Effect of Haart on Vitamin E Level of HIV Seropositive Women in Nigeria

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Abstract: The aim of this study is to determine the effect of highly active antiretroviral therapy (HAART) on the level of vitamin E in HIV seropositive women in their reproductive ages. These are women who notably have diverse experiences with HAART. The study was conducted in University of Benin Teaching Hospital (UBTH), a tertiary healthcare institution in mid-western part of Nigeria. Patients recruited into the study were women who have been confirmed HIV positive. Age ranges of 18 to 40years, to exclude women who have reached menopause, were considered. One hundred seropositive women with fifty HIV negative women of same age group as control, were recruited. The level of Vitamin E was obtained before commencement of HAART as baseline value. Monitoring was every three months for nine months period. Results showed a progressive rise in Vitamin E from the first month to the sixth month of therapy with a sharp drop in level by the ninth month. This phenomenon was observed in all the phases of menstrual cycle – follicular, luteal and even amenorrhea phases. **Conclusion:** Administration of vitamin E after the sixth month of therapy is strongly recommended since the level of vitamin E reduced subsequently in all phases.

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Introduction

Human immunodeficiency virus (HIV) is a retrovirus that attacks or infects essentially vital organs of the human immune system such as the CD_4^+T-cells, macrophages and the dendritic cells (Alimonti et al., 2003). These cells are required for the proper functioning of the immune system. HIV infection, if not treated, will progress to acquired immune deficiency syndrome (AIDS) and the median time of progression from HIV to AIDS is estimated at nine to ten years with median survival time after AIDS at only 9.2 months (Morgan et al., 2002). This condition leaves the individual susceptible to opportunistic infections and tumors (Morgan et al., 2002).

In the last two decades, there have been abundant reports in the literature on the HIV pandemic, and the treatment trials to reduce morbidity and mortality in people living with HIV/AIDS (Piwoz et al.,2004; Fawzi 2003;Harakeh et al., 1994). In recent times, the use of highly active antiretroviral therapy (HAART) for the treatment of HIV infected individuals have generated some research findings as regards its effects on micronutrients and some essential vitamins. This study aims to determine the effects of HAART on vitamin E, a major fat soluble antioxidant of the body, in reproductive aged seropositive women at different phases of their menstrual cycle.

Vitamin E is part of the immune system and has anticancer properties that maintains the integrity of cell membrane and essential for the maintenance and proper functioning of the heart, the skin and the sex organs in both males and females. Vitamin E also protects red blood cells and helps prevent destruction of Vitamins A and C. The determination of the effects of HAART on such an important vitamin that has both immune and regulatory properties in the body cannot be over emphasized especially on a target population of HIV seropositive women in their menstrual cycle and this is what makes this vitamin unique.

Materials and Methods

This study was done in University of Benin Teaching Hospital (UBTH) in Benin City, Nigeria. UBTH is a tertiary healthcare institution which has bed compliment of over eight hundred (800). It is a referral center for the whole state and the neighboring states. The subjects were patients confirmed HIV positive and referred to the infectious diseases clinic of UBTH for monitoring and treatment. One hundred

(100) of these HIV seropositive women between the ages of 18years and 40 years were recruited into the study. Fifty seronegative women of the same age group were used as control. Blood specimens were collected from these patients before and after initiation of HARRT therapy with all required ethical standards observed. Verbal and written consents were sought from willing patients before recruitment for the study using prepared forms which were attached to their case notes.

Ethical standards permission was obtained from the hospital management committee before commencement of the program. It is pertinent to stress that only willing patients were used in the study. These female patients selected were not above 40years old to eliminate menopausal women. Information on age, marital status, last menstrual period (LMP) were obtained with the weight and height measured with standard scales, using prepared data forms.

Blood collection was done using appropriate vacutainers. Separation of blood specimens using centrifuge was done as soon as registration was completed and the plasma stored in the refrigerator which is maintained at -70^{0}C.

Estimation of Vitamin E was according to method of Sauberlic et al. (1974). Based on the reduction of ferric ions to ferrous ions by tocopherols after xylene extraction of blood sample, the ferrous ions react with α-α-dipyridyl to give a red color which is measured at 520nm. To 1.5ml of plasma, standard or water (blank) is added 1.5ml absolute alcohol and 1.3ml xylene, bringing total volume to 4.3ml. These were mixed thoroughly for 2min and centrifuged for 10min.

0.25ml of each supernatant was placed in a fresh tube and 1ml of α-α-dipyridyl added and mixed. Reading was taken at 460nm using reagent blank. 0.33ml ferric chloride was then added to each tube and second reading taken immediately within 30 second at 520nm.

Calculation

$$\left[\frac{Test\ A_{520nm} - (0.29 \times A_{460nm})\ test}{Std\ A_{520nm} - (0.29 \times A_{460nm})Std}\right] \times 2 = (mg/dl)Vit.E$$

A_{520nm} − $absorbance\ \ at\ 520nm$
A_{460nm} - $absorbance\ \ at\ 460nm$
Ref. range = 5 – 18mg/dl

Statistical Analysis

Mean values and their standard error of means (SEM) were computed on Microcal origin 5.0 statistical software. Data analysis and graphic presentations were done using this package. Values are presented as Mean ± SEM and comparisons of the means were done using ANOVA and student t-test. ANOVA was used since number of samples (n) was greater than 50 and P-value <0.05 was considered significant for two independent variables.

Results

The baseline value of Vitamin E was significantly reduced when compared with the seronegative control women (Table 1 Fig 1.) The pattern of change at the baseline level shows that the highest depletion was at the luteal phase. At the follicular phase, HAART led to an initial drop by the third month of therapy with a sharp recovery at the sixth month (Fig.2). At the luteal phase, there was same drop by the third month with a significant elevation at the sixth month and was followed by a significant decrease by the ninth month (Fig.3).

The pattern of change in the amenorrhea phase was same as in the luteal phase (Fig.4). The comparative analysis shows that by the sixth month, all phases showed peak values of Vitamin E that became reduced significantly by the ninth month (Fig.5). The reductions observed in the study were below the negative control values.

Results were grouped into three different phases namely: Follicular (F), Luteal (L) and amenorrhea (A) phases for those experiencing loss of menstrual periods at any point. The following codes were used to denote the results as follows:

BT: Mean of baseline total (N = 100)
BF: Mean of baseline follicular phase (N = 53)
BL: Mean of baseline luteal phase (N = 38)
BA: Mean of baseline amenorrhea phase(N = 9)
F1: Mean follicular after 3months on HAART
F2: Mean follicular after 6months on HAART
F3: Mean follicular after 9months on HAART
L1: Mean of luteal after 3months on HAART
L2: Mean of luteal after 6months on HAART
L3: Mean of luteal after 9months on HAART
A1: Mean of amenorrhea after 3months on HAART
A2: Mean of amenorrhea after 6months on HAART
A3: Mean of amenorrhea after 9months on HAART

All monitoring were done after embarking on highly active antiretroviral therapy (HAART). Comparative analysis of the HIV-negative patients with the HIV positive patients (Baseline) that have not commenced therapy is shown in table 1.

Table 1: Commenced therapy

Parameter	**Negative Control (n = 50) Mean ± SEM**	**Baseline (n = 100) Mean ± SEM**	**Comparative Analysis Neg. Vs Baseline**
			P < 0.05 (significant)
Vitamin E	6.23±0.41	3.50±0.30	significant

Comparative analysis of HIV negative patients with HIV positive patients (Baseline) that have not commenced therapy.

NB: P < 0.05

The measured value of Vit. E was significantly reduced in HIV infection though.

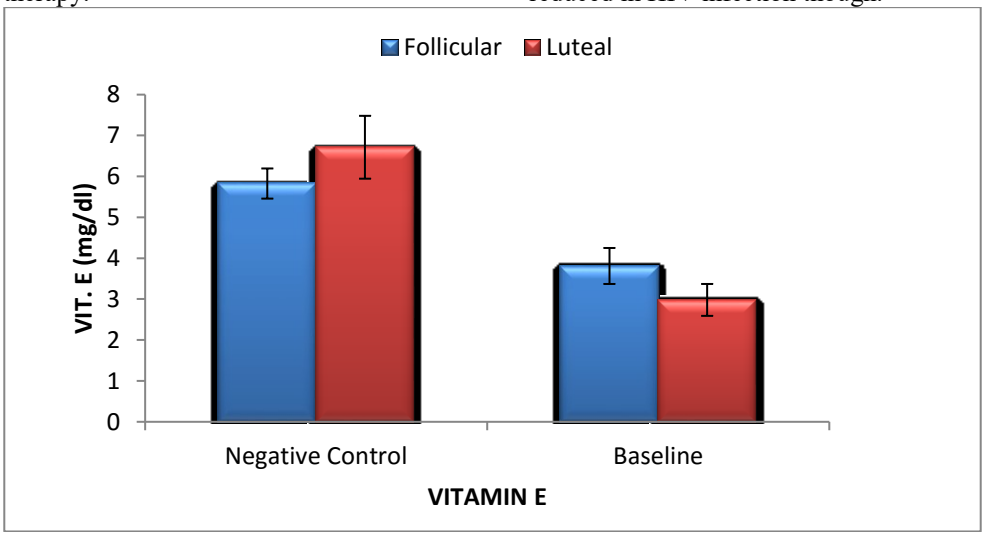

FIG. 1: Vitamin E Statistical Analysis Of Hiv Positive And Hiv Negative Controls At The Follicular And Luteal Phases.

HIV infection significantly reduced Vitamin E at both phases.

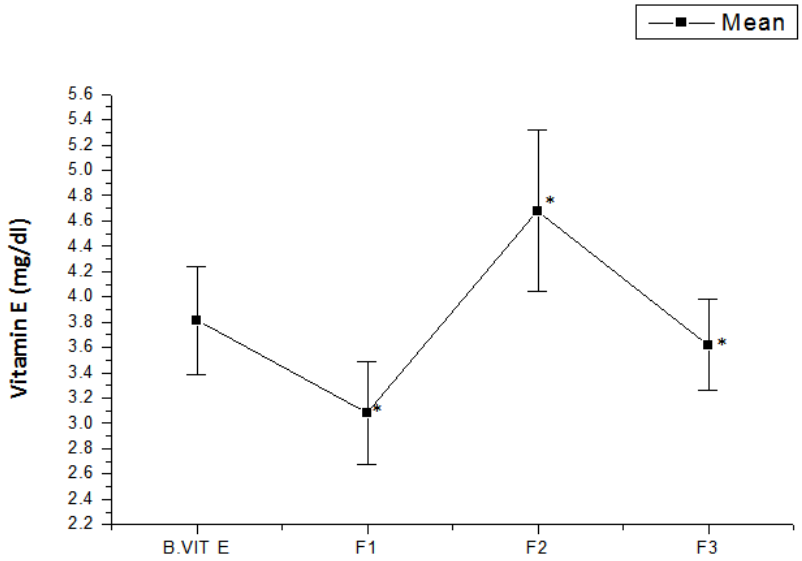

FIG. 2: Vitamin E Changes At Different Periods Of Haart Administration During The Follicular Phase.

There was a statistical significant decrease at the 1st follow-up, which later picked up statistically at the 2nd follow-up but decreased further by the end of the study period (9th month).

N.B: *P < 0.05
B.VIT. E: BASELINE VITAMIN E
F1: 1ST MONITORING AT 3-MONTHS
F2: 2ND MONITORING AT 6-MONTHS

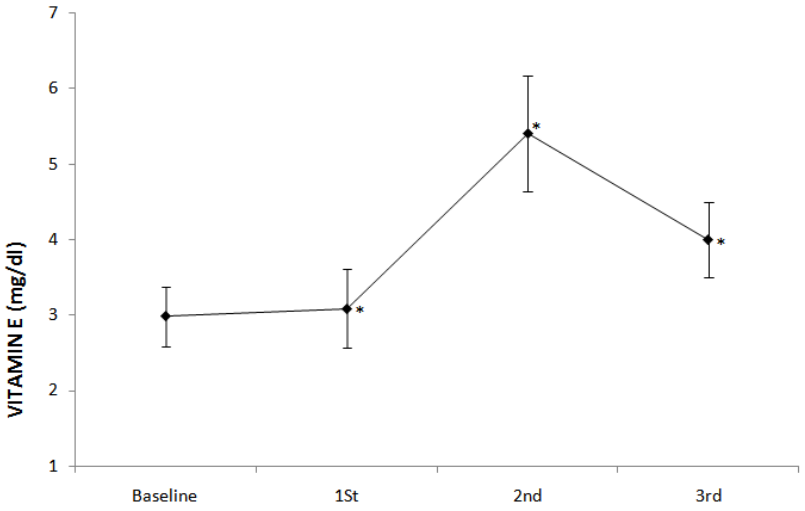

FIG. 3: Vitamin E Changes At Different Periods Of Haart Administration During The Luteal Phase.

There was a significant decrease recorded at the 3rd monitoring after peaking at the second monitoring.

N.B: *P < 0.05
B.VIT. E: BASELINE VITAMIN E
L1: 1ST MONITORING AT 3-MONTHS
L2: 2ND MONITORING AT 6-MONTHS
L3: 3RD MONITORING AT 9-MONTHS

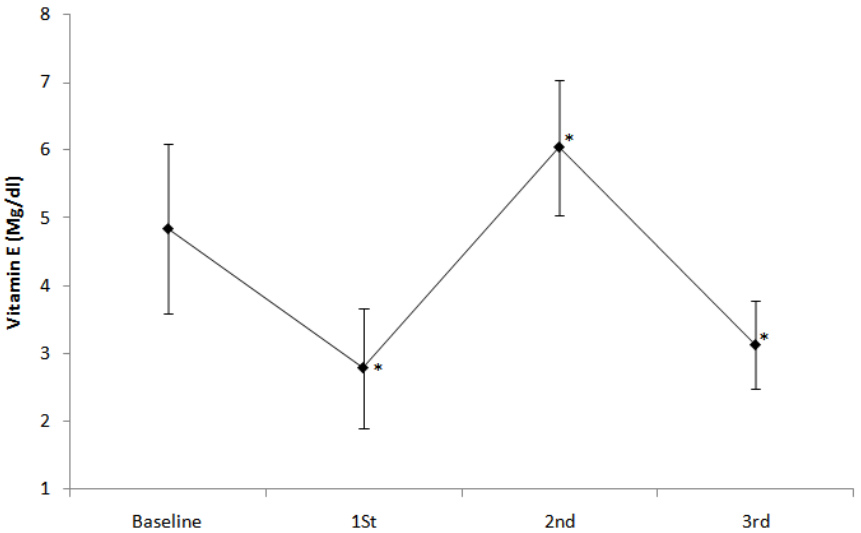

FIG. 4: Vitamin E Changes At Different Periods Of Haart Administration During The Amenorrhea Phase.

There were significant decreases recorded at the 1st and 3rd monitoring after peaking at the second monitoring.

N.B: *P < 0.05
B.VIT. E: BASELINE VITAMIN E
A1: 1ST MONITORING AT 3-MONTHS
A2: 2ND MONITORING AT 6-MONTHS
A3: 3RD MONITORING AT 9-MONTHS

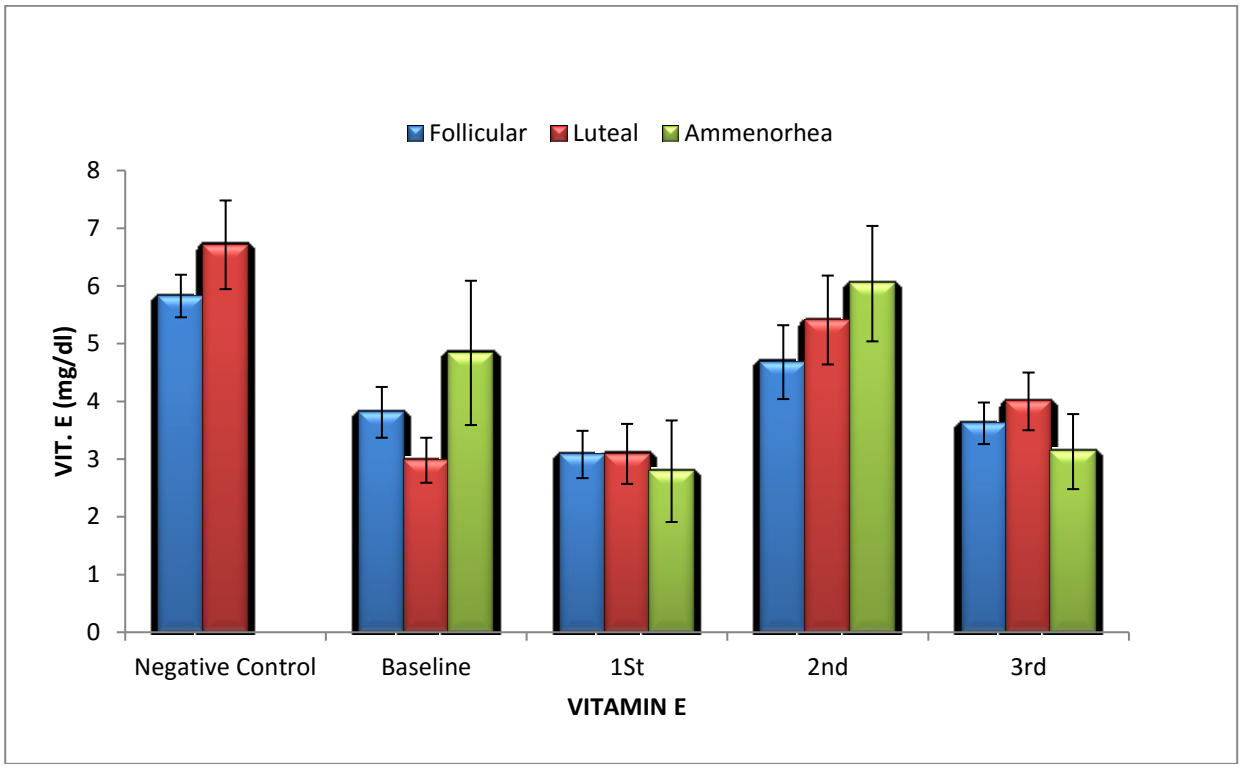

FIG.5: Vitamin E Statistical Analysis Of All Phases

There were statistically significant alterations in Vitamin E at the different phases and levels as HAART progressed. Note the significant reduction in all phases at the 3rd monitoring.

N.B: *P < 0.05
1ST MONITORING AT 3-MONTHS
2ND MONITORING AT 6-MONTHS
3RD MONITORING AT 9-MONTHS

Discussion

Vitamin E plays both immune and regulatory roles in the body (Sauberlic et al., 1974). It is a fat-soluble anti-oxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation (Jiamton et al., 2003), α-Tocopherol, the most biologically active form of Vitamin E has a regulatory effect on enzymatic activities (Herrera 2001). Vitamin E also has effects on gene expression and connective tissue growth factor (Herrera 2001) and plays a role in neurological functions (Schneider 2005) as well as inhibition of platelet aggregation (Vilar et al., 2002). Its most important function though is in antioxidant metabolism (Fawzi et al., 2004; Vilar et al., 2002; Muller 2010). Clinical studies have been conducted to test the health benefits of micronutrients, including Vitamin E, on AIDS patients (Fawzi et al., 2004; Herrera 2001; Atkinson and Epang 2008). The critical roles of vitamins and other trace elements have been established (Herrera 2001).

In this study, it has been established that HIV infection leads to a significant reduction in the levels of Vitamin E. This is in consonance with the work of various authors Piwoz et al., 2004; Fishman et al., 2004; Herrera 2001; Fawzi et al., 2004, Azzi, 2007). Subsequent follow-up results with HAART in this

study produced statistically significant increases in the level of Vitamin E in the first six months in all phases (Fig.5). It is to be noted however, that HAART generally led to decreased levels in all phases by the ninth month of monitoring (Fig.5).

Patients infected with HIV present with a progressive increase in the plasmatic levels of HIV-RNA and compromising of the cellular immune defense and system mainly as a result of apoptosis (Zingg and Azzi 2004).This phenomenon together with a deficiency in antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and gluthathione peroxidase and a decrease in the plasmatic levels of antioxidant vitamin E and C, carotenoids and selenium, may lead to oxidative stress and an accelerated progression to AIDS in HIV-seropositive patients (Fawzi et al 1998; Bradley et al., 2000). Reactive oxygen species (ROS) formed, cause damage to DNA through base modifications and deletions. Use of Vitamin E and C was shown to be effective in decreasing the levels of DNA base modifications in lymphocytes of HIV-seropositive patients (Fawzi et al, 1998).

In response to high levels of cytokines such as TNF-α, IL-1, IL-6 and IL-8, the cells of the immune system, principally neutrophils and macrophages, produce free radicals which may cause cell damage, triggering a cascade of events and consequently apoptosis. Apoptosis is a physiological form of cell death which performs an important role in homeostatic maintenance of tissues. However, under pathological conditions, such as HIV infection, the cell death levels are increased contributing to the weakening of the immune system and AIDS progression. This process is associated with structural alterations which include increased membrane permeability, chromatin configuration and DNA fragmentation as a result of the activation of endonucleases (Jaruga 1999).

Studies carried out using Vitamin E and C in association with ART showed increased cellular viability of lymphocytes and decreased levels of apoptosis of these cells in patients infected with HIV (Gill et al., 2003). Increase in pro-oxidant conditions in HIV along with increased apoptosis levels lead to increased viral replication through the activation of the nuclear transcription factor NF-kb (Jaruga 1999). NF-kb (NF Kappa b) is found in the cytoplasm, in its inactive form, bound to the inhibitory protein Ikb (I Kappa b). Factors such as TNF-α and ROS are capable of causing the disassociation of this complex.

Nutritional deficiencies, common in HIV-infection, render HIV-seropositive patients more susceptible to opportunistic infections and accelerate the progression to AIDS, along with reducing the success of ART. Function of the immune system is directly linked to the nutritional state of the patient. Nutrient requirement may be increased during acute and chronic infections, including HIV infection (Dror and Allen 2011). Supplementation with Vitamin E amongst other micronutrients can reduce the incidence of infections such as oral ulcers, oesophageal candidiasis and other inflammatory conditions (Herrera 2001). Vitamin E supplementation is also associated with increase in the expression of CCR5 co-receptor in HIV-subjects (Gill et al 2003).

Vitamin E, independent of its antioxidant action, α-tocopherol acts on the immune system decreasing HIV-replication through the regulation of such processes such as:

- Reduction in levels of the pro-inflammatory cytokines such as IL-IB and TNF-α via inhibition of the 5-lipoxygenase pathway.
- Reduction in the levels of cytokines such as IL-4, IL-5 and IL-6.
- Modulation of the production of prostaglandin E_2 (PGE_2) high levels of which decrease the production of IL-2, a cytokine important for the growth and differentiation of T and B cells. PGE_2 also inhibits the activation of natural killer (NK) cells, decreasing the production of interferon-γ, an important cytokine in cell-mediated immunity.
- Inhibition of interaction between endothelial cells and monocytes through the negative regulation adhesion molecules.

It is suggested that Vitamin E is capable of normalizing the alterations in immune functioning observed during HIV infection. Vitamin E supplementation is capable of re-establishing the plasmatic levels of this anti-oxidant, reducing oxidative stress, increasing cell viability and consequently reducing the risk of AIDS progression (Dror and Allen 2011).

Conclusion: Vitamin E plays a very vital role in the maintenance of the immune system and slows down progression to AIDS in HIV-infected patients. It is therefore necessary to encouraged vitamin E supplementation after the sixth month of HAART as the level of this vitamin decreases after the sixth month of HAART administration.

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