

Effects of Mega Dose Micronutrient Supplementation On Serum Zinc, Retinol and Immune Status of Adult Males and Females Diagnosed with and Without HIV, Malaria and TB in Western Kenya – An Unpublished Perspective as at The Year 2004

Mbakaya Charles^{1,2*}, Nyambaka Hudson³, Waudo Judith³, Nawiri Mildred³, Amukoye Evans¹, Orege Patrick⁴, Kisingu Wilfred⁵, Koech Davy¹, Ndiege Isaiah³, Mpoke Solomon³, Mwangi Moses⁵, Wanzala Peter⁵, and Muniu Erastus⁵

¹Kenya Medical Research Institute Nairobi County, Kenya

²Rongo University College (RUC), School of Physical Sciences, Technology and Engineering

³Kenyatta University, School of Pure and Applied Sciences, Nairobi, Kenya

⁴National AIDS Control Council, Office of the President, Nairobi County, Kenya

⁵Center for Public Health Research, KEMRI, Nairobi County, Kenya

***Corresponding Author:** Mbakaya Charles, Rongo University College (RUC), School of Physical Sciences, Technology and Engineering and Kenya Medical Research Institute (KEMRI), P. O. Box 54840 Nairobi 00200, Nairobi County, Kenya.

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Abstract

Background: The role of micronutrients in management of HIV/AIDS, malaria and TB remains poorly understood worldwide.

Objectives: To assess differences in mega dose nutritional management between HIV-seronegative and seropositive adult males and females diagnosed with HIV at Voluntary Testing and Counseling Centers (VCT) in Western Kenya.

Methods: This was a randomized controlled study in which 90 subjects were recruited on the basis of an HIV-seropositive result from a voluntary and counseling center (VCT) using rapid HIV test kits. They were evaluated at baseline and every 4 weeks for 3 months to establish their clinical, biochemical and immunological status. After 12 weeks, 74 clients were still in the study, 9 were lost to follow-up while 7 had died. Of the 74 who completed the study, confirmation of baseline HIV status by ELISA revealed that 63 were HIV-seropositive while 11 were HIV-seronegative despite losing spouses to HIV/AIDS. Correlations between parameters at baseline, during and after intervention were determined; Spearman's Rho Coefficients indicating the level of significance. Group means were used to compare continuous data while categorical data was compared using Chi-Square.

Results: Significant reductions in the clinical manifestation of disease were noted in the cohort after intervention for 12 weeks. Despite the large and different micronutrient dosages used between the two study arms, the only difference by arm of intervention was in the serum vitamin E level at 4 weeks which was much higher in arm 1 than it was in arm 2 of the study ($p = 0.005$). This might have been occasioned by the significant repletion of zinc in both arms, probably because use of citric acid in both arms improved zinc up-take from the supplements, food and/or reserves enabling other nutrients to be appropriately restored in both arms, these supporting the decision to pool the study arms and compare differences by HIV-seronegative and seropositive, notwithstanding the small sample sizes recruited but which nonetheless were our study limitation. Independent of the intervention arms, reduction of viral load by more than $0.5 \log_{10}$ copies/ml correlated with higher baseline optical densities of HIV antibodies ($P = 0.016$) and higher baseline viral loads ($p = 0.0001$). A lower optical density of HIV antibodies at baseline correlated with higher serum zinc levels at 12 weeks ($p = 0.008$) and a lower Body Mass Index (BMI) at baseline ($p = 0.029$). Independent of the arm of study, a significant increase in CD4 cells counts post intervention correlated with lower baseline viral loads ($p = 0.010$), lower baseline NK cell counts ($p = 0.007$)

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($p = 0.007$) and HIV-seronegativity ($p = 0.001$). Malaria parasitaemia at baseline correlated with higher baseline CD4 cell counts ($p = 0.019$), HIV-seronegativity ($p = 0.002$), lower WHO clinical staging of disease ($p = 0.024$) and higher serum zinc at 12 weeks ($p = 0.054$). Infection with HIV at baseline correlated with lower NK cell counts at 12 weeks ($p = 0.003$), higher CD8 counts at baseline ($p = 0.0001$), lower CD4 cell counts at baseline ($p = 0.0001$), lower serum zinc at baseline ($p = 0.050$) and lower serum zinc at 12 weeks ($p = 0.0001$). A higher baseline blood glucose level, often associated with hypercortisolism (mal functional endocrine system), correlated with lower baseline CD4 cell counts ($p = 0.002$) and infection with TB at 12 weeks ($p = 0.049$).

Conclusions: This study has unearthed critical differences in susceptibility to malaria, HIV/AIDS and TB that were significantly correlated to nutritional and immune parameters at baseline, during and post nutritional supplementation. Though preliminary, the data suggest the need for a new paradigm shift in the strategies for combating these killer diseases, especially in Sub-Saharan Africa. In the circumstances, innovative application of Nutraceutical Chemistry has an unexploited potential to contribute new insights into difficult health problems afflicting society and cannot be overstated.

Keywords: Analytical; Nutraceutical Chemistry; Immunity; HIV/AIDS; TB; Malaria

Introduction

Although HIV/AIDS is among the leading killers of workers in Sub-Saharan Africa and poses major worksite challenges impacting on work productivity, medical confidentiality, employment, insurance and Medicare [1], occupational health and safety specialists worldwide are yet to treat this disease with the seriousness it deserves. In the circumstances, there is increasing evidence that vitamin and mineral deficiencies may play an important role in HIV transmission and progression [2,3]. Notably, HIV-patients are under oxidative stress from loss of CD4 counts and infection while several micronutrients are required to combat disease [4,5]. Furthermore, a number of HIV-associated illnesses interfere with appetite as others result in a greater need for balanced nutrition [6-8]. Evidence from several studies has shown that micronutrient deplete patients experience faster progression of HIV to AIDS. While low serum vitamin A level is a known risk factor for mortality during HIV-infection, supplementation with high doses of micronutrients reduces progression to AIDS; resulting in higher survival rates [9-12]. For instance, other studies have shown that repletion of plasma levels of selenium and zinc decreases mortality due to HIV/AIDS [13-15]. In one controlled study, the average viral load of HIV/AIDS patients was 1.0 log copies/ml (SD 0.4) lower than in patients on vitamin supplements and comparable to the reduction in viral load obtained with AZT in 12 weeks [16]. Other researchers have demonstrated the importance of micronutrient zinc in HIV/AIDS and other clinical conditions [17,18]. In view of the foregoing literature we conducted an assessment on some novel antioxidant micronutrient combinations in the management of HIV/AIDS subjects with a view to improving the chances to sustain occupational health and productivity among infected workers in Kenya and beyond, given some of the challenges of using ARVs such as resistance and adverse effects.

In this paper, which is the first in a series of other pending publications, we provide a general description of the biodata of the subjects and give a comparison of the means of their biochemical data obtained at baseline and after 12 weeks of supplementation. However, it is noteworthy that upon confirmation of HIV status of the subjects recruited in this study on the basis of a positive VCT result, 11 subjects were found to be discordant while 79 were HIV-seropositive but nonetheless both groups received the supplements for 12 weeks. The 11 study participants found HIV seronegative had lost spouses to HIV, yet they had sexually related with them without protection; making them unique and worthy of a comparison with the seropositive subjects, notwithstanding the small sample size that could be considered as a limitation to this study. Since HIV-seronegative and HIV-seropositive subjects were both randomized to receive RDA and 7xRDA micronutrients, this paper attempts to compare trends in the pooled data between the two categories of subjects by HIV serostatus at baseline, during and post intervention., given that the seronegative subjects were few but with a unique immune system worthy of investigation, yet could not be split into the two respective study arms and compared meaningfully with the seropositive subjects. However, subsequent publications will elaborate on the differences between supplementation regimens for the HIV-seropositive subjects only.

Therefore, the aim of this study was to compare the benefits of managing HIV seronegative and seropositive subjects with micronutrients (pooled data by supplementation arms) with respect to nutritional and immunological restoration and reduction in disease burden.

Materials and Methods

The aim of this study was to evaluate the benefits of managing HIV/AIDS subjects on micronutrients with respect to nutritional restoration and reduction in disease burden. The research protocol was developed and subjected to the scientific and ethical clearance procedures at the Kenya Medical Research Institute (KEMRI) and approved to commence in June 2004 with KEMRI SSC Protocol No. 839. The study was conducted in the Western parts of Kenya where subjects were recruited from societies of people living with AIDS. The inclusion criteria were that a person was HIV-1 infected as diagnosed from a local VCT center and aged 18 - 60 years and living in proximal reach of the study area. Subjects using antiretroviral drugs or other nutritional supplements were excluded from the study. We neither monitored the sexual behavior nor provided the subjects with condoms during the study. Consenting HIV positive subjects were recruited into the study. Eleven subjects were subsequently confirmed HIV-seronegative but allowed to stay on the supplements as more confirmatory tests were done to establish their true status. The subjects were guided through the study objectives and benefits and written informed consent obtained. Study subjects were informed that their participation in the study had no collateral effects; that the supplements had no demonstrated incompatibility with other types of treatments; that they should follow treatment as advised without interruption and that they should not suspend treatment without consulting the investigators.

The project clinicians screened and enrolled subjects who met the inclusion criteria. The subjects were examined and their biological and clinical review data obtained and recorded on specified forms at baseline and at 12 weeks' post intervention. Blood (20 ml) was obtained from the subjects and dispatched to KEMRI laboratories for analyses. After obtaining baseline data, subjects were put on the intervention and provided with their monthly dose of supplements. Multi-micronutrient supplements used in this study consisted of:

Formulation A: A daily dose comprising malic acid (2.0 g), arginine (2.0 g), glucosamine (2.0 g), glycine (1.0 g), pyridoxal (1 mcg), vitamin C (0.06 g), folic acid (200 mcg), glycirrhizinic acid (0.1 g), elemental zinc as zinc sulphate (15 mg), honey and citric acid.

Formulation B: A daily dose comprising vitamins E (1000 IU), B₁₂ (1000 IU), C (1000 mg), DHEA (25 mg) and vitamin A (200,000 IU as a monthly dose) and

Formulation C: A daily dose comprising zinc (100 mg), selenium (200 µg), vitamin C (1000 mg) and honey.

Subjects randomized in arms 1 and 2 both received **Formulation A** (1 X RDA) for 12 weeks. Those in arm 2 received **Formulations B and C** for 8 weeks (6 X RDA) while those in arm 1 received matched placebos of **Formulations B and C** for 8 weeks. Effectively, between weeks 8 and 12, the subjects in both arms were on a 1 X RDA micronutrient supplements without citric acid to minimize any possible toxic effects from nutritional overload. The rationale for using mega-doses in this intervention was premised on the fact that an earlier study in Kenya that used 1x RDA doses of micronutrient zinc was ineffective in raising serum zinc levels during the three years of nutrition supplementation to HIV/AIDS patients.

At baseline and 12 weeks, subjects were clinically examined and details of HIV-associated opportunistic infections recorded and treated where necessary. Blood samples were collected every 4 weeks and analyzed in KEMRI Hematology, Nutrition, Immunology (baseline and 12 weeks), Virology (baseline and 12 weeks), and Biochemistry laboratories. Briefly, the specific tests undertaken were as follows:

- 1. HIV status & HIV-1 RNA viral load tests:** Blood (5 ml) was collected in EDTA vacutainers and delivered to KEMRI for analysis. Both rapid and ELISA tests were performed for confirmation of the HIV status of the subjects and the viral load determined using Roche kits (Amplicor version 1.5).

2. **CD4 & 8 counts and CD4/8 ratios:** Blood (2 ml) was collected in EDTA vacutainers and stored at room temperature and analyzed using a Cytometer (FacsCalibur, Becton & Dickson International, Belgium).
3. **Full haemogram:** Blood (2 ml) was collected in EDTA vacutainers and for determination of white blood cell count, red blood cell count, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration. Erythrocyte sedimentation rate (ESR) was performed using the Wintrob method.
4. **Serum zinc and retinol:** Blood (5 ml) was collected in an acid washed screw-cap glass tubes covered with aluminium foil and closed tightly. Serum was separated by centrifugation at 3000 rpm for 10 minutes. The serum zinc levels were determined using a Flame Atomic Absorption Spectrometer (FAAS). Serum retinol was determined using a High Performance Liquid Chromatograph (HPLC) at the Nutrition Laboratory at KEMRI.
5. **Vital signs:** The subjects' vital signs of temperature, pulse rate and respiratory rate were determined at baseline and 12 weeks.
6. **Liver function tests:** This were performed using a Clinical Chemistry Analyzer (Dionex, Buenos Aires, Argentina).
7. Data coding, entry and analysis were undertaken at the Centre for Public Health Research (CPHR, KEMRI). The SPSS/PC+ Vers. 11.5 programme was used for data entry and analysis. Variables that did not conform to normality such as viral load were transformed using $\log_{10}(x+1)$ transform. To test for continuous variables such as serum retinol and zinc between HIV-seronegative and HIV-seropositive subjects at baseline and 12 weeks' post-intervention, the un-paired t-test was used. Correlations between variables at all stages of the study period were made and Spearman's Rho Coefficients used to test for significance. Furthermore, non-parametric tests were undertaken to compare categorical data relating to prevalence of clinical signs and symptoms associated with HIV/AIDS both at baseline and after 12 weeks of intervention using McNemar's Chi-square tests.

Results

The biodata of the patients was as provided.

Parameter	Number (%) n = 90
Sex	
Female	63 (70.0)
Male	27 (30.0)
Marital status	
Single	5 (5.9)
Married	35 (41.2)
Widow	40 (47.1)
Widower	3 (3.5)
Divorced	2 (2.4)
Client Locale	
Chemelil	16 (17.8)
Kisumu City	32 (35.6)
Busia	13 (14.4)
Kakamega	29 (32.2)
Dietary Habits	
Eats 3 meals/day	51 (56.7)
Eats < 3 meals/day	39 (43.3)

Occupation	
Small scale farmers	25 (27.8)
Informal sector (Jua Kali)	57 (63.3)
Teachers	8 (8.9)
HIV status	
Confirmed HIV-seropositive	79 (88)
Confirmed HIV-seronegative	11 (12)

Table 1: Characteristics of study cohort at baseline.

The study population of 90 subjects was evenly distributed across the age bracket of 18 - 60 years, with females constituting 70% of the cohort. By marital status, 41% of the subjects were married while widows constituted 47% of the cohort. Majority of the subjects (63%) were employed in the informal (Jua kali) sector while 37% were either peasant farmers or teachers. However, 16 subjects did not complete the study as 7 succumbed to the disease while 9 dropped out of the study citing stigma, loss of interest and relocation from the study area as reasons. Of the 11 HIV-seronegative subjects, seven were randomized into arm 1 while 4 were allocated to arm 2. Compliance with supplementation was high as 79% of the subjects reported using the supplements as instructed.

Analysis of pooled clinical data of the HIV-seronegative (n = 11) and HIV-seropositive (n = 63) subjects suggested significant reductions in the prevalence of headache (p = 0.003), skin rash (p = 0.0001), pneumonia (p = 0.002) and weight loss (p = 0.005), while a significant increase was noted in pallor (p = 0.0001) between baseline and after 12 weeks of supplementation.

Clinical Signs Symptoms/Illnesses	Prevalence, Number (%)		P-Value 2 Tailed
	Week 0 (N = 74)	Week 12 (N=74)	
Headache	44 (62.9)	27 (37.0)	0.003
Skin rash	29 (41.4)	13 (17.8)	0.0001
Diarrhea	13 (18.6)	10 (13.7)	0.454
Cough	23 (32.9)	26 (35.6)	0.824
Fever	20 (28.6)	12 (16.4)	0.201
Oral thrush	13 (18.6)	14 (19.2)	1.000
Loss of appetite	24 (34.3)	21 (28.8)	0.405
Fatigue	30 (42.9)	27 (37.0)	0.458
Pneumonia	14 (20.0)	2 (2.7)	0.002
Boils	11 (15.7)	5 (6.8)	0.118
Itchy genitals	19 (27.1)	10 (13.7)	0.019
Pallor	3 (4.4)	16 (21.9)	0.0001
Loss of weight	29 (41.4)	12 (16.7)	0.005

Table 2: Prevalence of HIV/Aids related clinical signs/symptoms/illnesses in cohort before and after 12 weeks of nutritional supplementation.

As these are some of the key signs and symptoms associated with HIV/AIDS, the intervention had the overall effect of reducing the burden of disease in the cohort, though an elevation in pallor might allude to increased anaemia. There was some reduction in subjects with loss of appetite and fatigue between baseline and post intervention, the reductions were not statistically significant. Indeed, 88% of the clients reported that the interventions were beneficial to them in as far as improving their energy levels, capacity to work and appetite.

While mean serum zinc initially increased and declined in both study arms during supplementation, haemoglobin levels changed conversely. Notably, the mean serum ferritin levels of a randomly selected sub-population of the subjects changed from 62 ng/ml at baseline to 124 ng/ml at 4 weeks and declined to 109 ng/ml at 12 weeks. The only difference by arm of intervention was in the serum vitamin E level at 4 weeks which was much higher in arm 1 than arm 2 of the study ($p = 0.005$). Reduction of viral load by more than $0.5 \log_{10}$ copies/ml correlated with higher baseline optical densities of HIV antibodies at baseline ($p = 0.016$) and at 12 weeks of intervention ($p = 0.013$), higher baseline viral loads ($p = 0.0001$), higher serum vitamin E at 4 weeks ($P = 0.028$), older subjects ($p = 0.001$), higher BMI at week 12 ($p = 0.023$) and higher serum copper levels at 8 weeks ($p = 0.088$). A lower optical density of HIV antibodies at baseline correlated with higher serum zinc levels at 12 weeks ($p = 0.008$), a lower Body Mass Index (BMI) at baseline ($p = 0.029$) and lower blood total protein at baseline ($p = 0.034$). An increase in CD4 cells of greater than 50 counts in 12 weeks correlated with lower baseline viral loads ($p = 0.010$), lower baseline NK cell counts ($p = 0.007$), HIV-seronegativity ($p = 0.001$) and lower WHO clinical staging of the HIV disease ($P = 0.003$). Malaria parasitaemia at baseline correlated with higher baseline CD4 cell counts ($p = 0.019$), HIV-seronegativity ($p = 0.002$), lower WHO clinical staging of disease ($p = 0.024$), higher serum copper at 4 weeks ($p = 0.057$) and higher serum zinc at 12 weeks ($p = 0.054$). Infection with HIV at baseline correlated with lower NK cell counts at 12 weeks ($p = 0.003$), higher CD8 counts at baseline ($p = 0.0001$), lower CD4 cell counts at baseline ($p = 0.0001$), lower serum zinc at baseline ($p = 0.050$) and lower serum zinc at 12 weeks ($p = 0.0001$). A higher baseline blood glucose level correlated with lower baseline CD4 cell counts ($p = 0.002$), infection with TB at 12 weeks ($p = 0.049$) and oral thrush at baseline ($p = 0.039$). Consumption of a diet that was not balanced was associated with high production of eosinophils at 12 weeks ($p = 0.004$), higher serum copper levels at baseline ($p = 0.033$) and 4 weeks ($p = 0.005$), and lower BMI at baseline ($p = 0.068$).

It was notable that 33% of the subjects reported having sought medical attention for malaria related morbidity between 8 and 12 weeks of nutritional supplementation. Taking various biochemical factors into consideration, significantly high baseline serum zinc levels were noted among subjects who subsequently sought treatment for malaria during follow-up. Subjects seeking malaria treatment had a higher baseline mean serum zinc level compared to those who did not seek treatment within the third month of supplementation ($p = 0.016$). While 36% of the HIV-seronegative subjects were positive for malaria parasites by blood smear and microscopy at baseline, only 6% had malaria parasites among the HIV-seropositive subjects ($p = 0.002$). Notably, HIV-seronegative subjects had higher mean CD4 counts ($p = 0.002$) and lower mean CD8 counts ($p = 0.0001$) compared to HIV-seropositive ones.

Interestingly, subjects with malaria parasites at baseline had significantly lower mean serum retinol levels of $0.8544 + 0.221 \mu\text{mol/L}$ compared to $1.0527 + 0.3834 \mu\text{mol/L}$ for those without parasites ($p = 0.035$). Like HIV-seronegative subjects who had a high prevalence of malaria parasites at baseline, subjects infected with malarial parasites at baseline had higher baseline mean CD4 counts ($p = 0.055$) and lower mean CD8 counts ($p = 0.844$) than those without. Furthermore, subjects with malaria parasites at baseline had lower mean respiratory rate ($p = 0.0001$), pulse rate ($p = 0.010$) and temperature ($p = 0.0001$) than those without.

Eleven subjects found to be HIV-seronegative despite losing their spouses to HIV/AIDS had baseline mean serum zinc level of $165 \mu\text{g/dL}$ that was significantly higher than that of the HIV-seropositive ones at $128 \mu\text{g/dL}$ ($p = 0.041$). Even after 12 weeks of follow-up, the mean serum zinc levels of HIV-seronegative subjects remained significantly higher than that of the HIV-seropositive ones. Notably, the mean serum zinc levels in this cohort increased from baseline levels reaching a maximum at 4 weeks of zinc supplementation and declining again despite continued supplementation with zinc at the same dosage for another 4 weeks. On the contrary, the trend in haemoglobin levels was the reverse of that of zinc levels with time.

Parameters	Mean \pm SD ₊₊₊					
	Week 0			Week 12		
	HIV Seronegative (n = 11)	HIV Seropositive (n = 63)	P-Value 2-Tailed	HIV Seronegative (n=11)	HIV Seropositive (n=63)	P-Value 2-Tailed
Biochemical Variables						
ESR (mm/Hr)	27.8 \pm 19.6	44.2 \pm 18.5	0.022	26.0 \pm 17.1	39.0 \pm 22.1	0.041
Eosinophil (%)	-	-	-	3.6 \pm 2.5	4.2 \pm 3.0	0.496
Hb (μ g/dL)	12.4 \pm 2.5	11.5 \pm 2.0	0.304	11.7 \pm 2.0	10.3 \pm 1.9	0.056
Zinc (μ g/dL)	164.5 \pm 47.7	127.8 \pm 72.6	0.041	178.0 \pm 53.8	110.9 \pm 36.6	0.002
Retinol (μ mol/L)	1.16 \pm 0.42	1.01 \pm 0.37	0.288	0.98 \pm 0.51	0.92 \pm 0.38	0.750
Vital Signs						
Respiratory rate (breathes/min)	18.60 \pm 0.97	20.45 \pm 3.61	0.001	19.82 \pm 1.89	19.73 \pm 3.99	0.913
Pulse rate (beats/min)	74.40 \pm 3.86	80.48 \pm 4.37	0.004	80.00 \pm 8.00	73.3 \pm 12.24	0.126
Temperature ($^{\circ}$ C)	36.06 \pm 0.19	36.38 \pm 0.62	0.001	36.25 \pm 0.62	36.22 \pm 0.63	0.896
Immunological/Virological						
Total Lymphocytes ($\times 10^6$ /L)	2069 \pm 897	1830 \pm 725	0.415	2778 \pm 829	2005 \pm 793	0.013
CD4 counts ($\times 10^6$ /L)	870 \pm 443	320 \pm 211	0.002	1093 \pm 306	376 \pm 258	0.0001
CD8 counts ($\times 10^6$ /L)	458 \pm 243	986 \pm 501	0.0001	661 \pm 346	1098 \pm 562	0.0002
CD4/8 ratio ($\times 10^6$ /L)	1.97 \pm 0.46	0.38 \pm 0.33	0.0001	1.81 \pm 0.51	0.39 \pm 0.29	0.0001
NK counts ($\times 10^6$ /L)	414 \pm 312	341 \pm 213	0.465	530 \pm 322	282 \pm 152	0.029
Viral load (\log_{10} copies/ml)	0.000 \pm 0.000	4.394 \pm 0.642	0.0001	0.000 \pm 0.000	3.960 \pm 1.502	0.0001

Table 3: Selected biochemical parameters for HIV – seronegative and HIV – seropositive subjects on nutritional supplementation in western Kenya.

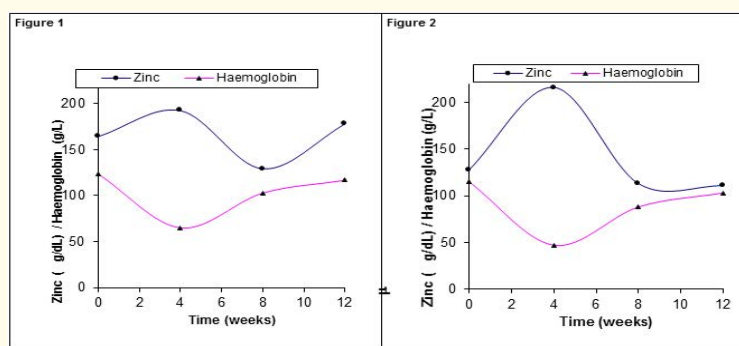


Figure 1: Trends in mean haemoglobin and mean serum zinc levels in HIV seronegative during 12 weeks of dietary supplementation with zinc.

Figure 2: Trends in mean haemoglobin and mean serum zinc levels in HIV seropositives during 12 weeks of dietary supplementation with zinc.

While the mean ESR values of HIV-seropositive subjects were significantly higher at baseline than for the HIV-seronegative ones ($p = 0.022$), there was a decrease in mean ESR values for both groups after supplementation. Biochemically, there was no difference in liver function tests between baseline and post intervention values for both the seronegative and seropositive study participants, probably suggesting that the interventions were not toxic. With respect to vital signs, there were significant differences in the baseline respiratory and pulse rates of the subjects; higher rates being noted in the HIV-seropositive ones. The same was observed with temperature with the HIV-seropositive subjects manifesting slightly higher body temperatures at the baseline. However, after 12 weeks of dietary intervention, the differences in these vital signs were not significantly different between the two groups! Immunologically, there were clear differences between the two groups with respect to the baseline CD4 counts ($p = 0.002$), CD8 counts ($p = 0.0001$) and CD4/CD8 ratio ($p = 0.0001$). The HIV-seronegative subjects experienced substantial absolute changes in their mean CD4 cell counts of $223 \times 10^6 /L$ compared to $56 \times 10^6 /L$ in the HIV-seropositive ones. Similarly, the mean total lymphocyte counts of HIV-seronegative subjects increased by $709 \times 10^6 /L$ counts compared to $175 \times 10^6 /L$ cell counts for the HIV-seropositive ones. Overall, there was a near significant change in the viral loads of the HIV-seropositive subjects of -0.434 (\log_{10} copies/ml) after the 12 weeks of dietary supplementation ($p = 0.088$).

Discussion

The observation that eleven subjects were HIV-seronegative, despite losing their spouses to HIV/AIDS points at the continued strange phenomenon of discordant couples' world over. High body temperatures, pulse and respiratory rates could be an indication of on-going inflammatory processes, making the vital signs a useful guide in assessing trends in disease progression; especially in resource constrained settings. Since, infection with HIV at baseline correlated with lower serum zinc at baseline ($p = 0.050$), lower serum zinc at 12 weeks ($p = 0.0001$) and that a lower optical density of HIV antibodies at baseline correlated with higher serum zinc levels at 12 weeks ($p = 0.008$), it is now apparent that micronutrient zinc status may be a critical modifier of susceptibility to HIV infection. This phenomenon is not entirely new as classical text books of environmental epidemiology do recognize that nutrition is one of the many factors (age, sex, genetics and physical condition etc.) that can modify exposure outcomes to environmental hazards (e.g. bacteria, fungi, viruses and parasites etc.). Simply put, high serum zinc levels appear not to favor antibody (humoral immunity). Thus, depending on one's zinc status at the time of exposure to HIV, one may mount humoral immunity if one is zinc deficient in which case they will become HIV positive upon testing or one may mount cellular immunity if one is zinc sufficient and thereby remain HIV-seronegative. This might be the most probable explanation of HIV discordancy among couples and alludes to a great potential of using zinc nurture in containment of new HIV infections in vulnerable populations across the globe. No wonder, some studies have reported that zinc has useful antiviral, antibacterial and anticancer properties and that there are similarities in symptoms of HIV/AIDS and serious zinc sufficiency [17]. Other studies have even been bolder in alluding that zinc may be a possible co-factor in the transmission and progression of HIV/AIDS in Sub-Saharan Africa.

Clinically, improvements noted with reduction in proportion of study subjects with loss of weight, headache, itchy genitals and skin rashes suggested that the nutritional interventions were beneficial. However, the decimal improvements in the proportion of subjects with reduced loss of appetite, fatigue and even the increased proportion of those with pallor might suggest that nutritional interventions were not effective on these parameters or that there might be a possibility of nutritional overload from the mega supplements; warranting more care during extended periods of intervention. However, increase in pallor is explainable, given that as serum zinc levels increased an inverse trend was observed with Hb which is a marker of iron status, this probably confirming that these two micronutrients compete during replenishment. It was of interest that the HIV-seronegative subjects had a higher baseline prevalence of malaria parasites (36%) than the HIV-seropositive ones (6%) ($p = 0.002$), this being contrary to what is known in the literature [19]. However, since infection with HIV at baseline correlated with lower NK cell counts at 12 weeks ($p = 0.003$), higher CD8 counts at baseline ($p = 0.0001$), lower CD4 cell counts at baseline ($p = 0.0001$), lower serum zinc at baseline ($p = 0.050$) and lower serum zinc at 12 weeks ($p = 0.0001$), this suggested presence of a functional humoral immunity that was effective in clearing malaria parasites from peripheral blood. This may have contributed to the high susceptibility of HIV-seronegative subjects to infection with malaria parasites. However, due to the efficient zinc-primed cell-mediated immunity that protected the HIV-seronegative subjects from infection, the malaria parasites may not have been a serious

clinical threat to them; given their normal levels of vital signs. Furthermore, the HIV-seropositive subjects predominantly mounted humoral immunity as shown by their elevated mean CD8 cell counts and higher optical densities of HIV antibodies at baseline ($p = 0.0001$).

Thus, it is possible that once a human host is in a high gear of production of antibodies to HIV, appropriate antibody immunity is also mobilized against other pathogens such as malaria parasites. It would therefore appear that control of malaria parasitaemia is best achieved with a functional humoral immunity. This might also suggest that in malaria endemic areas, cellular immunity may be down-regulated physiologically to prime humoral immunity that is acquired against malaria parasites. However, this may be a double jeopardy as exposure to HIV in such populations could lead to infections of epidemic proportions since the efficiency of cellular immunity may be down-regulated. Interestingly, malaria and HIV co-infections in malaria endemic regions are a fairly common phenomenon, especially in Sub-Saharan Africa. In a previous study testing malarial antigens in pregnant women, maternal antibodies to erythrocyte binding antigen (EBA-175) was the only highest antigenic malarial response in HIV-seropositive women as compared to HIV-seronegative ones ($p = 0.08$). The rest of the responses were found to be much higher in HIV-seronegative women [19]. This raises the possibility that antibodies to EBA-175 may, among other factors, have contributed to the low prevalence in baseline malaria parasitaemia in HIV-seropositive subjects in the present study. It is therefore possible that life threatening malarial attacks in the HIV-seropositive subjects may only occur when both cellular and humoral immunity have collapsed as is the case with the terminal stages of this disease when CD4 cell counts drastically fall below the $200 \times 10^6 /L$ cut-off. This is supported by the fact that at between 8-12 weeks of dietary intervention, when zinc levels were generally low while haemoglobin and by proxy serum iron levels were rising in both HIV-seropositive and HIV-seronegative subjects, a large proportion of the cohort (33%) reportedly had sought clinical management for malaria [20].

In the Papua New Guinea, studies have shown that zinc may play a role in reduction of morbidity related to *Plasmodium falciparum* infections as a 69% reduction was noted in clinic-based malarial episodes following supplementation with zinc [21]. In another randomized study conducted in the Gambia, 32% reduction in clinic visits due to *P. falciparum* infections was demonstrated among those given 70 mg zinc twice weekly for 18 months [22]. Thus, more elaborate studies are needed to better evaluate and understand interactions between HIV infection, haemoglobin levels, serum zinc and iron levels and malaria infectivity. Probably by building on these preliminary findings, initiation of new strategies based on the observed dynamics in serum zinc and haemoglobin levels may provide additional arsenals with which to combat malaria and HIV/AIDS. This is particularly important considering the gravity of these two killer diseases and their adverse impact on the population and healthcare delivery systems in Sub-Saharan Africa and other developing countries.

The high baseline means serum zinc and low mean serum retinol status noted in subjects with malarial parasites and clinical malaria in this study was also associated with type 1 (cellular-immunity) while the low mean serum zinc and high mean serum retinol levels were associated with type 2 (humoral immunity) and agrees to some extent with observations from other studies [20]. Consequently, subjects with low mean serum zinc and high mean serum retinol at baseline may have mounted efficient humoral immunity and were less likely to get infected with malaria parasites and progressing to clinical malaria during the third month of dietary intervention. Alternatively, subjects with high baseline mean serum zinc levels were more likely to reach peak mean serum zinc levels during supplementation and experience an early serum iron recovery flux, thereby increasing their susceptibility to malaria parasites progressing to clinical malaria as their acquired immunity might not have been fully developed; coming from a state of efficient cellular-immunity. The low mean serum retinol levels at baseline in subjects with malaria parasites ($p = 0.035$) indicates the susceptibility to malaria infection in subjects with cell-mediated immunity as alluded to by other studies [20]. Furthermore, the low body temperature ($p = 0.0001$) at baseline, respiratory ($p = 0.0001$) and pulse rate ($p = 0.010$) in subjects infected with malaria parasites confirmed absence of clinical malaria and suggested that cellular immunity was effective in controlling infection of cells by malaria parasites. However, this form of immunity appeared to be inadequate in clearing malaria parasites from peripheral blood.

The observed increase in the mean serum zinc levels in this cohort from baseline levels reaching a maximum after 4 weeks of zinc supplementation and declining thereafter despite continued supplementation at the same dosage for another 4 weeks may be due to the existence of a physiological process by which the body sequesters zinc after a critical concentration is achieved. It is possible that after adequate levels of zinc have been attained, the body may reduce absorption, increase excretion or increase storage; thereby leading to the decline in mean serum zinc levels even during a time of mega dose supplementation. The trend in mean serum zinc obtained in this study during and after supplementation with zinc in HIV-seronegative subjects in Figure 1 is similar to the one observed in blood levels of cadmium during the first year of occupational exposure and stoppage, suggesting similarities in metabolism. Therefore, the return of mean serum zinc and haemoglobin levels to baseline or higher than baseline levels upon reduction of zinc supplementation to RDA levels (15 mg/day) between week 8 and 12 in HIV-seronegative and not HIV-seropositive subjects in Figure 2 suggests the existence of a physiological mechanism that probably primes humoral immunity among subjects already infected with HIV. This is given that significant viral load reductions were associated with higher HIV optical densities of antibodies at baseline (0.016) and post intervention (0.013). Furthermore, this phenomenon may be occasioned by the need to prime humoral immunity which this study has shown to be most effective in reducing viral loads among HIV-infected subjects. Thus, the low zinc levels seen among HIV infected subjects might be physiologically controlled to enable them effectively deal with the prevailing environmental challenges by supporting acquisition of humoral (antibody) immunity against HIV, malaria and other pathogens. This may further explain why HIV-seropositive subjects had lower baseline malaria prevalence compared with HIV-seronegative subjects ($p = 0.002$).

Nonetheless, the interaction between zinc and haemoglobin, possibly suggests that in the initial stages of zinc supplementation, iron gives way, allowing antioxidant zinc to flash through the circulatory system. This was confirmed by the observed trend in mean serum ferritin which moved from 62 ng/ml at baseline to 124 ng/ml at 4 weeks, declining to 109 ng/ml post intervention. The initial increase in serum ferritin suggested increased iron storage at the time serum zinc levels were rising. However, the later decline in ferritin suggested release of iron from stores. Both trends, though, explain the trends seen in haemoglobin levels during supplementation in corroborating this findings, substantial lowering of haemoglobin levels following consumption of large doses of zinc gluconate (1000 mg/day for one year) have been reported. Thus, when serum zinc levels reach a critical peak value, the oxidant wave takes over and flashes the peripheral blood with oxidants, iron included. When the body is sufficient in both oxidant and antioxidant micronutrients, it is possible that oxidants flash the peripheral blood causing production of free radicals that are in turn mopped out following a subsequent antioxidant wave. Consequently, in an efficient human redox system, this cycle may be frequent and efficient as to alter the pathology of HIV/AIDS and other diseases that thrive on the presence of free radicals and oxidative stress. It is now apparent that an efficient human immune system may consist of Sine-Cosine waves of oxidant and antioxidant fluxes that repeatedly sweep the humoral system when adequate in key immune micronutrients. This might explain the many difficulties encountered in the scientific literature about interpreting zinc status and beneficial supplementation effects with this key- micronutrient in human populations worldwide. Nonetheless, misconceptions about benefits of micronutrient zinc in human immunity have not been helped by the lukewarm role played by chemists in applying the knowledge of chemistry in demystifying stubborn healthcare problems. This situation should now change, with chemistry starting to assume a central role in biomedical research in the 21st Century and beyond. The Vision and Mission of the Chemistry Central Journal, among other stakeholders, clearly crystallizes this research agenda.

It was notable that reduction of viral load by more than 0.5 log₁₀ copies/ml correlated well with higher baseline optical densities of HIV antibodies at baseline ($p = 0.016$) and at 12 weeks of intervention ($p = 0.013$), higher baseline viral loads ($p = 0.0001$), higher serum vitamin E at 4 weeks ($P = 0.028$), older subjects ($p = 0.001$), higher BMI at week 12 ($p = 0.023$) and higher serum copper levels at 8 weeks ($p = 0.088$). The observed reduction in mean log₁₀ viral load copies/ml in subjects using dietary supplements in 12 weeks that is equivalent to half the reduction that could be obtained by using a daily single therapy of AZT for 12 weeks [16] suggests that dietary supplements have a role to play in management of HIV/AIDS. The observed decline in mean retinol levels in both groups, despite administration of a monthly dose of vitamin A (200,000 IU) for 12 weeks to 50% of the cohort can be explained by mobilization of retinol by zinc. It is known

that zinc and vitamin A mobilize each other to and from liver stores and therefore as serum zinc levels improved, retinol was mobilized to the liver stores and possibly to cells and tissues, resulting in the decline in serum levels characteristic of type 1 immunological responses [20]. It is also possible that improvement of zinc levels resulted in increased synthesis of proteins, some of which may have included retinol binding proteins that sequestered retinol from the serum; retaining it in cells and tissues. Other researchers have shown that low retinol levels are associated with the more efficient cellular immunity while higher retinol levels are associated with the less efficient humoral immunity [20]. Thus, presence of adequate zinc supplies may have promoted utilization of retinol, creating a decline in its levels in peripheral blood while boosting cell-mediated immunity that is associated with higher NK cell counts. This phenomenon was observed in the HIV-seronegative subjects whose cellular immunity was boosted as manifested in the significant increase in their NK cell counts between baseline and 12 weeks of supplementation.

However, the increase in NK cell counts was not observed in the HIV-seropositive subjects. Instead, the NK cell counts decreased in HIV-seropositive subjects following 12 weeks of nutritional therapy, yet they also experienced a decrease in serum retinol. While there was a substantial increase in absolute CD4 and CD8 T-lymphocyte counts in both the HIV-seropositive and HIV-seronegative subjects, the differences between the groups remained large and significant both at baseline and after 12 weeks of dietary intervention. The large increase in immunochemical parameters in HIV-seronegative subjects suggests that nutritional support especially where populations are malnourished have beneficial effects in providing them with additional capacity to fight HIV/AIDS, other pathogens and occupational diseases that often take advantage of compromised immune systems. The large and very significant improvements noted in immunological markers following nutritional intervention might suggest as has been observed in other studies that nutrition has a critical role to play in human immunity and disease management, yet this has not been given its due attention and should now be the case going forward well into the 21st Century. Micronutrient zinc sufficiency, has in many publications been associated with an improved immune system, particularly because it upregulates innate immunity needed in disease prevention and combat, yet its use with other micronutrients with which it works in concert is only starting to be mainstreamed globally [17-23].

Conclusion

From the preliminary results of this study, it is possible that keeping serum zinc levels of humans at ~200 µg/dL coupled with provision of adequate vitamins, other related macro- and micro-nutrients could alleviate HIV and malaria complications leading to substantial reduction in morbidity and mortality due to these diseases as observed in other studies [24]. Furthermore, we believe that the nutrition and dietetics approach for the containment of HIV/AIDS and malaria should be mainstreamed into the overall health and safety management strategy, especially in developing countries with low capacity, low productivity, low income, mal-nutrition and disease [25]. The ILO and WHO recognize that the HIV/AIDS pandemic and malaria are eroding the human resource capacity by reducing the labor force in all sectors thereby posing a major burden on the basic health care systems [26]. It is our hope that nutritional support for workers in general, and particularly the HIV/AIDS infected workforce in Africa and the rest of the developing world will get expedited attention. Finally, the results of this study, though preliminary, are indeed remarkable and allude to the high potential that nutritional strategies hold in management of HIV/AIDS and malaria not only in Kenya, but also in other Sub-Saharan Africa and developing countries where nutritional deficiencies are rampant and could, over and above other occupational concerns, be responsible for the many observed disorders of physical and mental health among workers. Previously, it has been observed that management of HIV/AIDS could remain extremely elusive, and for a very long time to come, if the dual health effects of dioxins and zinc as well as other pertinent micronutrients are to continue being relegated to the periphery of biomedical research. Thus, as the majority of persons infected with HIV/AIDS are those in their productive working years, appropriately designed nutritional supplementation programmes may indeed provide an additional tool by which to manage work productivity in this era of HIV/AIDS. The results of this study corroborate and are apparently even more remarkable than those recently reported by other researchers on the benefits of using micronutrients to improve clinical presentation, immunity and reduce viral load in HIV/AIDS patients; thereby delaying the usage of ARVs [27,28]. However, larger and multi-center studies are clearly indicated with a view to refining and optimizing observations of this small study while targeting vulnerable populations such as working children, track drivers, women and commercial sex workers in Kenya and beyond.

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