EFFECTS OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART), ON SOME MICRONUTRIENTS IN HIV POSITIVE PATIENTS AT NSUKKA.

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ABSTRACTS

Some biological changes are observed during human immuno deficiency virus infection. Once $CD4^+$ count decreases to 500 cells/mm^3 highly active antiretroviral therapy (HAART) is initiated.

This work, seeks to elucidate the effect of HAART on some of the biochemical changes caused by HIV infection. 63 subjects comprising, 20 apparently healthy control subjects and 43 HIV positive subjects ready to be placed on HAART were recruited for the study. Concentrations of the following biochemical parameters: zinc, selenium, vitamin C, vitamin E and also CD4+ count was determined. The results showed that $CD4^+$ count increased significantly from baseline to 8 months into treatment ($p < 0.05$), zinc and selenium increased significantly from baseline to 8 months into treatment, but vitamin C increased up to the 4^{th} month but deteriorated by the 8^{th} month of initiation into HAART. Vitamin E showed no significant change in concentration ($p > 0.05$). There were significant positive correlations between the mean $CD4^+$ count and the mean concentrations of zinc ($p < 0.05$).

In this study, only zinc was significantly positively correlated with CD4+ count.

Key words: HIV/AIDS, HAART, CD4+, micronutrients.

1. INTRODUCTION

The HIV/AIDS (human immunodeficiency virus/ acquired immunodeficiency syndrome) pandemic has been terrorizing humanity over the past three decades. It has undermined the health of so many people, consequently affecting adversely, the work force and the economic stability of so many countries all over the world. World Health Organization (WHO) and United Nations Program on AIDS (UNAIDS) (2009), estimated that about 33.4 million people worldwide were living with AIDS, with 2.7 million new infections per year. Some biochemical abnormalities accompany infection with human immunodeficiency virus. These changes occur as a result of the complications of the disease itself, for example the body's normal response to infection

depletes nutritional stores. Furthermore metabolic stress responses cause catabolism of protein stores, consequently depleting protein stores (Drain *et al*., 2007; Zaneta *et al*., 2012). Studies have shown that the antioxidant system of the body is adversely affected in HIV infection, and changes in the activities of its components have been documented by several workers (Pasupathi *et al*., 2009). Other studies have also stated that ALT and AST activities usually increase in asymptomatic HIV sero-positive patients, signaling liver involvement in HIV infection. Patients especially at the final stage of AIDS may develop, HIV associated nephrophathy (HIVAN) which leads to an increase in their serum creatinine levels (Rudolf and Rodriguez, 2003). There are also changes in protein concentration especially reflecting a decrease in albumin and an increase in C-reactive protein (Drain *et al*., 2007; Sarro *et al*., 2010). The current treatment for HIV infection consists of highly active anti retroviral therapy. These drugs which are classified into, Nucleoside Reverse Transcriptase Inhibitors (NRTI), Non nucleoside reverse transcriptase inhibitors (NNRTI), Protease inhibitors (PT), and Fusion inhibitors (FI) were introduced in 1996 to improve the patients' quality of life, reduce HIV viraemia, and possibly prolong the life of the patient. They do not cure the patient of HIV or prevent the return once treatment is stopped (Drain *et al*., 2007).

Since infection with HIV resulted in the deterioration of the general well being of its patients, giving rise to many adverse biochemical changes, it became pertinent that research be geared towards examining the biochemical effects of HAART (Highly active antiretroviral therapy) with a view to determining whether they exert positive or negative effects on the parameters or systems under study, with respect to the population under consideration. A closer look at the micronutrient status of the patients following treatment became important as these are usually

adversely affected by the infection. Specifically, the trace elements, zinc and selenium which are involved in immune responses and in the antioxidant system (Kassu *et al*., 2006; Khalili *et al*., 2008; Muthuraj *et al*., 2010; Baum *et al*., 2010) also vitamins, C and E, which are antioxidant vitamins needed to be studied in these patients, following treatment in order to appreciate the effects of these drugs as these have also been observed to be affected negatively by the infection. Part of what propelled this work was an assertion by Drain *et al*. (2007) that there is paucity of data as regards determining whether HAART ameliorates micronutrient deficiency or to recommend or refute the benefit of providing micronutrient supplements to HIV-positive persons receiving HAART. Because of this, the workers recommended that future research should try to determine whether or not HAART initiation restores micronutrient concentrations. Based on the fore going, the aim of the research then is to assess the changes in the following antioxidant micronutrients: zinc, selenium, vitamin C and vitamin E in HIV positive patients on HAART.

2. MATERIALS AND METHODS

2.1 Subjects

Forty three (43) HIV/AIDS subjects, attending the AIDS clinic of Bishop Shanahan Hospital, Nsukka south east Nigeria and twenty (20) apparently healthy subjects who served as controls were recruited for the study. Informed consent was obtained from the participants and ethical clearance was sought for and obtained from Annunciation Hospital ethical clearance committee, Emene Enugu Nigeria.

2.2 Inclusion Criteria

HIV positive patients not yet on antiretroviral therapy but are due to be placed on it by virtue of their CD4+ counts (patients with CD4+ count 500/mm3 of blood and below were placed on HAART).

2.3 Design of the Experiment

All subjects were tested at presentation (after being confirmed positive), 4 months after the initiation of the antiretroviral therapy (Combivir N is the HAART in use in Bishop Shanahan Hospital, it contains zidovudine, lamivudine and nevirapine) and 8 months after. Their $CD4^+$ counts were also estimated, at all the presentations. The subjects were divided into 2 major groups, while group 2 is made up of 3 sub- groups as follows:

Group 1 (H1): HAART naïve subjects (forty three HIV positive individuals ripe for HAART initiation).

Group 2 (H2): The same HIV positive subjects four months into treatment with HAART.

Group 3 (H3): The same HIV positive subjects eight months into treatment

Group 4 (H4): Control (twenty apparently healthy individuals).

2.4 Specimen Collection, Processing and Storage

Venous blood, five milliliter (5 ml), was aseptically collected from each subject, three milliliter (3 ml) aliquot was allowed to clot and centrifuged at 3000 rpm for 5 minutes, to separate serum from erythrocytes. The serum was pipetted into a clean serum bottle and either analyzed immediately or stored at -4°C for a maximum of 48 hours. A two milliliters (2 ml) amount of the

sample was emptied into a sodium EDTA container for $CD4^+$ count. Retroviral screening was done using the requisite methods. CD4+ enumeration was done by the principle of flow cytometry using partec cyflow machine. The micronutrients were all assayed using Randox kits that utilize spectrophotometric methods.

2.5 Statistical Analysis

The results were presented as mean ± standard deviation. Differences between the results of the control subjects, and those of HIV positive subjects, before the commencement of HAART, 4 Months and 8 months into treatment, were analyzed using Student's t test. Effects of HAART on the biochemical parameters of HIV positive patients' were analyzed using ANOVA. Pearson correlation was employed in analyzing the relationship between $CD4^+$ count and the biochemical parameters.

3. RESULTS

Table 1 showed the mean ± SD of zinc and selenium concentration of control subjects (group 4) 795.50µg/dl (SD=30.70) and 4.97mg/dl (SD=8.08) were significantly higher than group 1, 2 and 3 HIV positive patients ($p< 0.05$). In the same table, mean± SD of zinc, selenium and CD4+ count of group 1 HIV positive patients; 160.64µg/dl (SD=47.69), 2.04mg/dl (SD=0.09) and 246.51 cells/mm^3 (SD=71.30) were significantly lower than group 2 and 3 HIV positive patients ($p< 0.05$). Mean± SD of CD4+ count of control subjects (group 4) 1023 cells/mm^3 (SD=45.40) was significantly higher than CD4+ count of group 1, 2 and 3 HIV positive patients ($p< 0.05$). The between group comparison of vitamin E showed no significant difference between the control subjects and HIV positive patients ($p> 0.05$). Mean± SD concentration of vitamin C in group 4

(control) 2.35mg/dl (SD=0.04) was significantly higher than group 1, 2 and 3 HIV positive patients. The between group comparison of vitamin C concentration among group 1, 2 and 3 showed no significant difference ($p > 0.05$) (Table 1). Only zinc was significantly positively correlated with CD4+count in this study.

4. DISCUSSION

The results of the work showed that the use of highly active antiretroviral therapy (HAART) led to a steady increase in CD_4^+ count from the HAART naïve stage to eight months into treatment. This agrees with the work of Ibe *et al.*, 2013, which showed that exposure to HAART led to an improvement in the $CD4^+$ count of HIV patients in south eastern Nigeria. The antioxidant micronutrients, zinc and selenium, showed significant improvement from baseline stage to eight months into treatment. The positive effect of HAART on zinc agrees with the work of Ndeezi *et al.*, 2010 which reflected that access to HAART in a population studied in Uganda, reduced the prevalence of zinc deficiency in this population while the effect seen in selenium concentration was also seen in the work of Akinjinmi *et al.,* 2013 which showed an improvement in the selenium concentration of a population of HIV positive subjects in Osun state once they started using HAART. Vitamin C on the other hand showed appreciable improvement between baseline stage and 4 months into treatment, but deteriorated by the eighth month. There is dearth of literature as regards the time dependent effect of HAART on the plasma vitamin C concentration of HIV positive individuals exposed to HAART, but the most likely thing should be a positive effect since HAART is supposed to improve the patient's condition. The fact the HAART is a drug that has to be metabolized with the resultant oxidative effect of such metabolism may explain the drop in the mean vitamin C concentration at the 8^{th}

month of HAART consumption. Vitamin E on its own showed no significant change in concentration during the period of treatment. This is similar to the observations made in a work reflected in a write up compiled by Drain *et al*., 2007 which showed that the population studied exhibited no significant differences between their mean vitamin E concentrations pre and post HAART. Positive correlation was observed only between zinc and $CD4^+$ count and this agrees with the work of Olaniyi and Arinola, (2007) which showed a positive correlation between zinc and $CD4^+$ count in a Nigerian HIV positive population that was involved in their study.

5. **CONCLUSION**

The commencement of HAART for the study population led to an increase in their CD_4+ count, zinc, selenium, and to some extent vitamin C, but there was no effect on vitamin E. All these are indicative of a positive prognosis with regard to HIV/AIDS infection which resulted from the initiation of HAART. Furthermore the effect of HAART on micronutrients like zinc and selenium can be used to monitor prognosis after the initiation of treatment.

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TABLE 1

Mean ± std of zinc (µg/ml), selenium (mg/ml), vitamin c (mg/ml), vitamin E (mg/ml) and CD4+ count (cells/mm^3) of HIV patients

Groups	zinc	selenium	vitamin C	vitamin E	$CD4^+$count
H1 (43)	160.64±47.69	2.04±0.09	2.01±0.63	0.82±0.93	246.51±71.30
H2 (43)	295.60±169.26	2.58±0.31	2.11±0.41	0.80±0.20	310.05±106.60
H3 (43)	633.02±103.63	3.46±0.45	1.98±0.47	0.97±0.36	319.02±138.68
H4 (20)	795.50±30.70	4.97±8.08	2.35±0.04	0.89±0.06	1023±45.40
F(P)value	180.2(0.00)	216.60(0.00)	0.67(0.51)	1.10(0.34)	5.65(0.00)
H1 vs H2	0.00*	0.00*	0.39	0.90	0.01*
H1 vs H3	0.00*	0.00*	0.81	0.23	0.00*
H2 vs H3	0.00*	0.00*	0.27	0.18	0.70
H1 vs H4	0.00*	0.00*	0.02*	0.74	0.00
H2 vs H4	0.00*	0.00*	0.02*	0.15	0.00*
H3 vs H4	0.00*	0.00*	0.00*	0.36	0.00*