrition and over-nutrition [1]. Under-nutrition describes growth failure often associated with macronutrient and micronutrient deficiencies as a result of poor nutrition. Over-nutrition represents excess nutrient and energy intake that results in adverse health outcomes. The global prevalence of stunting, underweight and wasting in under-five children has been declining since 1990 [2] contrasting with the prevalence of overweight which has increased during the same period. Almost a quarter of the under-five children in the world are stunted [2]. Under-nutrition leads to delayed motor development, cognitive impairment, behavioural problems, social development deficiency, immunodeficiency, increased morbidity and mortality [3, 4].

Africa and Asia carry more than 90% of the burden of under-nutrition in under-fives globally [2]. For Africa this means that the high burden of under-nutrition is co-existing with a high prevalence of HIV/AIDS [5] since the majority of HIV infected people are found on this continent. HIV infection and under-nutrition are two important factors that contribute to a significant proportion of under-five mortality. Under-nutrition underlies almost half of the under-five childhood deaths [6] while HIV/AIDS is in the top 10 causes of under-five deaths globally and contributes about three percent to childhood deaths in sub-Saharan Africa [7]. In Africa, the prevalence of HIV infection is highest in the sub-Saharan region [5].

1.1.2 Malnutrition in Zimbabwe

Zimbabwe is a land locked country situated in sub-Saharan Africa (See to Figure 1). It has a population of approximately 13 million people [8]. The country has experienced economic problems since the last two decades which peaked in 2008 with the country experiencing phenomenal inflation rates. A multi-currency system was introduced in 2009 and helped stabilize the economy [9]. Zimbabwe has a low human development index and was ranked 156 out of 187 countries in the 2014 United Nations Development Programme (UNDP) report [10]. Currently, the national prevalence of poverty is 63% with 16% of the population experiencing extreme poverty. People living in the rural areas experience more poverty (76%) compared to urban dwellers (38%) [9]. Approximately a fifth of households in rural areas experience moderate to severe hunger. In April 2014, 35% of children 6 to 59 months were reported to have less than 3 meals a day [9]. A quarter of the households in Zimbabwe also look after orphaned children.

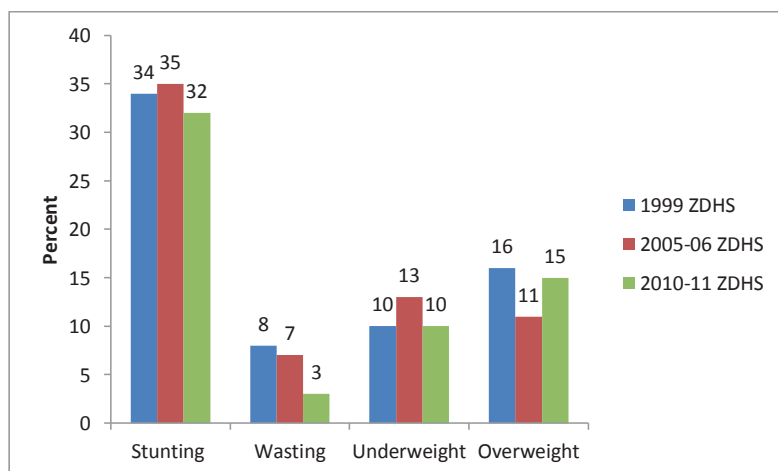


Figure 1 World Atlas Map showing location of Zimbabwe (Google Maps)

Under-nutrition is a key problem in Zimbabwe and is aggravated by the economic decline experienced since 1990 and the HIV/AIDS epidemic [11]. The Zimbabwe Demographic Health Survey (ZDHS) 2010/2011 showed that for under five children,

32% were stunted, 3% were wasted, 10% were underweight and 15% were overweight [12] as shown in Figure 2. The prevalence of stunting and underweight has remained unacceptably high in the past two decades but prevalence of wasting has declined in the same period of time. Overweight trends have not changed much since 1999 but dropped to 11% during the time of economic meltdown in 2005 to 2006. These prevalence figures are based on the 2006 World Health Organization (WHO) growth charts for children under 5 years [13]. Of note is that the ZDHS does not assess the nutritional status of children above 5 years. While the problem of under-nutrition has been prioritized and well defined for under-fives in low income countries with a high prevalence of HIV infection, school going children remain a low priority in nutrition programmes from these settings.

Figure 2 Trends in Nutritional Status of Under 5 Children from Zimbabwe

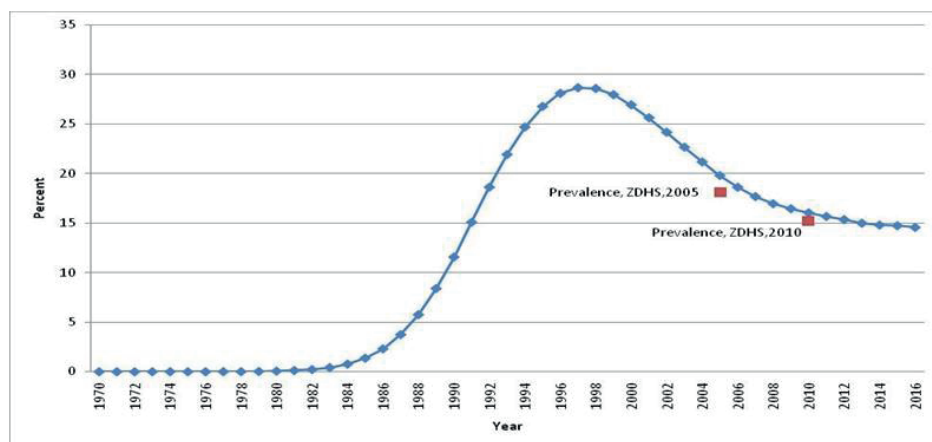


Source: ZDHS 2010/2011

1.1.3 The HIV Epidemic in Zimbabwe

Zimbabwe is located in the heart of the HIV epidemic and it is the only country in Southern Africa that has recorded a substantial decline in the prevalence of HIV infection in adults (see Figure 3). The adult HIV prevalence has been declining in the last decade from 27% but still remains high at 15% for the year 2014 [14]. The new HIV infections in adults have decreased from above 230 000 annually (1997) to below 60 000 (2014) [15]. The number of new HIV infections have also declined from 37 000 (1999) to 3 500 (2014) in children below 15 years of age. Prevalence of HIV infection in children below 15 years is currently 2.7% (see Table1).

Figure 3 Trends in the adult prevalence of HIV infection in Zimbabwe



Source: Zimbabwe Global AIDS Report 2015

Life expectancy at birth has increased from 36.5 years in the year 2002 to 59.9 years in 2014 [10]. Under 5 mortality rate for the year 2014 was 75 deaths per 1000 live births [16] and this represents a decline from 102 deaths per 1000 live births recorded in 1999 during the peak of the HIV epidemic [12]. HIV/AIDS contributes 21% to under-five mortality [12] in Zimbabwe. In response to the HIV epidemic the prevention of mother-to-child transmission of HIV (PMTCT) programme was initiated in 1999 using single dose nevirapine. The government of Zimbabwe declared a state

of emergency in 2002 [17] and the anti-retroviral therapy (ART) programme was initiated in 2004 [17]. The multiple ART for PMTCT was adopted by the country in 2008 using the WHO option A (see Table 2). Option B+ was launched in November 2014. It is estimated that in 2015 approximately 70 000 pregnant women and 113 000 children will require PMTCT and ART services respectively [15].

Table 1 Zimbabwean HIV/AIDS Statistics: 1999 and 2014

Characteristic	Year	
	1999	2014
Total Number People living with HIV	1,758,402	1,420,604
Number of Children <15 years living with HIV	141,109	156,718
Adult ≥15 years HIV Prevalence	25.72 %	14.80 %
HIV Prevalence Children	3.05 %	2.66 %
New HIV infections Adults ≥15 years	120,289	59,339
New HIV infections Children <15 years	36,778	3,587
Total Annual AIDS Deaths (Adults and Children)	117,768	42,534
Annual AIDS Deaths Children < 15 years	22,332	6,176
AIDS Orphans	1,057,494	841,939
Life Expectancy	37.8 years	59.9 years
Under Five Mortality	102	75

Source: Zimbabwe National HIV and AIDS Estimates 2013; Global AIDS Response Progress Report 2015 and UNDP Report 2014

Table 2 Summary WHO recommendations for PMTCT and ART Treatment Guidelines

Year Launched	2001	2004	2006	2010	2013
PMTCT	4 weeks AZT; AZT+ 3TC, or sdNVP	AZT from 28 weeks + sdNVP	AZT from 28weeks + sdNVP +AZT/3TC 7days	Option A (AZT +infant NVP) Option B (triple ARVs)	Option B or B+ Moving to ART for all Pregnant and breastfeeding women
Adult ART	No recommendation	CD4 <200	CD4 <200	CD4 ≤350	CD4 ≤500
Paediatric ART	No recommendation	CD4 guided treatment of advanced disease	CD4 guided treatment of advanced disease	Treat all infants below 2 years	All children<5 years

sdNVP Single dose Nevirapine; AZT Zidovudine; 3TC Lamivudine; ARVs Antiretrovirals; ART Anti-retroviral Therapy

Source: WHO <http://www.who.int/hiv/pub/guidelines/en/>

1.2 Background

Malnutrition can be acute, chronic or both. The indices that combine weight with height [weight-for-height (wasting) and Body Mass Index (BMI) (thinness)] are used to define acute under-nutrition and over-nutrition (overweight/obesity) for different age groups while height for age describes chronic under-nutrition (stunting). Acute under-nutrition is important since it results in a limited ability to respond to stresses such as infection. Acute under-nutrition has been reported to increase risk of dying with moderate and severe acute under-nutrition having mortality rates of 30-115 and 73-187/ 1000/year respectively according to data from Africa as well as Asia [4]. Consequences of stunting include cognitive impairment [18-22], short stature in adults [23-25], poor reproductive outcomes [26], reduced economic productivity [27], and later risk of obesity as well as chronic diseases [28]. Weight for age indicator is a

composite representation of both wasting and stunting [29]. Mid-upper-arm-circumference (MUAC) indicates wasting while head circumference is a proxy for brain growth. MUAC identifies under-nourished children at high risk of dying [30]. MUAC performed as well as weight-for-height Z score in predicting inpatient mortality in 12 to 59 months old children with SAM in a rural malaria endemic coastal area in Kenya [31]. It also has the advantage that it is cheap and easier to perform compared to measuring height and weight as well as being easier to interpret. In rural South Africa, MUAC was noted to have potential use in identifying obesity in children 5 to 14 years old [32]. Therefore, besides MUAC use in identifying under-nourished children below 5 years at high risk of dying, it also may have a role in screening for over-nourished older children.

Management of acute malnutrition should be proactive and short duration highly intensive regimes are used for treatment. Currently, the WHO recommends a programme of using community based approach [33] for treatment of both acute moderate and severe malnutrition using ready-to-use-supplementary-food (RUSF) or ready-to-use-therapeutic-food (RUTF) respectively for children above 6 months (see Figure 4). This programme consist of inpatient stabilisation care for management of complicated severe acute malnutrition (SAM), outpatient therapeutic component for uncomplicated SAM and supplementary feeding for moderate acute malnutrition (MAM) [34]. The RUTF and RUSF are lipid rich pastes enriched with powdered ingredients producing a high energy food with low water content. The low water content in RUTF and RUSF discourages microbial contamination and growth; hence they can be stored at ambient temperature even in the tropics for a few months [35]. The constituents of RUTF and RUSF include milk powder, vegetable oil, sugar, peanut butter and powdered vitamins and minerals. Zimbabwe adopted and is

implementing this integrated approach to management of acute malnutrition. Active identification of acutely malnourished children in the community is done using MUAC and screening for pedal oedema. However, effectiveness of the intervention on catch up growth of acutely malnourished children may depend on the specific context of implementation [36].

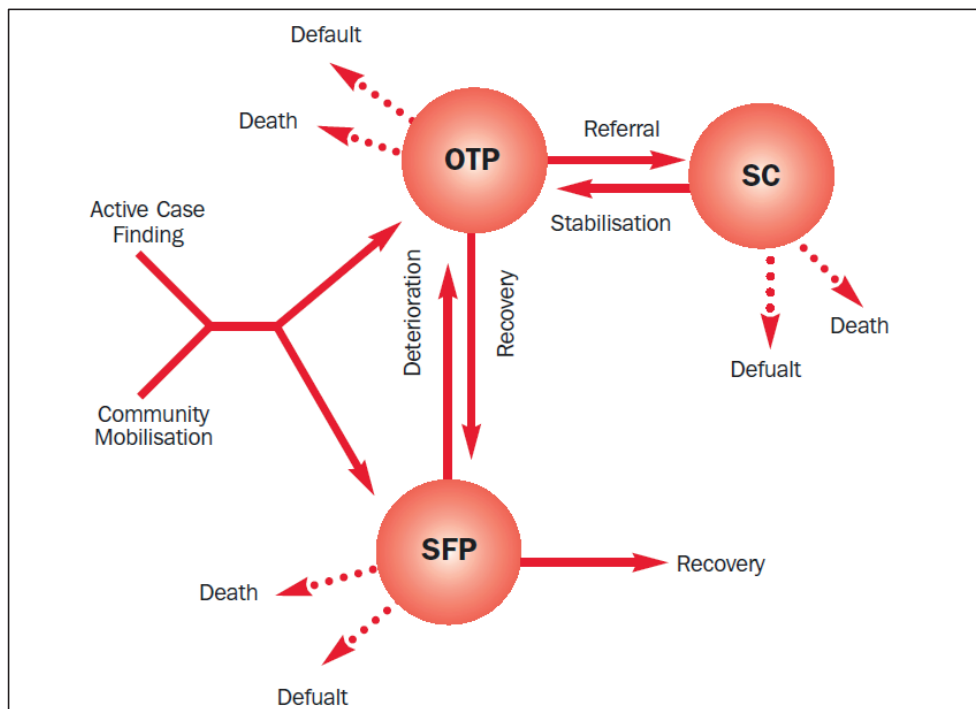
Long term preventive programmes to improve nutrition, maternal and infant health are needed to address chronic malnutrition. Adequate maternal nutrition in pregnancy including micronutrient supplementation; promotion of exclusive breastfeeding; provision of adequate complementary feeds; proper water, sanitation and hygiene to reduce infections; poverty alleviation programmes; health services improvement for both women and children as well as improving maternal education are some of the multi-sectorial evidence based interventions for reducing stunting in children [37, 38].

1.2.1 Nutritional Status of Children Under-five and School Aged HIV Exposed and Unexposed Children from Low Income Countries

High levels of stunting, wasting and underweight have been reported in the postnatal period in children from areas with increased burden of HIV infection. In India [39] and South Africa [40], researchers have reported early occurrence of under-nutrition before age three months in HIV infected infants [39]. However, in Tanzania, researchers reported later occurrence of under-nutrition in children born to HIV infected women with median time to first episode of stunting, wasting and underweight all occurring after six months of age [41]. Under-nutrition occurs more frequently in HIV infected infants as reported in South Africa [40] and in children below two years as reported in the Democratic Republic of Congo [42]. Under-

nutrition has also been noted to be frequent in HIV exposed but uninfected infants in Kenya [43].

Figure 4 Conceptual framework Integrated Management of Acute Malnutrition



SC Stabilisation care; OTP Outpatient therapeutic programme; SFP Supplementary feeding programme
 Source: Community Therapeutic Care Field Manual, 2006

Multiple factors have been associated with occurrence of under-nutrition in the postnatal period which includes socio-demographic, immunological and environmental factors. The maternal factors include advanced HIV infection, young age and low maternal education. Poverty, not breastfeeding, early weaning and residing in the rural areas are some of the socio-environmental factors contributing to the under-nutrition. The child factors associated with under-nutrition included HIV

infection, anaemia, low birth weight, prematurity, male gender as well as repeated infections such as malaria and diarrhoea [39-41, 44, 45] in these settings with high burden of HIV infection. Regarding all children despite their HIV infection status, a window of opportunity has been described for interventions to prevent under-nutrition in the first 1000 days of life as it has been noted that under-nutrition particularly stunting begins in utero [37]. Once children become stunted in early life it is very difficult for catch up growth to occur as long as the child remains in the same environment [46]. The children that become stunted before 24 months of age tend to remain stunted into adult hood and suffer the long term consequences of stunting which tend to affect even the next generations [47, 48].

Stunting and wasting remain problematic in school-aged children from Africa [49-52]. High prevalence of stunting has been documented in rural school children 8 to 11 years old from South Africa [53]. There are reports on paradoxical co-existence of over-nutrition and under-nutrition in 5 to 14 year old children from Pakistan [54]. Malnutrition affects growth, development, mental and physical capacity in school-aged children [55]. It is essential to offer interventions to correct and prevent under-nutrition especially stunting in school aged children since it is associated with cognitive deficits [56-58]. Despite the fact that stunting is very difficult to reverse especially if children remain in the same environment [59], Tanzanian children demonstrated considerable catch-up growth in the pubertal period [60]. Catch up growth in Ethiopian, Indian, Vietnamese and Peruvian school aged children followed up between 8 and 15 years was associated with improved cognitive function [57, 61]. Factors associated with catch-up growth of children above 5 years include birth length, less severe stunting, gender, household wealth, modernization, maternal

education and maternal height [61-63]. It is important to identify and correct malnutrition in school-aged children.

1.2.2 Nutrition and HIV

HIV infection can result in malnutrition by reducing nutrient intake, increasing nutrient requirements of the body, altering metabolism and increasing nutrient losses from the body [64-68]. Nutrient intake is reduced through several mechanisms. HIV infection affects human capacity to work and therefore causes food insecurity at the household level [69]. The opportunistic infections (OIs) in HIV infected individuals can cause painful oral conditions that reduce intake of nutrients. Both HIV and OIs cause fever which is associated with reduced appetite [67]. HIV infection alters metabolism [65, 66] by increasing the resting energy expenditure [70, 71]. Increased susceptibility to OIs will also increase metabolic requirements and result in increased nutrient demand which worsen the nutritional status of individuals with HIV infection [67]. Furthermore, HIV infection effects on the gut include increased diarrhoea episodes and malabsorption [72-75] which result in nutrient losses from the gut. HIV and malnutrition both depress the cellular as well as humoral immunity [67] resulting in enhanced susceptibility to OIs therefore increasing morbidity and mortality. HIV infected children with severe acute malnutrition (SAM) have more than 3- fold risk of mortality compared to HIV uninfected children with SAM [76].

In both HIV infected adults and children various macronutrient as well as micronutrient interventions have been studied to assess their effects on morbidity and mortality. However, currently no firm conclusions from intervention studies can be made on impact of macronutrient supplementation on morbidity and mortality in HIV infected individuals [77]. HIV infection is associated with multiple micronutrient deficiencies [78] such as vitamins, essential and trace element deficiencies. These

micronutrient deficiencies affect growth negatively [79] and have been demonstrated even in apparently ambulant HIV infected children in Nigeria [78]. Micronutrient supplementation has been demonstrated to be safe and effective for reducing morbidity and mortality in HIV infected children [80]. Vitamin A supplementation reduced all-cause mortality by half in hospitalized Tanzanian HIV infected children with pneumonia [81] and Zinc reduced morbidity from diarrhea in HIV infected South African children [82]. In contrast, multiple micronutrient supplementation which contained several vitamins and micronutrients (Vitamins A, B1, B2, niacin, B6, B12, C, D and E, folate, zinc, copper, iodine and selenium) failed to reduce mortality, alter growth nor CD4 counts after 12 months in children below 5 years from Uganda [83]. Comparison was made of children on ART versus those who were not on ART. Multiple micronutrients however, reduced duration of hospital stay, micronutrient deficiencies and improved appetite in these children. Multiple micronutrient supplementation improved vitamin B12 and folate status in HIV infected children in Uganda [84]. As for iron supplementation, another common micronutrient deficiency, no conclusion has been reached yet on its impact on clinical, immunological and virologic outcomes in children infected with HIV [85].

1.2.3 Micronutrient Deficiencies

Various micronutrient deficiencies accompany malnutrition [86, 87]. Iodine, Vitamin A, Iron, Zinc and folic acid are some of the common micronutrient deficiencies which can contribute to morbidity and mortality in under-nourished children. Studies have demonstrated that supplementing these deficient micronutrients is beneficial to both HIV infected and uninfected children under 5 years from the low income countries [88-90]. However, it has been demonstrated that HIV infected children from these

low income countries have a higher burden of multiple micronutrient deficiencies compared to their HIV uninfected peers [91] and this could worsen their health outcomes. Fortunately, studies are showing that supplementation of these micronutrient deficiencies is also beneficial to the HIV infected children. Targeted micronutrient supplementation may be useful in HIV infected children since some micronutrients such as iron are beneficial but may be associated with increased risk for malaria in endemic areas [92]. Hence, identification and treatment of these micronutrient deficiencies is important for children living in low income countries with an increased burden of under-nutrition co-existing with high prevalence of HIV infection.

In Zimbabwe, the national micronutrient survey of 2012 [93] demonstrated quite significant multiple micronutrient deficiencies in children below 5 years of age. Nineteen percent of the under-fives had Vitamin A deficiency (retinol binding protein $<0.825 \mu\text{mol/litre}$ and C-reactive protein $\leq 3\text{mg/L}$), 72% had iron deficiency (ID) (soluble transferrin receptor (sTfR) $>8.3\mu\text{g/ml}$) and 24% had iron deficiency anaemia (IDA) (Haemoglobin $<11\text{g/dL}$ and sTfR $>8.3\mu\text{g/ml}$). Under-five children living with HIV had higher prevalence of Vitamin A deficiency compared to the HIV uninfected children. Anaemic HIV infected children similarly had a higher prevalence of iron deficiency compared with their anaemic uninfected peers. Of note is that children between 5 and 12 years were only assessed for iodine deficiency and 37% had inadequate urinary iodine levels. Anthropometric measurements or assessment for Vitamin A status, Iron deficiency and IDA were not done in the children aged 5 to 12 years. In addition, the ZDHS also restricts assessment of children's nutritional status to under-fives [12]. Notably, nutrition programmes in low income settings including Zimbabwe have been focusing mainly on under-five children nutritional status

consequently resulting in limited information on the burden of malnutrition and on micronutrient status of school-aged children. This information would help guide policy formulation for nutrition programmes for children above 5 years in areas with a high prevalence of HIV infection in addition to a high burden of under-nutrition.

1.2.3.1 Anaemia and Iron Deficiency

Globally, iron deficiency is the commonest micronutrient deficiency resulting in anaemia in both adults and children [94]. Iron deficiency is a multisystem disorder with important health and economic effects. It negatively affects psychomotor development and cognitive function [95-97]. Iron deficiency results in abnormal neurone formation, myelination, neurotransmitter metabolism and changes in the hippocampus explaining its effects on brain development. Iron deficiency will cause irreversible changes during critical periods of brain growth in the first year of life [98]. Despite this fact, research has shown that iron supplementation in non-anaemic iron deficient adolescent children improves verbal learning and memory [99]. It also has been associated with improved red cell indices and learning achievement in children of school age with iron deficiency anaemia [100].

HIV infection has become an important cause of anaemia through several mechanisms especially in high HIV burden areas such as sub-Saharan Africa [101-103]. The prevalence of anaemia and iron deficiency is unknown for HIV exposed and infected school children in Zimbabwe. Furthermore, the association of HIV infection or HIV exposure with anaemia in these school children is not well-defined. Improving iron status and general childhood nutrition would positively influence school performance [104].

1.2.3.2 Selenium Deficiency

Selenium deficiency is a less talked about micronutrient deficiency associated with under-nutrition and has not been clearly defined in school aged children from low income countries. Selenium is an essential micronutrient for human health [105]. It is an important component of the selenoproteins that have many functions in the body [105]. Selenium has an essential role in all aspects of the immune system: cell mediated and humoral functions [106, 107]. It is a potent antioxidant that protects the cell membrane and Deoxyribonucleic acids (DNA) [108, 109] safeguarding the body from many diseases. Animal studies on the mouse model have found a benefit of selenium on reducing Coxsackievirus B3 pathogenicity [110-113] and preventing cancer [114]. Observational studies in humans suggested a benefit of selenium in preventing cancer but randomized controlled trials have not demonstrated convincing evidence for selenium cancer prevention benefits [115]. Very few intervention studies found a benefit of selenium in preventing cancer in selenium deficient individuals [116, 117]. Selenium deficiency was associated with increased mortality and rapid disease progression in HIV infected adults who were intravenous drug users in the United States of America (USA) [118] and pregnant Tanzanian women [119]. Low serum selenium levels were associated with increased mortality of perinatally HIV infected children from the USA [120]. Low serum selenium levels were also associated with increased morbidity and mortality in Tanzanian children who were born to HIV infected women [121]. Higher baseline selenium levels were associated with lower HIV ribonucleic acid (RNA) in antiretroviral therapy (ART) naïve HIV infected children [122]. Selenium supplementation in HIV infected adults was also associated with suppression of HIV viral replication in a double blind randomized control trial [123]. Selenium daily supplements were associated with

reduced rates of hospitalisation and health related costs in HIV infected adults [124]. In contrast, selenium supplementation has been associated with negative outcomes in HIV infected individuals. Kupka et al reported increased HIV shedding in the cervico-vaginal secretions of Tanzanian women with serum selenium higher than 114 micrograms per litre while Sudfeld et al reported increased HIV detection in breast milk of primiparous Tanzanian women not on HAART [125, 126]. Further research is needed to define the impact of these findings on HIV transmission. However, since there are clear benefits of selenium, it then becomes important to assess selenium levels in children from settings with high burden of HIV infection and under-nutrition as this may provide evidence for a possible need of supplementation with this micronutrient.

1.2.4 Macronutrient Deficiencies: Omega 3 Long Chain Polyunsaturated Fatty Acids (LCPUFA)

Macronutrients are nutrients required in large quantities and provide energy for the human body. They include carbohydrates, proteins and fats. Fats are classified as saturated, monounsaturated and polyunsaturated. Long chain polyunsaturated fatty acid (LCPUFA) deficiency is an important macronutrient deficiency that accompanies malnutrition and may have a synergistic effect with the various micronutrient deficiencies on growth and neurocognitive development of children. Omega 3 fatty acids are LCPUFA essential to human health that are derived from alpha linoleic acid which comes from the diet [127]. The important metabolites of alpha linoleic acid include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [128]. They are anti-inflammatory [129-132], have an essential role of brain function, growth and development [133, 134]. Omega 3 fatty acid deficiency present with

symptoms that could partially explain the symptoms of under-nutrition such as growth failure, immunodeficiency and neurocognitive defects [135].

Docosahexaenoic acid and arachidonic acid which is an omega 6 fatty acid derivative are the main fatty acids in the brain [136]. DHA is an important constituent of the cell membrane and hence deficiency will affect many cellular functions especially in the brain [137]. Essential fatty acid deficiency can occur in under-nutrition because of reduced intake, poor absorption, and reduced endogenous production of the LCPUFA from the parent molecule alpha linoleic acid resulting from poor enzyme activity [135]. In addition, the increased peroxidation that occur with under-nutrition because of associated antioxidant deficiencies especially selenium and vitamin E deficiency result in reduced levels of LCPUFA [135]. Under-nutrition in HIV uninfected children was associated with lower linoleic acid, arachidonic acid, DHA and reduced plasma plus red blood cell membrane LCPUFA [138-140]. Both under-nutrition and HIV infection may deplete LCPUFA from the cell membranes [141]. Reduced levels of DHA have been reported in under-nourished HIV infected children compared to under-nourished HIV uninfected children [140]. This could potentially exacerbate neurodevelopmental outcomes of malnourished children in association with zinc, iron, iodine and protein deficiencies. Moreover, the poor socio-economic and psychological background that under-nourished children often live in worsen the neurodevelopmental outcomes [135]. HIV infection in well-nourished children on highly active ART (HAART) was associated with reduced alpha linoleic acid, high DHA and increased turnover of LCPUFA [142].

Intervention studies have shown benefits of maternal and infant supplementation of omega 3 LCPUFA on neurocognitive development of younger children below five years [143]. This is most likely because the brain is rapidly growing and developing

before 2 years of age [144, 145]. Further brain development and maturation continues in childhood especially in the frontal lobe and the retina [145], therefore, omega 3 LCPUFA supplementation could also benefit older children. However, research in well children above 5 years has been equivocal in terms of improving neurocognitive outcomes. A single blinded randomised placebo controlled trial in south African children 7-9 years old who were supplemented with bread spread with marine fish flour giving a total DHA per week of 892mg for 6 months showed improvement in learning ability and memory [146]. However, the control group were more stunted, underweight and wasted than the intervention group and this possibly confounded the results as stunting is known to reduce cognitive function in children. In a double blind randomised placebo controlled trial which investigated effect of omega 3 supplementation (520mg) for 16 weeks in main stream school children from Wales who were aged 8-9 years and had intelligent quotient ≥ 70 showed that omega 3 fatty acid status improved with minor differences in cognitive and behavioural outcomes [147]. The intervention group had greater accuracy on a test of impulsivity and supplementation had a protective effect on pro-social behaviour. However, the supplementation period may have been too short to show significant differences in children now experiencing slow brain growth and the cheek cell polyunsaturated fatty acids (PUFA) status used to assess omega 3 status may reflect recent intake but not mirror well the brain PUFA status. In contrast, the CHAMPION study which investigated 6 to 10 year old Indian children who were supplemented with LCPUFA and micronutrients found no effect on cognitive performance [148]. However, the children had high prevalence of micronutrient deficiencies including IDA, too low doses of DHA below the recommended daily allowance were used and no placebo was used. Australian and Indonesian children (the NEMO study) children were

randomised to micronutrients, LCPUFA, both or placebo. There were no effects attributed to LCPUFA supplementation though the micronutrients improved height and short term memory. Too low doses of DHA and EPA were administered and there was a high prevalence of IDA [149]. DHA did not modify cognitive performance in 10 to 12 year old children from Newcastle, England. The children were recruited in a double blind placebo controlled randomised trial and were supplemented with 1000mg DHA, 400mg DHA or placebo for 8 weeks. Limitations included a small sample size, lack of biochemical confirmation of adherence and the short duration of intervention period [150]. Nevertheless, in children above 5 years who are unwell with phenylketonuria and psychiatric disorders, supplementation with omega 3 LCPUFA has been shown to be beneficial [151-153]. In addition, an Italian study that pooled data from four cohorts of participants showed that from the neonatal period to adulthood, children had the lowest level of LC-PUFA [154]. It then becomes very important to determine the levels of omega 3 LCPUFA in children from settings with dual burden of under-nutrition and HIV infection as omega 3 LCPUFA supplementation could potentially mitigate some of the effects of micronutrient deficiencies such as iron deficiency on cognitive function and the immune system in malnourished HIV infected, HIV exposed and HIV uninfected school aged children from resource limited settings.

1.3 JUSTIFICATION

Under-nutrition remains a problem in school aged children from low income countries particularly those with a high burden of HIV infection. Under-nutrition is associated with multiple micronutrient deficiencies that result in short and long term effects of great consequence to the individual affected and the society.

Zimbabwe just like many low income countries in Sub-Saharan Africa is affected by the dual burdens of under-nutrition and HIV infection. Nutrition programmes in Zimbabwe are focusing on the first 1000 days of life as evidence has shown that interventions targeting this age group are cost effective in reducing the burden and consequences of under-nutrition. However, this has resulted in nutrition programmes overlooking children above 5 years. Importantly, nutritional surveys including the Zimbabwe National Demographic Health and the Micronutrient Surveys do not assess nutritional status of children above 5 years. This has resulted in limited information on children above 5 years' nutritional and micronutrient status.

Information on the nutritional and micronutrient status of children above 5 years may assist policy makers when designing their nutrition programmes and will allow targeted interventions to address micronutrient deficiencies especially for HIV exposed and infected children in this age group. This becomes extremely important especially with the growing numbers of HIV exposed children in the country following the success of the prevention of mother-to-child transmission of HIV infection (PMTCT) programmes. In addition, assessing the nutritional and micronutrient status of HIV uninfected children above 5 years in low income countries will also provide useful information for planning appropriate nutrition programmes for these children.

1.4 THE RESEARCH QUESTION

What is the iron, selenium, omega 3 fatty acid and nutritional status of the children aged 7 to 10 years who were born in the Better Health for African Mothers and Children (BHAMC) study, a PMTCT cohort?

1.4.1 Objectives

Primary: To measure prevalence of malnutrition, anaemia, iron deficiency, selenium deficiency and describe the omega 3 fatty acid status in HIV unexposed and HIV exposed (infected and uninfected) children 7 to 10 years old who were born in a national PMTCT programme (BHAMC study) from a peri-urban setting in Zimbabwe, a low income country with a high burden of HIV infection.

1.4.2 Secondary Objectives:

1. To measure the prevalence and factors associated with stunting, thinness, overweight, underweight and selenium deficiency in children 7 to 10 years old.
2. To determine the prevalence and factors associated with anaemia and iron deficiency in 7 to 10 years old HIV infected and uninfected children using haemoglobin, serum ferritin and soluble transferrin receptor levels.
3. To determine and compare omega 3 fatty acid levels in 7 to 10 years old HIV unexposed and exposed children using whole blood dry blood spot samples.

1.4.3 The Study Factors

The HIV status (infected, exposed uninfected and unexposed uninfected) of the 7 to 10 year old children is the main study variable. The children's gender, age, orphan hood status, household income and clinic site were the other study factors.

1.4.4. The Outcome Variables

The main outcome measures were the nutritional status of the children (stunting, thinness, underweight, overweight), haemoglobin, serum Ferritin, soluble transferrin receptor (sTfR), serum selenium and whole blood omega 3 fatty acids [Eicosapentaenoic acid (EPA), Docosapentaenoic acid (DPA) and docosahexaenoic acid(DHA)].

2. MATERIALS AND METHODS

2.1 Study Population and Setting

This cross-sectional study was carried out from August 2011 to June 2012 at 3 peri-urban primary care clinics (Epworth, St Mary's and Seke North Clinics) in Zimbabwe. Epworth clinic is located in a much poorer community compared to Chitungwiza community where St Mary's and Seke North Clinics are located. Epworth is also in a different geographical location and falls under a separate administrative area from the Chitungwiza sites. The participants were 7-10 years old school aged children whose mothers had been recruited into a PMTCT programme from 2002 to 2004. This was a sub-study in the BHAMC study which investigated the role of sexually transmitted infections on vertical transmission of HIV and is described elsewhere [155]. Briefly, the BHAMC study recruited women 36 weeks pregnant coming through the national PMTCT programme who underwent voluntary counselling and HIV testing. Hence the cohort of children comprised of HIV negative unexposed, HIV negative exposed and HIV infected children. HIV negative unexposed children are HIV uninfected children who were born to HIV negative women. HIV negative exposed children were born to HIV positive women but are HIV uninfected. HIV infected children were born to HIV infected women and became HIV infected. HIV test results available from a previous study were used to define the HIV infection status of the children [156]. Children were tested for HIV infection using HIV DNA PCR at birth, 6 weeks, 4 months and 9 months. After 18months, testing was done using Enzyme linked immunoassay (ELISA) and Western Blot.

2.1.1 Inclusion and Exclusion Criteria

All children between 7 and 10 years who were still alive from the BHAMC cohort at the time of the study, whose care givers consented to be included in the study, were eligible for recruitment. The second siblings to the original cohort of children were excluded.

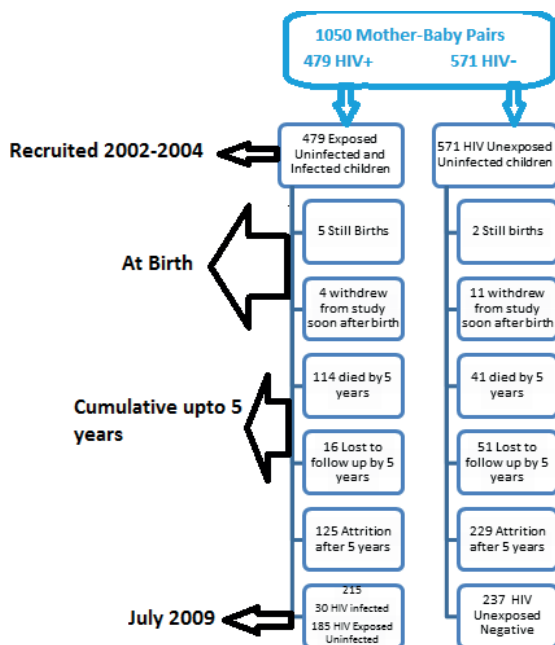
2.1.2 Sample Size Calculation

The sampling frame is shown in figure 3. The prevalence of malnutrition (p): stunting, thinness, overweight and underweight in this group of children was unknown and estimated to be 50%. The minimum sample size required for estimating the prevalence of malnutrition in this population of children with a confidence interval of 95% (significance level; z) and a margin of error (se) of 0.05 was calculated to be 208 using the Raosoft® sample size calculator. The calculation is based on the following formula:

$$n = z^2[p(1 - p)]/se^2$$

It was also assumed that the prevalence of anaemia and selenium deficiency were 50% since they were unknown for this particular population of children between 5 and 10 years of age from Zimbabwe. The minimum sample size required for estimating both the prevalence of anaemia and selenium deficiency in this population of children was calculated to be 208.

Figure 5 The Sampling Frame for 7 to 10 year old children from the 1050 mother-baby pairs in the BHAMC cohort, Zimbabwe



Adapted from Kurewa et al, 2011

The original birth cohort had 1050 babies born to 571 HIV negative and 479 HIV positive mothers. However, during the planning stage of this study, only 237 babies born to HIV negative mothers and 215 born to HIV positive mothers could be traced for possible recruitment into the study. The rest had either been lost to follow up or had died [155]. A decision to include more than the 208 children was made to aid the assessment of iron, omega 3 fatty acids and selenium status of the children whose prevalence's were unknown for this Zimbabwean population of children and had been estimated to be 50%. Caregivers in the community were contacted by peer counsellors and were informed about the study. Participants were consecutively recruited as they presented to the local clinic every Thursday between 8am and 6pm.

2.2 Data Collection

A questionnaire designed to collect data was piloted, modified and then was administered to collect socio-demographic data including age, gender, household monthly income, primary care giver (defined as person who took care of the child during most of the day) and whether parents of the child were alive. All the study personnel were trained to use the data collection tool in order to standardize data collection. All the children had a complete physical examination performed by the study paediatrician. Children were treated for illness identified during the physical examination. Height, weight, occipital-frontal (head) circumference and MUAC were measured. The children's weight was measured once while they put on light clothing with a Seca digital scale (manufactured in Germany model: 881 1021659) which was calibrated daily. A stadiometer was used to measure height once. The children took off their shoes, stood feet together with the heels against the wall and looked straight ahead. One person would ensure the correct head position and another person ensured that the knees were extended while the heels touched the back of the wall. The head circumference was measured in the occipital-frontal diameter with a non-stretchable tape measure. The measurement was repeated three times and the largest measurement was taken as the head circumference. The MUAC was also measured once in the non-dominant arm. The midpoint between the shoulder and the elbow was determined with flexible non-stretchable tape. The MUAC was then measured with the limb hanging down and relaxed. The WHO's Anthroplus software (version 1.0.4) was used to calculate Z scores for body mass index, weight for age and height for age. The WHO recommended Z score cut-offs for underweight, stunting and thinness were used to define under-nutrition. Z scores below -2 and -3 were considered as moderate and severe under-nutrition respectively. Children with

a BMI Z-score below -3 for age were classified as SAM. Overweight and obesity definitions used are as shown in Table [29].

Table 3 Definition of Terms

Characteristic	Indicator
Stunting	Height-for-age Z score < -2
Severe Stunting	Height-for-age Z score < -3
Underweight	Weight-for-age Z score < -2
Severe Underweight	Weight-for-age Z score < -3
Thinness	BMI-for-age Z score < -2
Severe Thinness	BMI-for-age Z score < -3
Overweight	BMI-for-age Z Score ≥ 1 and <2
Obesity	BMI-for-age Z Score ≥ 2
Selenium deficiency	Serum Selenium <0.89 μ mol/L
Anaemia	Hb <11.5 g/dl
Iron Deficiency	Serum Ferritin (F) 15 μ g/L
Iron Deficiency Anaemia	Hb <11.5 g/dl AND F <15 μ g/L OR sTfR >8.3 μ g/L

BMI-for-age Body mass index-for-age; Hb Haemoglobin; F Ferritin;

sTfR Soluble Transferrin Receptor

Source: Cole et al, 2007; WHO, 2008; Amare et al, 2012

2.2.1 Blood Samples Collection

2.2.1.1 Haemoglobin Determination

Participants' hands were washed thoroughly with soap, rinsed well with warm water and dried with paper towel. The middle finger was massaged and a lancet was pressed on the fleshy part of the finger tip. The finger was gently squeezed to

produce a drop of blood. The initial drop of blood was wiped away with cotton wool. Blood was collected in a micro cuvette slide and the haemoglobin level was determined using a battery powered HemoCue photometer. The photometer uses the azide-methemoglobin method for determining haemoglobin concentration [157]. Anaemia was defined as a haemoglobin level of less than 11.5 grams per decilitre (g/dl) and a classification proposed by WHO was used to interpret the public health significance of the prevalence as shown in Table 4.

Table 4 Classification of Prevalence of Anaemia in Populations Based on Haemoglobin Level[158]

Prevalence of Anaemia	Category of Public Health Significance
≤4.9 %	No Public Health Problem
5.0-19.9 %	Mild Public Health Problem
20.0-39.9 %	Moderate Public Health Problem
≥40.0 %	Severe Public Health Problem

Source: WHO, 2001

2.2.1.2 Dry Blood Spot for Omega 3 Fatty Acids Determination

The subsequent drops of blood were collected on filter paper by allowing blood to touch the filter paper inside a marked circle without smearing it so that blood was drawn onto filter paper by capillary action. This was repeated until four circles were filled with blood. The back was checked to ensure it looked like the front. This procedure can be viewed on the web page as described by the Institute of Aquaculture, University of Stirling in Scotland [159]. The filter paper was then air dried away from the sun at room temperature for at least 3 hours. It was then

packaged in foil paper with desiccant and sealed. The filter papers were then stored in a freezer at -20 °C until shipping under the same conditions to a laboratory testing site in Scotland.

2.2.1.3 Blood Collection for Serum Ferritin, Soluble Transferrin Receptor and Selenium Levels Determination

The skin was wiped with a methylated spirit-soaked swab and allowed to air-dry for one minute. A tourniquet was applied above the elbow joint and using appropriate protective equipment, blood was collected by venipuncture into a red top plain tube (4ml) and a royal blue top trace element tube (5ml). Care was taken to prevent haemolysis of the samples. The specimens were immediately stored in a cooler box at a temperature between 2 and 8°C for transportation to the laboratory where they were spun for 10 minutes at 1100 revolutions per minute. At least two 1ml aliquots of serum from both the red and royal blue top tubes were stored at -80 °C. The specimens were temporarily stored in a local bio bank. One aliquot from the blue top tube was shipped on blue ice to Fürst Medical laboratory in Norway for selenium determination. Determination of serum ferritin and soluble transferrin receptor (sTfR) levels was done in a local laboratory: the University of Zimbabwe - University of California, San Francisco (UZ-UCSF) laboratory.

2.3 Laboratory Analyses

2.3.1 Serum Ferritin and Soluble Transferrin Receptor Levels

Enzyme immunoassays for the quantitative analysis of serum ferritin [RAMCO LABORATORIES INC., Stafford; USA] [160] and sTfR levels [RAMCO LABORATORIES INC., Stafford; USA] [161] were used to determine serum ferritin

and soluble transferrin receptor levels in the children respectively. The manufacturer provided software to calculate the actual amount of serum ferritin and serum transferrin receptor levels from the derived absorbance. ID was defined as a serum ferritin level of less than 15 micrograms per litre ($\mu\text{g/L}$). IDA was defined as a haemoglobin level of less than 11.5 g/dl and either ferritin below 15 $\mu\text{g/L}$ or a soluble transferrin receptor level of more than 8.3 $\mu\text{g/L}$ (above manufacturer' reference limit). Serum ferritin levels were measured in all children. Only children with ferritin levels ranging between 15 and 50 $\mu\text{g/L}$ and who had haemoglobin less than 11.5 grams per decilitre with ferritin above 15 $\mu\text{g/L}$ had their sTfR level measured. This was done to identify children who had ID but had ferritin above the cut off value raised falsely because of inflammation, since the study setting is an area of high infectious disease burden.

2.3.2 Serum Selenium Determination

Automated inductively coupled plasma mass spectrometry was used to measure serum selenium levels. A PerkinElmer Sciex; Elan® DRC™ II (Manufactured 2005 Shelton, USA) spectrometer was used. The mass spectrometer generated single charge ions from elements in the sample. The ions were then separated based on mass-to-charge ratio and then detected. Internal and external calibrators were used for quality control as recommended by the manufacturer. There is no clearly set definition for selenium deficiency in this age group of children. However, in this study selenium deficiency was defined as a value below 0.89 $\mu\text{mol/ litre}$ and this was based on studies from Vietnam [162] and Ethiopia [163] in children of a similar age group. These studies' definition was used to enable comparison of results since

there are limited studies of selenium deficiency in children and selenium levels above this cut off are associated with favourable health outcomes [164].

2.3.3 Omega 3 Fatty Acids Determination

Omega 3 fatty acids analysis was performed at the Nutrition Group Laboratory at the Institute of Aquaculture, University of Stirling in Scotland. A method described by Bell et al [165] was used to measure whole blood phospholipids from dry blood spots on filter paper treated with butylated hydroxytoluene (Sigma Aldrich Limited, Gillingham Dorset, United Kingdom). Automated gas liquid chromatography was used for analysis. Results were reported as percent by weight of each fatty acid to total fatty acids. The omega 3 polyunsaturated fatty acids: EPA, Docosapentaenoic acid (DPA) and DHA were selected for data analysis.

2.4 Ethics Statement

This study was approved by the local authorities in Chitungwiza (Seke North and St Mary's Clinics) and Mashonaland East Province (Epworth clinic), Medical Research Council of Zimbabwe, Research Council of Zimbabwe and the Norwegian Research Ethics Committee. After the study was explained to the participants, the care givers gave written informed consent and the children gave written assent to be included in the study. It was clearly explained to caregivers that refusing to participate in the research would not prejudice them in anyway and they would still be able to get the best care for their children at the clinics. Consent to allow storage of specimens in a local bio-bank and shipping of specimens to laboratory testing sites outside Zimbabwe during the study period were also obtained from the care givers. It was

also highlighted that it was their right to refuse storage or shipment of specimens to another country. In addition, permission to ship specimens to Scotland and Norway for determination of omega 3 fatty acids and selenium levels respectively was obtained from the Research Council of Zimbabwe. International standards for shipping specimens were applied. Data collection tools and the consent forms were translated to the local Shona language. Data was made anonymous by using study identification numbers. The data forms were stored separately from the participants' identities and were only accessible to the study team.

Participants and their care givers had their travel costs fully reimbursed. They were offered standard of care for co-morbid conditions detected during the physical examination. The study provided essential drugs for community management of common childhood illnesses. Children with SAM were managed according to the Ministry of Health and Child Care protocol for treating SAM in children above 5 years with RUTF [166]. The children were discharged from follow up once their BMI Z-score was above -2 or after 4 months in the programme. Those who did not improve were referred to the provincial hospital for further investigation and treatment. Anaemic children were given iron supplements for two months and those with severe anaemia (below 7g/dl) were referred to the district hospital for further investigation and treatment. All HIV infected children were on trimethoprim-sulphamethoxazole prophylaxis. They were accessing care and treatment for HIV infection from the local governmental and non-governmental HIV clinics.

2.5 Statistical Analyses

Data was double entered into a computer using the CSPRO package. Data was cleaned and the SPSS statistical package [IBM SPSS Statistics version 20.0.0.1] was used for statistical analysis. A p-value less than 0.05 was considered statistically

significant. Descriptive statistics and prevalence calculations were used to describe the data. Correlation was used to describe continuous data. Chi square and Fisher's exact tests were performed to determine associations between categorical data for example; to investigate associations between anaemia and the categorical characteristics of the participants. Pearson's chi-square and risk estimates were used to determine the odds of detecting anaemia in HIV infected and HIV uninfected children. Spearman correlation was used to investigate relationships between haemoglobin and the participants' continuous characteristics.

The one-way Anova test and the Boniferroni post-hoc test were used to compare means of continuous outcome variables (anthropometric measurements and selenium) according to the HIV status of the children. These tests were used to describe the factors associated with malnutrition and selenium deficiency. The Kruskal-Wallis test as well as the Medians test were used to compare distribution and medians of omega 3 fatty acids: EPA, DPA and DHA among the HIV infected, HIV exposed uninfected and HIV unexposed uninfected children as the omega 3 fatty acid data was not normally distributed.

3. RESULTS

3.1 Demographic Characteristics of the Participants

A total of 318 children were recruited in the study out of a possible 452 children. Of the 134 children that were not recruited; the majority had been lost to follow up, 23 refused to participate in the study and 1 child from St Mary's clinic refused to assent as he did not want to have blood drawn from him. The 'lost to follow up' group included an unknown number of participants whose families had relocated from the area of study.

The primary caregiver was the mother for 71% of the children and 19% were being looked after by their grandmothers. Very few (4%) primary care givers had not gone to school while 64% had gone up to secondary school. Majority of the working mothers (93%) were informally employed with 38% working as vendors. Fifty seven per cent of the mothers were married, 9% had remarried and 9% were divorced. Seventy five percent of the children's fathers were alive and 52% of the children were living with their fathers in the same household. A third of the children were orphaned with 5% being double orphans (both parents deceased). Table 5 shows the socio-demographic features of the children.

Majority of the children were HIV negative unexposed (57%). One child had no confirmed HIV status hence was excluded from the analysis requiring HIV infection status. This child was lost to follow up soon after birth only to return to the study area during the study period. The child's caregiver was advised to get the child tested for HIV as the child was exposed at birth. The prevalence of HIV infection in the cohort was 7%. The 9 and 10 year age groups were combined because there were only three children in the 10 year age group. There were more children recruited from

Epworth (85%) and St Mary's (83%) clinics living in households with monthly incomes less than or equal to \$250 ($p < 0.0001$). However, there were no statistically significant differences in gender, orphan hood and HIV status distribution of the children by clinic site of recruitment.

3.2 Nutritional Status and Serum Selenium Levels

The anthropometric measurements of the participants are shown in Table 6. There were six children whose head circumference and mid-upper arm circumference values were missing. The mean Z scores of the weight for age, height for age and BMI for age were all between 0 and -1. Stunting was the commonest form of under-nutrition occurring in 12% of the children as shown in Table 7. Three children met the WHO criteria for diagnosis of severe acute malnutrition and were managed with a ready-to-use therapeutic food. Only one HIV uninfected child failed to recover after 4 months of community based treatment for severe acute malnutrition and required referral for further management at the provincial hospital. Only 269 out of 318 children had their serum selenium values determined because trace element tubes expired before the study was completed. Almost half of the children who had their serum selenium levels measured were classified as selenium deficient using the protocol definition.

Table 5: The Socio-demographic Characteristics of 318 Zimbabwean School Children Aged 7-10 years (Paper 1)

Characteristic	Description	Frequency
Age Group	7 years	21 (7%)
	8 years	105 (33%)
	9 years	189 (59%)
	10 years	3 (1%)
Gender	Male	137 (43%)
	Female	181 (57%)
HIV status	Negative unexposed	180(57%)
	Negative exposed	116(36%)
	Positive	21(7%)
	Unknown	1(0.3%)
Orphan hood	Double orphan	15(5%)
	Single orphan	79(25%)
	Not orphaned	224(70%)
Primary Caregiver	N=318	
	Mother	226 (71%)
	Grandmother	59 (19%)
	Sibling	5 (5%)
	Other	28 (9%)
Primary Caregiver's Education	No School	11 (4%)
	Primary level (≤ 7 years)	93 (29%)
	Secondary Level (8-13 years)	205 (64%)
	Diploma or Degree	7 (2%)
	Unknown	2 (1%)
Mother's Occupation	N=285	
	Formal	20 (7%)
	Informal	265 (93%)
Household		
Monthly income US\$ median(Q1;Q3)	N=268	150 (80;250)
Total number of people living in the household median(Q1;Q3)	N=318	5 (4;6)
Number children below 10 years median (Q1;Q3)	N=316	2 (1;3)

Table 6: The Mean Values of Anthropometric Measurements and Serum Selenium Levels of 318 7-10 Year Old Children from Zimbabwe (Paper 1)

Characteristic	Frequency N	Mean (\pm SD*)	Minimum	Maximum
Height cm	318	127(\pm 6.9)	104	149
Weight kg	318	25(\pm 3.8)	15	42
Head Circumference cm	312	52(\pm 1.6)	47	57
Mid-upper arm circumference mm	312	180(\pm 15.6)	120	230
Weight-for-age Z score	317	-0.77(\pm 0.93)	-4.97	1.72
Height-for-age Z score	318	-0.88(\pm 1.03)	-4.96	1.96
BMI-for-age Z score	318	-0.38(\pm 0.88)	-3.32	1.76
Serum Selenium value μ mol/L	269	0.85(\pm 0.16)	0.30	1.60

* Standard Deviation

Children from households with a monthly income of less than US\$250.00 and from Epworth clinic had a higher prevalence of selenium deficiency as shown in Table 8. There was no statistically significant difference in selenium levels among the children according to their HIV status. Stunting was associated with HIV exposure and orphan hood.

Table 7: Prevalence of Stunting, Underweight, Thinness, Overweight and Selenium Deficiency (Serum Selenium <0.89µmol/L) among 318 School Children from Zimbabwe (Paper 1)

Characteristic	Indicator	Frequency n (%)
Stunting	Height-for-age Z score < -2	37(12)
	Height-for-age Z score < -3	7(2)
Underweight	Weight-for-age Z score < -2	25(8) ¹
	Weight-for-age Z score < -3	4(1)
Thinness	BMI-for-age Z score < -2	11(4)
	BMI-for-age Z score < -3	3(1)
Overweight	BMI-for-age Z Score ≥1 and <2	15 (5)
Selenium deficiency	Serum Selenium <0.89µmol/L	129 (48) ²

¹ N = 317 children

² N = 269 children

Table 8: Factors Associated with Stunting, Thinness, Overweight, Underweight and Selenium Deficiency in 318 7-10 Year Old Children from Zimbabwe (Paper 1)

Characteristic	Frequency	Stunting	Thinness	Overweight	Underweight ¹	Selenium deficiency ²
		n (%)	n (%)	n (%)	n (%)	n (%)
Gender						
Male	137	19(14)	5(4)	8(6)	12(9)	54(45)
Female	181	18(10)	6(3)	7(4)	13(7)	75(50)
Age						
7 years	21	5(24)	2(10)	0	1(5)	8(38)
8 years	105	12(11)	4(4)	4(4)	9(9)	48(53)
9-10 years	192	20(10)	5(3)	11(6)	15(8)	73(46)
Clinic						
Epworth	104	14(13)	3(3)	7(7)	9(9)	45(62) *
St Mary's	104	12(12)	4(4)	3(3)	11(11)	40(41)
Seke North	110	11(10)	4(4)	5(5)	5(5)	44(45)
Household monthly income						
≤\$250	205	26(13)	8(4)	12(6)	3(5)	96(54)*
> \$250	63	6(10)	1(2)	1(2)	20(10)	15(33)
HIV status						
Negative Unexposed	180	12(7)	5(3)	10(6)	15(8)	63(43)
Negative Exposed	116	17(15) **	4(3)	5(4)	8(7)	53(52)
Positive	21	8(38) **	2(10)	0	2(10)	13(62)
Orphan hood						
Not orphaned	224	20(9)	6(3)	14(6)	18(8)	97(50)
Orphaned	94	17(18)*	5(5)	1(1)	7(7)	32(42)
Total	318	37(7)	11(4)	15(5)	25(8)	129(48)

*p value < 0.05

**p value <0.001

Analysis done with One-way ANOVA and Boniferroni post hoc test

¹ N=317

² N=269

Obesity was not detected in this group of children and no overweight children were stunted. Two of the stunted children also had thinness. Of the 37 stunted children, 16 (43%) were also underweight for age. HIV infected children were 7.5cm shorter than the HIV uninfected unexposed children ($p= 0.00$). The HIV infected children were also 5.4cm shorter than the HIV uninfected exposed children ($p=0.00$). There was no significant difference in mean weight of the HIV uninfected (unexposed and exposed) children. However, HIV infected children were 3.5kg lighter than the HIV uninfected unexposed children ($p=0.00$) and were 2.7kg lighter than the HIV uninfected exposed children ($p=0.01$). There was no statistically significant difference in head circumference among all the children according to their HIV status. HIV uninfected unexposed and HIV uninfected exposed children had MUAC size longer than that of children with HIV infection by 11 mm ($p=0.01$) respectively.

3.3 Prevalence of Anaemia and IDA

The mean haemoglobin and ferritin stratified for age, gender, maternal and children's HIV status are presented in Table 9. One child did not have results for haemoglobin. Two children did not have serum ferritin results. One hundred and ninety children who had met the study criteria had their sTfR levels measured. The prevalence of all-cause anaemia in the children was 15% ($n= 48$) and that of IDA was 2%. There was no difference in the prevalence of all-cause anaemia between the girls and boys ($p = 0.94$). Only one child had severe anaemia of 4.4 g/dL and further investigation at the provincial hospital showed that this child had sickle cell anaemia. The prevalence of ID was 4%. The WHO recommends using a higher cut off level for ferritin of 30 $\mu\text{g/L}$ in children below five years in areas with a high infectious disease burden. However, there is no recommendation for children above five years in comparable

settings. When the higher cut off for ferritin was applied (Table 10), it resulted in higher prevalence of ID (32%) and IDA (5%). Anaemia was more likely to be present in HIV infected children (p -value <0.001) with an odds ratio of 4.9 (CI 1.9- 12.4). The maternal HIV status at birth was not related to presence of anaemia in the children aged 7 to 10 years.

Table 9: Haemoglobin, Ferritin, All-cause Anaemia (ACA) and Iron Deficiency Anaemia (IDA) Stratified for Gender, Age and HIV Status in Zimbabwean School-aged Children (Paper 2)

Characteristic	Subcategory	Frequency (n)	¹ Haemoglobin (g/dl) n = 317	² Ferritin (μ g/L) n = 316	ACA n (%)	IDA ³ n (%)
Gender	Female	181	12.5(\pm 1.10)	46.1(\pm 26.5)	27(15)	2(1)
	Male	137	12.5(\pm 1.23)	47.2(\pm 39.5)	21(15)	5(4)
Age (years)	7	21	12.7(\pm 1.04)	48.3(\pm 25.2)	3(14)	0(0)
	8	105	12.4(\pm 1.08)	48.8(\pm 41.7)	21(20)	2(2)
	9	186	12.6(\pm 1.19)	45.3(\pm 27.7)	23(12)	5(3)
	10	6	12.3(\pm 1.42)	41.8(\pm 24.5)	1(17)	0(0)
HIV status	Negative unexposed	179	12.7(\pm 1.0)	49.5(\pm 37.6)	18(10)	2(1)
	Negative exposed	116	12.4(\pm 1.3)	42.1(\pm 24.0)	21(18)	4(3)
	Positive	21	11.9(\pm 1.3)	51.0(\pm 34.0)	9(43)	1(5)
	Total	316	12.5(\pm1.16)	46.6(\pm32.7)	48(15)	7(2)

¹ Mean haemoglobin (standard deviation)

² Mean ferritin (standard deviation)

³ IDA defined as Hb < 11.5 g/dl and either F < 15 μ g/L or sTfR level > 8.3 μ g/L

Table 10: Prevalence of Iron Deficiency (ID) and Iron Deficiency Anaemia (IDA) Using a Ferritin Cut-Off Value of 15µg/L and 30µg/L Respectively in 7-10 Year Old Zimbabwean Children Stratified by Age, Gender and HIV Status (Paper 2)

Characteristic		Frequency n	ID F<15µg/L n (%)	ID F<30µg/L n (%)	IDA ¹ F<15µg/L n (%)	IDA ² F<30µg/L n (%)
Gender	Female	179	7(4)	50(28)	2(1)	6(3)
	male	137	6(4)	52(38)	5(4)	10(7)
Age group						
	7 years	20	0(0)	5(25)	0(0)	0(0)
	8 years	105	5(5)	31(30)	2(2)	5(5)
	9-10 years	191	8(4)	66(35)	5(3)	11(6)
HIV Status						
	Negative unexposed	179	5(3)	57(32)	2(1)	8(4)
	Negative exposed	115	8(7)	40(35)	4(3)	7(6)
	Positive	21	0(0)	4(19)	1(5)	1(5)
Total		316	13(4)	102(32)	7(2)	16(5)

¹ IDA defined as Hb < 11.5 g/dl and either F < 15 µg/L or sTfR level > 8.3 µg/L

² IDA defined as Hb < 11.5 g/dl and either F < 30 µg/L or sTfR level > 8.3 µg/L

3.4 Omega 3 Fatty Acid Levels

There were no statistically significant differences among the children's EPA, DPA and DHA levels (medians and distribution) according to their HIV status, gender and nutritional status as shown in Table 11. However, there was a statistically significant difference in the EPA medians by age group ($p=0.02$). The 7 year old age group had the lowest median compared to the 8 and 9 year old age groups. However, this age group only had 21 participants.

Table 11: The Whole Blood EPA, DPA and DHA Levels of 7-10 Years Old Children from a Peri-Urban Area in Zimbabwe Stratified by HIV Status, Age, Gender and Nutritional Status (Paper 3)

Characteristic	N	EPA ¹ Median(IQR)	p- value ²	DPA ¹ Median(IQR)	p- value ²	DHA ^{1,2} Median(IQR)	p- value ²
Age group							
7 years	21	0.17 (0.03)	0.02 [*]	0.75(0.18)	0.38	2.09(0.49)	0.56
8 years	105	0.20 (0.10)		0.81(0.21)		2.18(0.59)	
9-10 years³	192	0.18 (0.08)		0.78 (0.18)		2.11 (0.52)	
Gender							
Female	181	0.19 (0.08)	0.37	0.78 (0.20)	0.26	2.16 (0.50)	0.82
Male	137	0.18 (0.08)		0.81 (0.18)		2.11 (0.60)	
Stunting							
Yes	37	0.18 (0.84)	1.00	0.80 (0.24)	1.00	2.14 (0.54)	0.73
No	281	0.19 (0.09)		0.79 (0.18)		2.12 (0.52)	
Underweight							
Yes	25	0.17 (0.07)	0.99	0.77 (0.13)	0.99	2.04 (0.52)	0.22
No	292	0.19 (0.08)		0.79 (0.19)		2.14 (0.55)	
Thinness							
Yes	11	0.17 (0.07)	0.54	0.71 (0.18)	0.54	2.08 (0.39)	0.54
No	307	0.19 (0.09)		0.79 (0.19)		2.14 (0.56)	
HIV Status							
HIV unexposed uninfected	180	0.19(0.08)	0.40	0.80(0.18)	0.76	2.11(0.55)	0.44
HIV exposed uninfected	116	0.18(0.09)		0.79(0.18)		2.16(0.50)	
HIV infected	21	0.19(0.12)		0.79(0.25)		2.07(0.65)	
Total	317⁴	0.19 (0.09)		0.79 (0.19)		2.14 (0.54)	

^{*}Significant p-value less than 0.05

¹Measured as % weight of Total serum phospholipids (% wt/wt)

²The Median test was used to compare medians across groups

³Combined the 9 and 10 year olds because there were only 3 children 10 years old

⁴One child's HIV status from the 318 children was unknown.

4. DISCUSSION

Majority of the children who participated in this study were HIV negative either unexposed or exposed. The prevalence of HIV infection of 7% documented in this study is above the reported national prevalence for children below 15 years from Zimbabwe, which for the year 2014 was 2.7% [15]. The highest recorded prevalence of HIV infection for children below 15 years (3.05%) was reported in the 2005 [15]. The BHAMC cohort is a highly selected population of children whose prevalence of HIV infection cannot be generalized to the prevalence of HIV for children below 15 years in Zimbabwe. This can be explained by the methodology of the BHAMC cohort [156] where active recruitment of HIV infected pregnant women was deliberate so that there were adequate numbers to address questions on mother-to-child transmission of HIV.

4.1 Paper 1- Nutritional Status and Selenium Deficiency

4.1.1 Stunting, Wasting, Underweight and Overweight/Obesity

The prevalence of stunting of 12% among 7 to 10 years old HIV exposed (infected and uninfected) and HIV unexposed uninfected children in this study demonstrated is regarded as of mild public health significance according to WHO [167]. This result is similar to findings from Burkina Faso [168] where prevalence of stunting of 8.8% in a similar peri-urban and urban setting were documented. However, the children from Burkina Faso included older children of up to 14 years and their HIV infection status was not determined. The stunting prevalence from our study is lower than the prevalence of stunting from Tanzania, another sub-Saharan African country, which reported stunting rates of 42.5% in children 7 to 18 years old who had a high prevalence of anaemia (Hb <11g/dL) of 31%, high burden of parasitic infections with 57% suffering from *Schistosoma Haematobium*, however HIV status was not

documented [169]. Prevalence of anaemia from our study is also lower compared to results from studies from Asia [170-172]. Most of these studies included children above 5 years up to 18 years who were slightly older than the children who participated in our study. The exception was a study from Vietnam [162] with an almost identical age group (6-9 years) but slightly less number (n=292) of children with unknown HIV status and the study reported quite a high figure of stunting of 33%. The Vietnamese children demonstrated gender differences in prevalence of stunting and under-weight which were absent in our study. Boys were more under-weight and stunted compared to girls. In our study, when stunting was stratified by HIV status, the prevalence was highest in HIV infected children (38%). This finding is similar to a research from Malaysia [172] in HIV infected children, but this particular study included children up to 18 years and had a much higher prevalence of severe stunting. In addition, orphaned children were more stunted in our study and this could be explained by the difficult socio-economic status that these children often endure. The prevalence of underweight (8%) found in our study was much lower than that reported in Vietnamese [162] and South East Asian children [171] but similar to findings from Malaysia [172] in HIV infected children. The overall prevalence of thinness (4%) was also much lower compared to an Africa study from Burkina Faso [168] but was similar to findings from Italy [173] a developed country. The 5% prevalence of overweight was comparable to findings from Burkina Faso [168] and South east Asia [174] which are also low income countries. It was much lower compared to the high income countries in children of a similar age group [173, 175, 176] and it was also lower than findings from Malaysia [172] in HIV infected children on antiretroviral therapy. Some children from our study had more than one nutritional deficit and this has been shown to increase risk of mortality in under-five

children [177]. The HIV uninfected (unexposed and exposed) children above 5 years had better nutritional status than the HIV infected children. Prevalence of stunting has been shown to be high in HIV infected children on [178] and off [179] antiretroviral treatment. Prevalence of underweight and stunting have been reported to be higher among HIV infected children compared to HIV negative children [180, 181]. Though the HIV uninfected exposed group of children were slightly shorter than the unexposed children this was not statistically significant and their weight was also comparable. This finding from our study is in contrast to what has been described for younger children where HIV uninfected exposed children have been shown to have a high prevalence of under-nutrition [182, 183] compared to HIV uninfected unexposed children. The head circumferences of the children 7 to 10 years old were not statistically different when stratified by HIV status. This could be due to survivor bias where many HIV infected children with advanced disease who may have had smaller head sizes from HIV encephalopathy died before two years of age [184].

4.1.2 Selenium Deficiency

This is the first study that has assessed children's selenium status in Zimbabwe. Overall, the prevalence of selenium deficiency (48%) was high in all the children and this could be reflecting the selenium soil status of the region. The prevalence of selenium deficiency was comparable to results from Ethiopia (62%) [163] and Vietnam (76%) [162]. These two studies had almost similar sample sizes, used a similar cut-off value to define selenium deficiency, had similar mean selenium levels but the HIV status of the children were not determined. The prevalence of selenium deficiency in our study was much higher than that obtained in HIV infected children on antiretroviral treatment from Malaysia (12.5%) [172] who also had a higher mean

selenium level (1.13 $\mu\text{mol/L}$). The Malaysian study had a smaller sample size compared to our study. There was no gender difference in the levels of selenium in our study and this was in contrast to results from Iran [185] which demonstrated that girls had higher serum selenium levels. In our study selenium deficiency occurred more frequently among children from households with a monthly income of less than USD\$250 and from children from one site, Epworth which is in a different geographical location from the other two sites in Chitungwiza. The prevalence of selenium deficiency did not differ according to HIV status although there was a tendency for frequency of selenium deficiency to be higher in the 7 to 10 year old HIV infected children but this did not reach statistical significance. Though some studies have shown lower selenium levels in HIV infected individuals [186], there was no difference among the three groups in our study and this is similar to results found in Rwandan children younger than 5 years [187]. It is important to note that selenium levels in different regions are influenced by the soil content of the element [188-190]. Some regions are well known for low selenium content for example areas in China [191] and Malawi [189].

4.2 Paper 2- Anaemia and Iron Deficiency

This is one of the few studies that have evaluated anaemia and ID in children above 5 years born in a national PMTCT programme from an area with a high prevalence of HIV infection. According to WHO, the 15% prevalence of anaemia has a mild public health significance as it falls between 5 and 19.9% [192]. In spite of this, anaemia has important effects at the individual level in school going children. Our study concurs with other African studies that have shown that HIV infection

increased the odds of anaemia in children [193-195]. However, in our study the maternal HIV status at birth was not related to presence of anaemia in the children after five years of age.

Very few children had IDA but when a higher ferritin cut off of 30 µg/L was applied the prevalence of IDA became of mild public health significance in this cohort. A small proportion of anaemia was caused by ID in these children. ID increased almost 8 fold when the higher cut off of 30 µg/L was used. This study documented a much lower prevalence of anaemia, ID and IDA anaemia compared to a study done by Midzi et al in a rural area in Zimbabwe with a high prevalence of malaria, schistosomiasis and soil helminths [196]. The prevalence of anaemia and IDA reported by Midzi et al were 48% and 38% respectively. Our study was conducted in a peri-urban area around Harare, the capital city of Zimbabwe, with no malaria [197, 198], schistosomiasis and a very low soil helminths burden [199]. Another difference is that Midzi et al included preschool children below five years. Our study also differs from other African studies done in areas with high parasite burden which also reported high prevalence of anaemia (26-46%) and IDA (16-18%) [200-202]. Researchers from Africa and Europe have found that boys had more ID compared to girls [201, 203] in a similar age group to our participants, while in an Asian study ID was higher in girls [202]. In our study there was no gender difference in the occurrence of anaemia or IDA. However, our result on prevalence of anaemia concurs with the global estimates from WHO where Zimbabwe is estimated to have an anaemia prevalence of mild public health significance [192].

Anaemia is important in school going children as it has been associated with reduced physical work capacity, reduced cognitive function and reduced intellectual performance[204]. Iron deficiency has also been shown to negatively affect physical growth [205], since iron is necessary for growth and metabolism[206]. Furthermore, ID negatively affects learning, memory, affective and social behaviour [95, 207, 208]. It also affects physical work performance, cognitive function, language development and causes poor grades at school [209-214]. ID is associated with increased gut uptake of other heavy metals like lead [215]. Programmes to prevent ID occurring in children must be in place as ID causes changes in the brain that persist for a long time [208]. The importance of identifying individual children including those above 5 years with anaemia, ID and IDA should be emphasized as they all can result in children failing to achieve their full potential at school. This impacts on their future economic performance and can result in poverty. Programs should be in place to identify school children with anaemia, ID and IDA for intervention purposes. These should be continuous with the programs for infants and children under five years so as to have an impact in preventing the long term consequences of anaemia, ID and IDA.

4.3 Paper 3- Omega 3 Fatty Acid Levels

Our study happens to be the first study to determine children's whole blood fatty acid levels in Zimbabwe, a low income setting with a significant burden of chronic malnutrition and HIV infection. We did not find any association of age and gender with EPA, DPA and DHA levels except that EPA median was lower in the 7 year old age group compared to the 8 and 9-10 year age groups. This finding differs from

results of a heterogeneous cohort of children 3 to 8 years old from 8 European countries who were well nourished where the median EPA levels in the age groups 7 to 9 years were higher [216]. However, the median DPA and DHA levels were lower by almost half the quantities found in our study. This European study had a much larger sample size and there were subtle differences in the analytical method. In this European study both capillary and venous whole blood samples were used for determining omega 3 LCPUFA levels but we only used capillary blood samples collected on a dry blood spot. Another difference was the fact that 7% of the children who participated in our study were HIV infected, 12% chronically malnourished and 8% underweight for age. In addition, our study also had fewer children in the 7 year age group and had a homogenous population of children from one peri-urban setting. Eicosapentaenoic acid, DPA and DHA levels documented in our study were lower compared to another study done in the United Kingdom (UK) in school children 6 to 10 years old who were under performing in reading [217]. Whole blood fatty acid levels were determined from dry blood spot samples with minimal differences in the laboratory protocols. Notably, these UK 6 to 10 year old children's omega 3 fatty acid levels were also higher than in the 8 European countries [216]. DPA and DHA were higher in boys and DPA was lower in the 7 year age group. In contrast, we found lower EPA in the 7 year age group. A cross-sectional study done on Danish children 8 to 11 years old [218] who were well nourished reported higher medians than ours for the 3 omega fatty acid levels. This Danish study used heparinized whole blood samples instead of capillary DBS to determine the fatty acid levels. It is a challenge to compare fatty acid analysis across studies because of analytical methodological differences, different range of fatty acids measured, and the dietary diversity among the different populations. However, we have compared our study findings to studies

that used whole blood to measure fatty acids. Plasma has less fatty acids compared to erythrocyte levels but whole blood fatty acid levels though lower have been found to correlate to erythrocyte levels [165].

4.3.1 Omega 3 Fatty Acid Levels and HIV Infection

HIV status was not associated with EPA, DPA and DHA levels in our study. In contrast, well-nourished Italian children one to 15 years old on highly active anti-retroviral drugs had higher EPA and DHA compared to HIV negative controls [219]. In these Italian children, there were also no age or gender differences. However, this was a very small study with 14 HIV infected children and 30 HIV negative controls. An earlier study in much younger children with a median age of 2 years in Rumania living in an orphanage [140], malnourished HIV infected children had lower plasma DHA and total omega 3 LCPUFA compared to malnourished HIV negative children. The 35 severely malnourished Rumanian children (19 HIV infected) were also compared to healthy German children and the malnourished children had lower DPA, DHA and total omega 3 LCPUFA levels. No studies comparing HIV exposed uninfected children to HIV infected or HIV unexposed uninfected children were identified.

4.3.2 Omega 3 Fatty Acid Levels and Nutritional Status

There was no difference in the EPA, DPA and DHA levels of the children in our study according to their nutritional status. This population of children was characterized by significant prevalence of under-nutrition, anaemia [220] and selenium deficiency [221]. Studies on association of omega 3 fatty acid levels and under-nutrition are limited especially studies that use capillary whole blood for fatty acid analysis.

According to a review by Smit and colleagues in 2004 essential fatty acid deficiency could play a role in protein energy malnutrition [135] as it is associated with low DHA levels. A case control study that compared children with non-organic failure to thrive reported higher erythrocyte EPA and DPA in well-nourished controls [222] . However, erythrocyte fatty acid analysis was done only in 51 cases and 9 controls. This unequal representation limits conclusions that can be drawn from this study. Nigerian severely malnourished children with kwashiorkor and marasmus defined using the Welcome classification [223] had low DHA levels [138]. An earlier case-control study in Nigerian children which looked at 8 healthy children compared to 17 severely malnourished children with either kwashiorkor or marasmus reported low DHA and DPA levels in the malnourished children [139].

4.4 Study Limitations

One of the major limitation in our study was the unequal numbers in the comparison groups. There was a much smaller sample size of HIV infected children (7%) compared to the HIV uninfected (exposed and unexposed) and the unequal numbers in the different age groups. We investigated serum selenium levels which reflect short term selenium status instead of red cell selenium levels which reflect long term selenium status [224]. Selenium was determined in 269 (85%) of the children. The children (15%) who did not have their selenium status assessed were mostly from Epworth (57%) and this site had more children with selenium deficiency from our analysis. Hence the prevalence of selenium deficiency we found is likely to be an underestimation of the actual value. We also did not assess the diet of the children which would have assisted in answering the question on why selenium and nutritional status were poor in this group of children. According to the 2014 Zimbabwe Vulnerability assessment committee report [9] most households

consumed maize, sugar, oil, vegetables and salt almost on a daily basis. The least consumed food groups were meat and pulses. Meat, poultry and fish are the foods that contribute most of the dietary selenium [105]. Maize has been described as a poor source of selenium in neighbouring South Africa [225] and Malawi [226]. Therefore the assumption is that most of these children from a poor peri-urban area have reduced access to selenium rich foods. In addition, the assessment of nutritional status of the children was done only once; the study design does not address risk factors associated with the under-nutrition in the study population.

We used haemoglobin level and serum ferritin complemented with sTfR levels to make a diagnosis of IDA, which is the WHO recommendation [227]. The recommended gold standard is bone marrow iron studies [228, 229] but this is invasive and difficult to justify in well children. Besides, some studies have shown that having unstainable bone marrow iron may not equal ID [230, 231]. C-reactive protein has been used by others to identify inflammation and improve reliability of ferritin in diagnosis of ID. Although thresholds of C-reactive protein levels that make ferritin unreliable are not clearly defined, thresholds of 10-30 nanograms per litre are used [232]. C-reactive protein levels were not measured in our study. The combination of ferritin and sTfR level may allow some differentiation between ID and inflammation [232]. Conversely, there are studies that found sTfR level to be affected by inflammation [233]. In contrast, there are also studies that have found sTfR to be relatively independent of inflammation [234, 235]. Not using C-reactive protein or sTfR level to define ID resulted in underestimation of prevalence of ID in our study. The fact that this was a cross sectional study meant we were unable to delineate the causality of anaemia in these children.

Our study is one of the few studies to report on omega 3 fatty acid levels in association with HIV and nutritional status. However, because the 7 years age group was relatively small compared to the other two age groups, limits conclusions that can be drawn for this age group. This study was a cross-sectional study where measurement of whole blood fatty acids was done only once. Whole blood fatty acids reflect both the short term intake (plasma) and long term intake (erythrocyte) of fatty acids and percent total fatty acid levels are lower than the erythrocyte fatty acids as they include a wider range of fatty acids [236]. However, whole blood fatty acids have been found to correlate well with erythrocyte fatty acid levels [237]. The population of children studied was a narrow age group constituting 7 to 10 year old children and was made up mainly of children from very low income peri-urban setting. These results therefore cannot be generalized to children outside this age group, from rural or urban setting and from higher income households. Lastly, because dietary habits of the children were not studied, it remains unclear why our results were lower than some of the studies quoted from Europe. It is however, important to note that Zimbabwe is a land-locked country with reduced access to sea food which is a good source of omega 3 fatty acids.

5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

1. Chronic malnutrition and selenium deficiency were prevalent in this 7-10 year old cohort of children who were born in a prevention of mother-to-child transmission of HIV programme in a peri-urban setting from a low income country with a high HIV burden.
2. The prevalence of anaemia and ID was of mild public health significance in the 7-10 year old children. HIV infection was an important determinant for the presence of anaemia. IDA constituted 15% of all cases of anaemia.
3. There was no difference by HIV status, gender and nutritional status in the capillary whole blood omega 3 fatty acid levels EPA, DPA and DHA in the children 7 to 10 years old from Zimbabwe.

5.2 Recommendations

1. We recommend setting up of nutrition programmes for school children above 5 years in our setting with a high burden of HIV infection. These programmes should be in continuity with the under-five nutrition programmes already in existence in this setting. Further and larger studies that will also include children from rural and urban areas are recommended so as to ascertain the selenium status of this population as this may guide policy on the need for supplementation of selenium in Zimbabwe.
2. HIV prevention programs for children should be strengthened to eliminate paediatric HIV infections as this may also reduce the cases of anaemia in school children in areas with high burden of HIV infection. IDA should be addressed easily and cheaply through iron fortification of basic food stuff such as mealie-meal and teaching the public to consume iron rich food.

3. Further studies assessing LCPUFA levels that include larger sample size, wider range of children from both urban and rural areas are recommended as this may clearly define the association of omega 3 LCPUFA with HIV status and under-nutrition in children from low income countries.

6 FUTURE RESEARCH

Our study found high prevalence of under-nutrition, selenium deficiency and anaemia in 7 to 10 year old children. We did not find an association between under-nutrition and HIV status with omega 3 fatty acid levels in these children but our study was confined to a peri-urban population of children. I hope to assess the selenium and omega 3 Fatty acid status of children from rural and urban areas in Zimbabwe in order to have a more representative view of the selenium and omega 3 fatty acids status of children in my country as this will better guide policy makers in decisions on supplementations of these two nutrients in our setting. There is also a need for research assessing the impact of iron supplementation and nutrition programmes in school aged children from Zimbabwe.

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ERRATA

Page 4, line 14: “Aquaculture, and University of Stirling in Scotland” to read “Aquaculture, University of Stirling in Scotland”

Page 6, line 6: “child health services.” to read “child health services from August 2011 to June 2012.”

Page 7, line 6: “children and therefore nutrition programmes” to read “children, therefore nutrition programmes”

Page 10, line 5: Formatted font from bold “Figure 4 Conceptual framework Integrated Management of Acute Malnutrition”

Page 10, line 8: Formatted font from bold “Table 1 Zimbabwean HIV/AIDS Statistics: 1999 and 2014”

Page 10, line 10: Formatted font from bold “Table 3 Definition of Terms”

Page 10, line 11: Formatted font from bold “Table 4 Classification of Prevalence of Anaemia in Populations Based on Haemoglobin Level [158]”

Page 12, line 14: “have more than 90% of the burden” to read “carry more than 90% of the burden”

Page 15, line 13: “PMTCT programme” to read “prevention of mother-to-child transmission of HIV (PMTCT) programme”

Page 15, line 14: “nevirapine, .the government” to read “ nevirapine. The government”

Page 15, line 15: “the ART programme” to read “the anti-retroviral therapy (ART) programme”

Page 18, line 24: “few months (Briend2001)” to read “few months [35]”

Page 39, line 5 and 6: “WHO used to interpret the public health significance of the prevalence as shown in Table 2.” to read “WHO was used to interpret the public health significance of the prevalence as shown in Table 4.”

Page 45, line 16: “Table 1 shows the socio-demographic features of the children.” to read “Table 5 shows the socio-demographic features of the children.”

Page 46, line 6: “shown in Table 2.” to read “shown in Table 6.”

Page 46, line 10: “children as shown in Table 3.” to read “children as shown in Table 7.”

Page 48, line 5: “ in Table 5.” to read “in Table 8.”

Page 51, line 15: 'in Table 5.' to read " in Table 9."

Page 52, line 2: "applied (Table 6)" to read " applied (Table 10)"

Page 55, line 4: "shown in Table 7." to read "shown in Table 11."

Page 67, line 8: "factors associated" to read "risk factors associated"

Page 69, line 5: "HIV transmission programme" to read "HIV programme"

Page 69, line 14: "1 We" to read "1. We"

Page 69, line 20: "2 HIV" to read "2. HIV"

Page 70, line 1: "3 Further" to read "3. Further"

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APPENDICES

Appendix 1 ENGLISH CONSENT FORM

BURDEN OF MALNUTRITION IN 7-10 YEAR OLD CHILDREN BORN IN A MOTHER-TO-CHILD TRANSMISSION OF HIV INFECTION PREVENTION PROGRAMME, ZIMBABWE

Principal Investigator: Patience Kuona [MBChB, MMED Paediatrics (UZ)]

Co-Investigator(s): Marshall Munjoma

Phone number(s): 0772396029 / 0734612649

What you should know about this research study:

- We give you this consent so that you may read about the purpose, risks, and benefits of this research study.
- Routine care is based upon the best known treatment and is provided with the main goal of helping the individual patient. The main goal of research studies is to gain knowledge that may help future patients.
- We cannot promise that this research will benefit your child. Just like regular care, this research can have side effects that can be serious or minor.
- You have the right to refuse to allow your child to take part, or agree for your child to take part now and change your mind later.
- Whatever you decide, it will not affect your child's regular care.
- Please review this consent form carefully. Ask any questions before you make a decision.
- Your choice to allow your child to participate is voluntary.
- If you have questions concerning this study, you can **contact: Dr Patience Kuona at 0772396029**

PURPOSE

Malnutrition and iron deficiency are major problems in our children. These problems affect children in the short and long term with a big effect on the involved child and the society. The levels of omega 3 fatty acids and selenium in our children are unknown. You are being asked to participate in a research study to find out how common malnutrition and iron deficiency are in a cohort of children who were born in a national PMTCT program in the Better Health for African Mothers and Children study. This research is also going to find out the levels of selenium and omega 3 fatty acids in the children who are now of school going age. The results of this study could assist us in knowing how children born in a national PMTCT program grow after birth and improve their care. Your child was selected because they were born in the BHAMAC study cohort. The research team is made up of doctors, nurses and scientists. The research is funded by The Lettern Foundation.

Procedures

If you allow your child to take part in this research, a few questions will be asked about your child and their health. A doctor will examine your child. We will collect a few drops of blood from your child by a needle prick on the thumb and blot it on a filter paper. We will also draw 5ml of blood for measuring selenium levels. This will only be done once. We also request your permission to store unused blood from your child. Omega 3 test and selenium are not available in the country currently. We also request your permission to take some of the blood outside the country so these tests can be done. If your child is wasted, you will be asked to give them a therapeutic food known as plumpy nut. We will then follow up your child for at most 4 months to see if their nutritional status recovers. We will measure their weekly weight gain.

Risk and discomforts

The blood testing may cause discomfort or a small bruise as with any other blood test. Plumpy nut is peanut based and may cause allergy to susceptible individuals.

Benefits and/ or compensation

The study result will help us understand the growth pattern and nutritional state of the children born in a national PMTCT program. It will define if there is significant iron deficiency in school going children and levels of omega 3 fatty acid levels and selenium. This could influence policy on supplementation of these nutrients. This will assist us in improving care to children born in PMTCT programs. By participating in this research you will have the advantage of knowing your child's nutritional status. Illness that occur during the study period will be treated free of charge. Taking part in this study will not cost you. All tests will be done free of charge. We will not pay you to take part in the study but we will refund transport cost for the study visits.

Confidentiality

Information about you will be stored using a study number in safe paper and computer files. No-one will be able to access the information about you except the research team. No-one will be able to identify your child from the information we will collect. Dr Patience Kuona will be responsible for keeping your personal information confidential.

Voluntary Participation and withdrawal

Participation in this research is voluntary. If you decide not to participate, your decision will not affect your future relationship with this clinic, and University of Oslo. If you decide to participate you are free to withdraw your consent and assent at any time and discontinue participation without penalty.

In the event of injury, contact Dr Kuona at 0772396029

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over.

AUTHORIZATION

YOU ARE MAKING A DECISION WHETHER OR NOT TO ALLOW YOUR CHILD TO PARTICIPATE IN THIS STUDY. YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTOOD THE INFORMATION PROVIDED ABOVE, HAVE HAD ALL YOUR QUESTIONS ANSWERED, AND HAVE DECIDED TO ALLOW YOUR CHILD TO PARTICIPATE.

The date you sign this document to enrol your child in this study, that is, today's date, MUST fall between the dates indicated on the approval stamp affixed to each page. These dates indicate that this form is valid when you enrol your child in the study but do not reflect how long your child may participate in the study. Each page of this Informed Consent Form is stamped to indicate the form's validity as approved by the MRCZ.

_____	_____
Name of Parent (please print)	Date
_____	_____ AM
Signature of Parent or legally authorized representative	Time PM

Relationship to the Subject	

_____	_____
Signature of Witness	Signature of Research Staff
(Optional)	

YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM TO KEEP.

If you have any questions concerning this study or consent form beyond those answered by the investigator, including questions about the research, your rights as a research subject or research-related injuries; or if you feel that you have been treated unfairly and would like to talk to someone other than a member of the research team, please feel free to contact the Medical Research Council of Zimbabwe on telephone 791792 or 791193.

Appendix 2 ASSENT FORM

BURDEN OF MALNUTRITION IN 7-10 YEAR OLD CHILDREN BORN IN A MOTHER-TO-CHILD TRANSMISSION OF HIV INFECTION PREVENTION PROGRAMME, ZIMBABWE

ASSENT FORM- MRCZ/B/222

My name is Patience Kuona. I am doing a research study to describe the growth pattern and nutritional status of children who were born in a national program to prevent mother to child transmission of HIV infection. I am going to ask you and your caregiver some questions, examine you and take some blood from you. You may be given a nutritional supplement called plumpynut depending on your nutritional status. You are allowed to refuse to take part and we will continue treating you as usual without prejudicing you. This research is going to assist in describing the growth, nutritional status, levels of omega 3 fatty acid levels and selenium in children born in national PMTCT programs.

I have discussed this clinical research study with the child using language which is understandable and appropriate. I believe I have fully informed this participant of the nature of the study and its possible risks and benefits. I believe the participant understood this explanation and assented to participate in this study

Ini ndinonzi Patience Kuona. Ndirikutarisa vana vakazvarwa muchirongwa chekudzivirira chirwere cheHIV ndakanangana nekutarisisa kukura kwavo. Izvi zvichabatsira kuti tizive kuti ropa ravo rine maomega 3 fatty acids, selenium uye iron yakakwana here. Zvichabatsira kuchengetwa kwevana ava pakukura kwavo.Ini ndichakubvunza mibvunzo nemubereki wako, ndokutarisa uye nekukutora ropa richanotariswa kulaboratory. Wakasununguka kuramba kutariswa kunyangwe wanga wambobvuma hako isu tinoramba tichikurapa semazuva ose.

Name (Zita rako)

Date (Zuva)

Signature of Research Staff

Date

Appendix 3 **SPECIMEN STORAGE INFORMED CONSENT FORM**

SPECIMEN STORAGE INFORMED CONSENT FORM

Chitungwiza (St Mary's and Seke North Clinics) and Epworth

BURDEN OF MALNUTRITION IN 7-10 YEAR OLD CHILDREN BORN IN A MOTHER-TO-CHILD TRANSMISSION OF HIV INFECTION PREVENTION PROGRAMME, ZIMBABWE

Protocol Version 2 08 July 2011

MRCZ/B/222

SPECIMEN STORAGE AND SHIPMENT

Consent Version

(English)

PRINCIPAL INVESTIGATOR: Patience Kuona

PHONE: 0772396029

INTRODUCTION:

You have decided to take part in the investigational research study named above, sponsored by the Lettern Foundation. While in this study, blood will be collected from your child. You are kindly being asked to agree to the storage of these samples for use during the study and after the study have ended. We are also asking to ship these samples to another laboratory outside Zimbabwe. This consent form gives you information about the collection, storage, and use of these samples. These samples may be useful for future research. The study staff will talk to you about this information. Please ask if you have any questions. You will be asked to sign or make your mark on this form to indicate whether you agree to have your child's samples stored and tested. You will be offered a copy of this form to keep.

YOUR PARTICIPATION IS VOLUNTARY:

Allowing your samples to be stored is completely voluntary. You may decide not to have any samples stored other than what is needed to complete this study and still be in this research study or any future study. Even if you decide now that your samples can be stored for future research, you may change your mind at any time. If this happens, you must tell the study staff that you have changed your mind. If you decide not to have your samples stored or used for future research, they will be destroyed at the end of the study.

PURPOSE:

The specific research to be done on the samples from your child include measuring your haemoglobin, selenium, omega 3 fatty acid, serum ferritin and serum transferrin receptor levels. Your child's samples will only be used for these tests only. No other kinds of tests

will be done by anyone on your child's stored specimens without first explaining the test to you and obtaining your permission.

The study researchers do not plan to contact you or your child's regular doctor with any results from tests done on the stored samples. This is because research tests are often done using experimental procedures, so the results may not help for making decisions on managing your health. In the case that a specific test result gives important information about your health, the researchers will tell the study staff and the study staff will try to contact you. If you wish to be contacted with this type of test result, you must give the study staff any change to your contact information. If your child has a regular doctor and you want the study staff to tell this doctor the test results, you must give the study staff the doctor's contact information. Your child's samples will not be sold or used directly to produce commercial products. Research studies using your samples will be reviewed by the Norwegian Ethics Board and a special committee at the Medical Research Council of Zimbabwe.

PROCEDURES:

We will collect a few drops of blood from your child by a needle prick on the thumb and blot it on a filter paper. We will also draw 5ml of blood for measuring selenium levels. This will only be done once. Each time your child's blood is drawn, up to 2mL (which is about half a teaspoon) of the sample may be stored. Your blood will be stored safely and securely in a storage facility at the University of Zimbabwe. Only the people who work at the facility and approved researchers will have access to your child's samples. The people who work at the facility will not have any information that identifies your child. The approved researchers may be given information about your child such as their age and sex, but they will not be given the child's name or any other information that identifies your child. Your child's samples may be shipped to approved researchers who work outside of Zimbabwe. There is no time limit on how long your samples will be stored.

RISKS and/or DISCOMFORTS:

There are few risks related to storing your samples. When tests are done on the stored samples there is a rare but possible risk to your privacy. It is possible that if others found out information about your child that is learned from tests (such as information about your genes) it could cause you problems with your family (having a family member learn about a disease that may be passed on in families or learning who the true parent of a child is).

POTENTIAL BENEFITS:

There are no direct benefits to you from having your samples stored. You and others could benefit in the future from research done on your blood.

CONFIDENTIALITY:

To keep your information private, your child's samples will be labelled with a code that can only be traced back to your study clinic. Your child's name, where they live, and other personal information will be protected by the study clinic. When researchers are given your child's stored samples, they will not be given your personal information. The results of future tests will not be included in your child's health records. Every effort will be made to keep your child's personal information confidential, but we cannot

guarantee absolute confidentiality. Your child's personal information may be disclosed if required by law.

Efforts will be made to keep your child's study records and test results confidential to the extent permitted by law. However, we cannot guarantee absolute confidentiality. Your child will be identified by a code, and personal information from their records will not be released without your written permission. Any publication of this study will not use your child's name or identify them personally. However, your child's records may be reviewed by the Norwegian Ethics Board, the Medical Research Council of Zimbabwe and the study staff.

In addition to the efforts made by the study staff to keep your child's personal information confidential, an Oath of Confidentiality was signed by all our staff working in this study. This Oath requires study staff not to tell people who are not connected with this study, information about your child or other study participants or any other information related to the study.

PROBLEMS OR QUESTIONS:

For questions about the storage of your samples, contact:

Patience Kuona 0772396029

For questions about your rights as a research subject, contact:

***The National Coordinator
Medical Research Council of Zimbabwe
National Institute of Health Research
Cnr Mazoe Street/ Josiah Tongogara
Avenue
Harare
Ph: +263 4 791792, 791193
Cell: +263 912 433 166***

CONSENT FOR SPECIMEN STORAGE AND SHIPMENT

Please carefully read the statements below (or have them read to you) and think about your choice. No matter what you decide it will not affect whether you can be in the research study, or your routine health care.

_____ I agree to have samples of my child's blood shipped outside the country, stored and used for future testing related to nutrition of children.

_____ I agree to have samples of my child's blood shipped outside the country but do not want it to be stored and used for future testing related to nutrition of children.

_____ I do not agree to have samples of my child's blood shipped outside the country, stored and used for future testing related to nutrition in children.

Participant Caregiver/ Parent Name (print)
Date

Participant Caregiver Signature or Mark and
Date

Study Staff Conducting
Consent Discussion (print)

Study Staff Signature and Date

Witness Name (print)

Witness Signature and Date

(As appropriate)

Appendix 4 **DATA COLLECTION TOOL**

Nutritional Assessment Questionnaire

1. Study ID No:.....
2. Date of interview:.....
3. Site:.....
4. Date of Birth:.....
5. Age (years):.....
6. Gender: 1. Female 2. Male
7. Birth rank:
8. HIV status: 1. Negative 2. Positive
9. At Birth: Height (cm)
10. At birth: Weight (kg)
11. At Birth: OFC (cm)
12. 4-6 weeks: Height
13. 4-6 weeks: Weight
14. 4-6 weeks: OFC
15. 3-4 months: Height
16. 3-4 months: Weight
17. 3-4 months: OFC
18. 9 months: Height
19. 9 months: Weight
20. 9months: OFC
21. Current: Height
22. Current: Weight
23. Current: OFC
24. Feeding method at birth: 1. Breast milk 2. Formula 3. Mixed
25. Exclusive breast feeding period (months) or age introduced other liquids and solids:
26. Age stopped breast feeding (months):
27. Primary care giver: 1. mother 2. Maid 3. Other
28. Mother: 1. Alive 2. Died
29. If alive how old is she (years):
30. Level of education attained: 1. primary 2. Secondary 3. diploma / degree
31. Occupation: 1. Formal 2. informal

