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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

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Abstract

Long chain polyunsaturated fatty acids are essential macronutrients that have several benefits which have been described for children's health. Omega 3 LCPUFA metabolism has been reported to be altered in under-nourished and in HIV infected children. Therefore, we describe Eicosapentaenoic acid, Docosapentaenoic acid and Docosahexaenoic acid levels of HIV infected, HIV exposed uninfected and HIV unexposed uninfected school aged children from a low income country with a high burden of HIV infection and under-nutrition. This cross-sectional study recruited children 7 to 10 years old. Capillary blood was collected on filter paper and whole blood fatty acid analysis done using automated gas liquid chromatography. Kruskal Wallis and Median tests were used to compare the distribution and medians of the Omega 3 LCPUFA among the children according to HIV status, gender, age and nutritional status. A total of 318 children were recruited with 21 (7%) being HIV infected and 116 (37%) being HIV exposed uninfected. Chronic malnutrition was present in 12% of the children. The omega 3 fatty acids were expressed as percent weight of total fatty acids. The medians (interquartile range) for EPA, DPA and DHA for all the children were 0.19 (0.09), 0.79 (0.19) and 2.14 (0.54) %wt/wt respectively. EPA, DPA and DHA levels were not associated with the HIV status of the children. EPA levels were much lower in the 7-year-age group compared with the 8 and 9 - 10-year-age groups. Further studies assessing LCPUFA levels that include larger sample size, children from both urban and rural areas are recommended as this may

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assist in clearly defining the association of LCPUFA with HIV status in children from low income countries with high burden of under-nutrition.

Keywords

Omega 3 LCPUFA, Under-Nutrition, HIV, Children

1. Introduction

Under-nutrition is associated with many macro- and micronutrient deficiencies. Long chain polyunsaturated fatty acids (LCPUFA) are important macronutrients whose deficiency can have a synergistic effect with 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 [1]. The important metabolites of alpha linoleic acid include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [2]. They are anti-inflammatory [3]-[6] and have an essential role of brain function, growth and development [7] [8].

Omega 3 fatty acid deficiency present with symptoms that can partially explain the symptoms of under-nutrition such as growth failure, immunodeficiency and neurocognitive defects [9]. Docosahexaenoic acid an omega 3 LCPUFA and arachidonic acid which is an omega 6 LCPUFA are the main fatty acids in the brain [10]. DHA is an important constituent of the cell membrane and hence deficiency will affect many cellular functions especially in the brain [11]. 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. 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 [9]. LCPUFA deficiency could potentially exacerbate neurodevelopmental outcomes of under-nourished children in association with zinc, iron, iodine and protein deficiencies that also occur with under-nutrition. Moreover, the poor socio-economic and psychological background that under-nourish children often live in worsen the neurodevelopmental outcomes [9]. Altered levels of omega 3 LCPUFA have been reported in under-nourished human immunodeficiency virus (HIV) infected and uninfected children [12] and in HIV infected well-nourished children on highly active anti-retroviral therapy [13].

Intervention studies have shown benefits of maternal and infant supplementation of omega 3 LCPUFA on neurocognitive development of younger children below five years [14]. This is most likely because the brain is rapidly growing and developing before age 2 years [15] [16]. Further brain development and maturation continues into childhood especially the frontal lobe and the retina [16]. Hence omega 3 LCPUFA supplementation could benefit older children above 5 years. However, research in well children above 5 years has been equivocal in terms of improving neurocognitive outcomes with some studies finding benefit [17] [18] while others did not [19] [20]. Nevertheless, in children who are unwell with phenylketonuria and psychiatric disorders, supplementation with omega 3 LCPUFA has been shown to be beneficial [21]-[23]. In addition, an Italian study did show that from the neonatal period to adulthood, children had the lowest level of omega 3 LC-PUFAs and this could potentially result in poor health outcomes for children [24]. Omega 3 LCPUFA supplementation could potentially mitigate the effect of micronutrient deficiencies on cognitive function and the immune system in under-nourished HIV infected, HIV exposed uninfected and HIV unexposed children from low income countries. Very few small studies have reported on association of HIV and omega 3 LCPUFA in children but more studies are needed to clearly define the interaction with bigger sample sizes. This short communication aims to add to the body of scientific knowledge by describing the omega 3 fatty acid levels of HIV infected, HIV exposed uninfected and HIV unexposed school aged children from a low income country with a high burden of HIV infection and under-nutrition.

2. Materials and Method

A total of 318 children were recruited. Care givers and participants gave written informed consent and assent respectively. This study was approved by the Norwegian Research Ethics Committee, The Medical Research

Council of Zimbabwe and The Research Council of Zimbabwe.

2.1. Study Design, Population and Setting

This cross-sectional study was performed between August 2011 and June 2012 in a research that has been described previously [25]. Briefly, children 7 - 10 years old who were being followed up in a prevention of mother to child transmission of HIV infection cohort known as the Better Health for African mothers and children (BHAMAC) [26] had their nutritional status, selenium and iron status assessed. The children were recruited from 3 primary care peri-urban clinics just outside Harare, the capital city of Zimbabwe. This cohort of children consisted of HIV exposed (infected and uninfected) and unexposed children. The HIV status of the children was confirmed with HIV DNA polymerase chain reaction before 18 months of age and thereafter by using enzyme linked immune-assay (ELISA) and Western Blot methods [26]. Only the index children above 5 years of age were eligible for recruitment from the original mother-baby pairs of the BHAMAC cohort. Subsequent siblings were excluded from this study.

2.2. Data and Blood Collection

Socio-demographic information was collected using a standardized questionnaire and complete physical examination was performed on all participants by the study paediatrician including nutritional assessment. The World Health Organization criteria for defining stunting, thinness and underweight using Z scores were applied. Recruitment and blood collection occurred every Thursday between 8 am and 6 pm. Participants were not fasted before blood collection as there is evidence that fasting is unnecessary [27]. Participants' hands were washed with soapy warm water and were then dried with a paper towel. A lancet was pressed on the fleshy part of the middle finger. The first drop of blood was wiped away with cotton wool. The subsequent drops of blood were collected on filter paper treated with butylated hydroxytoluene (Sigma Aldrich Limited, Gillingham Dorset, United Kingdom) using 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 [28]. 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. International standards were used for shipping the specimens from Zimbabwe to Scotland.

2.3. Laboratory Analysis

Omega 3 fatty acids analysis was performed at the Nutritional group laboratory at the Acquaculture Institute, University of Stirling in Scotland. A method described by Bell *et al*. [29] was used to measure whole blood phospholipids from dry blood spots. Automated gas liquid chromatography was used for analysis. Results were reported as percent 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. Statistical Methods

Statistical package for the social science (SPSS) version 22 was used to analyze the data. Descriptive statistics were used to describe the data. Kruskal-Wallis test as well as the medians test were used to compare distribution and medians of omega 3 fatty acids among the HIV infected, HIV exposed uninfected and HIV unexposed uninfected children as the omega 3 fatty acid data was not normally distributed. The significance level (α) was set at 0.05.

3. Results

Out of the possible 452 children who were eligible for recruitment, 318 children participated in the study. The 134 (30%) children who did not participate were either: lost to follow up, caregivers refused, did not have a legal guardian to consent or had died at the time of the study. Of note is that there was a programme carried out by the authorities in the areas where the three clinics were located that displaced many families to rural areas before the study commenced.

The socio-demographic characteristics and nutritional status of the participants are shown in **Table 1**. There were more females and majority of the participants had not been exposed to HIV infection. About a fifth of the children were chronically malnourished and almost two thirds were living in households with income less than USD$250 per month.

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 2**. However, there was statistically significant differences in the EPA medians by age group ($p = 0.02$). The 7-year-old age group had a lowest median compared to the 8- and 9-year-old age groups. However, this age group only had 21 participants.

4. Discussion

In a low income country with a significant burden of chronic under-nutrition and HIV infection children's whole blood EPA, DPA and DHA derived from dry blood spot (DBS) samples were determined. The omega 3 fatty acids levels did not differ among the children according to HIV status, gender and nutritional status. However, the 7-year-old children had lower median for EPA compared to the 8 and 9 - 10-year-old age groups.

Table 1. The socio-demographic characteristics and nutritional status of 7 - 10 years old children from Zimbabwe.

Characteristic	N = 318	Frequency
Age group		
7 years		21 (7%)
8 years		105 (33%)
9 - 10[1] years		192 (60%)
Gender		
Male		137 (43%)
Female		181 (57%)
HIV status		
Negative unexposed		180 (56%)
Negative exposed		116 (37%)
Infected		21 (7%)
Unknown		1 (0.3%)
Stunting		37 (12%)
Underweight	317[2]	25 (8%)
Thinness		11 (4%)
Household income		
≤USD$250		205 (64%)
>USD$250		63 (20%)
Missing		50 (16%)
Clinic site		
Epworth		104 (33%)
St Mary's		104 (33%)
Seke North		110 (34%)

[1]There were only 3 children aged 10 years old; [2]One child had a missing weight.

Table 2. 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.

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 years[3]	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	**317[4]**	**0.19 (0.09)**		**0.79 (0.19)**		**2.14 (0.54)**	

*Significant p-value less than 0.05; [1]Measured as % weight of Total serum phospholipids (% wt/wt); [2]The Median test was used to compare medians across groups; [3]Combined the 9 and 10 year olds because there were only 3 children 10 years old; [4]One child's HIV status from the 318 children was unknown.

4.1. Omega 3 Fatty Acid Levels and Age/Gender

Our study determined whole blood fatty acid levels and did not find any association of age and gender with EPA, DPA and DHA levels except that EPA was much lower in the 7-year-old age group. A heterogeneous cohort of European children 3 to 8 years old from 8 countries who were well nourished the median EPA levels in the age groups 7 to 9 years was higher compared with our study findings [30]. However, the median DPA and DHA levels were lower by almost half the quantities found in our study. The European study had a much larger sample size and there were subtle difference in the analytical method. In this European study, both capillary and venous whole blood samples were used but we only used capillary blood samples collected on a dry blood spot. Our study had 7% of the children 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. Compared to another study done in the United Kingdom (UK) in school aged children 6 to 10 years old who were under performing in reading and had slightly more boys [31], all 3 omega fatty acid levels were lower in our study. Whole blood fatty acid levels were determined from dry blood spot

samples with minimal differences in the laboratory protocol. Notably, these UK 6- to 10-year-old children's omega 3 fatty acid levels were also higher than the European study. 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 [32] 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 [29].

4.2. 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 [33] and selenium deficiency [25]. 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 [9] 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 [34]. 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 had low DHA levels [35]. 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 [36]. A rat model of protein energy malnutrition [37] found that omega 3 fatty acid may have a role in intestinal repair of malnourished rats with chronic diarrhoea.

4.3. Omega 3 Fatty Acid Levels and HIV Status

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 [38]. In this particular study 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 [12], malnourished HIV infected children had lower plasma DHA and total omega 3 LCPUFA compared to malnourished HIV negative children. In this study 35 severely malnourished children (19 HIV infected) were also compared to health 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.

4.4. Limitations

This study compared omega 3 fatty acid levels in children 7 to 10 years old by HIV status, age, gender and nutritional status. This is one of the few studies to report on this finding and adds to the body of scientific knowledge. However, the 7 years age group was relatively small compared to the other two age groups constituting only 7% of the whole group. This limits conclusions that can be drawn. This study was a cross-sectional study were 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 fatty acids [39]. However, whole blood fatty acids have been found to correlate well with erythrocyte fatty acid levels [40]. The population of children studied was narrow constituting 7- to 10-year-old children and was made up of mainly children from very low income peri-urban setting. These results therefore should not be generalized to children outside this age group, from rural or urban setting and from higher income households. Lastly, dietary habits of the children were not studied. This may have explained why our results were lower than some of the studies quoted from Europe. Zimbabwe is a land-locked country with reduced access to sea food which is a good source of omega 3 fatty acids.

4.5. Conclusion

Our study has found no difference by HIV status, gender and nutritional status in the capillary whole blood omega 3 fatty acid levels EPA, DPA and DHA in children 7 to 10 years old from a low income country peri-urban setting with a dual high burden of HIV infection and under-nutrition. 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 in under-nourished children from low income countries.

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Conflict of Interest

All authors declare no conflict of interest.

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