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Pre-treatment biochemical laboratory values in asymptomatic HIV-seropositive patients: Possible predictors of onset of HAART therapy.

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Abstract

Micronutrient deficiencies may be common during human immunodeficiency virus (HIV) infection. Malabsorption, diarrhea, impaired storage and altered metabolism can contribute to the development of micronutrient deficiencies. The present study is aimed at assessing pre-treatment biochemical parameters: serum iron, ferritin, TIBC, total protein, albumin, globulin, in addition to CD_4^+ cell count and viral load levels in asymptomatic HIV sero-positive patients. The test subjects comprised of 50 asymptomatic HIV sero-positive patients (25 males and 25 females) aged 18-35 years recruited from the PEPFAR unit, University of Nigeria Teaching Hospital (UNTH), Ituku Ozalla, Enugu State, whereas 60 apparently-healthy, age-matched, HIV-negative volunteers (30 males and 30 females) served as control subjects. There were significant increases (P<0.05) in total protein and globulin, with significant decreases (P<0.05) in albumin and CD_4^+ counts compared to the controls. There was also significant increase (P<0.05) in the CD_4^+ counts of the male test subjects, compared to females. The male and female test subjects, however showed significant increases (P<0.05) in total protein and globulin with significant decrease in the CD_4^+ counts when compared with their respective controls. The study suggests the need for checking pre-treatment biochemical parameters, in addition to viral load, in asymptomatic HIV patients, to help determine the appropriate time to initiate HAART therapy and also monitor disease progression.

Keywords: HAART, seropositive, pre-treatment, asymptomatic.

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Introduction

Human immunodeficiency virus (HIV) infection produces a slow but progressive and lethal immune suppression which can lead to opportunistic infections, neurologic disorders and malignancies. This results from the progressive depletion of the body's cells bearing the cluster of differentiation 4 ($CD4^+$) molecule particularly the helper/inducer subsets of T-lymphocytes, monocytes and macrophages [1,2,3]. Ultimately, the disease progresses to acquired immunodeficiency syndrome (AIDS), the terminal phase of the disease. With the advent of highly active antiretroviral therapy (HAART), viral replication can be effectively controlled and rate of disease progression decreased. Because the likelihood and timing of development of clinical AIDS following seroconversion, for any particular individual, are not readily predictable, the use of non-clinical disease markers has become critically important to patient management [4,5,6]. There have been several surrogate markers of HIV infection used for monitoring disease progression. Such markers include serologic T-cell activation markers like β2-microglobulin, neopterin, serum interleukin 2R (sIL-2R), and serologic B-cell activation markers like immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), and antibodies to HIV like anti-p24, anti-gp120 and anti-p17. Other antibodies used as HIV surrogate markers include: Lupus anticoagulant and anticardiolipin, antileukocyte antibodies, and anti $CD4^+$. Other serologic markers include tumour necrosis factor (TNF) and Acid-Labile Human Leukocyte. Other

groups of HIV surrogate markers include antigen markers such as p24 antigen as well as $CD4^+$ T cells and Cell surface activation markers [4]. Research is still going on to determine the best surrogate markers that can effectively predict the progress of HIV infection and the point of therapeutic intervention.

This study is therefore aimed at evaluating some biochemical parameters (nutrients such as serum iron, ferritin and protein) in asymptomatic HIV sero-positive patients in addition to viral load and CD_4^+ cell count as possible predictors of onset of HAART therapy in these patients, instead of using CD_4^+ cell count alone as practiced in most places.

The information obtained from this study, may also possibly lead to better understanding of the changes in micronutrient status with advancing HIV infection and the underlying mechanisms. This will go a long way to help enhance the health, quality of life and survival of those already infected with the virus.

Subjects and Method

Subjects

Fifty (50) asymptomatic HIV sero-positive patients (25 males and 25 females) aged between 18-35 years and yet to be placed on antiretroviral drugs at the PEPFAR Unit, University of Nigeria Teaching Hospital, UNTH, Ituku-Ozalla, Enugu, Nigeria were recruited for the study which lasted from January to May, 2010. The control subjects comprised of 60 age-matched, HIV negative volunteers (30 males and 30 females). Well-structured questionnaires were issued to the subjects.

The subjects were predominantly of the middle socio-economic class with very few of them in the high socioeconomic class. Their diet generally reflected an average balanced diet made up of common food items found in our environment, as captured in the questionnaire. The weight of the subjects was however not captured. Informed consent was given by all the subjects before they were recruited into the study. Approval was given by the institution's ethics committee before the commencement of the study.

Sample Collection and Preparation

Exactly 5 ml of whole blood was collected from the subjects, via clean vene-puncture from the ante cubital vein using sterile syringe and needle under aseptic conditions.

The blood samples were collected with subjects in the sitting position, without undue pressure to either the arm or the plunger of the syringe. After collection, 2 ml of blood were transferred into EDTA bottles and used for $CD4^+$ cell count. The remaining 3mls were transferred into plain bottles and allowed to clot and then centrifuged at 3000rpm for 5 mins to obtain the serum. The separated clear serum supernatants were then transferred into sterile tubes and used for the estimation of serum total protein, albumin, globulin, serum iron, total iron-binding capacity (TIBC) and viral load.

All the test subjects recruited for the study were asymptomatic HIV sero-positive patients who had not been placed on any medication (highly active antiretroviral therapy HAART). All subjects who had been placed on HAART medication were excluded from the study.

Analytical Methods

Serum iron, TIBC and Ferritin were estimated using the method of Tietz [7], whereas total protein, albumin and globulin estimations were done by Biuret reaction [7]. $CD4^+$ cell count was done by the method of Fryland *et al* [8] using partial flow cytometer while the viral load estimation was done using the method of Stevens *et al* [9].

Statistical Analysis:

The data was analyzed with the Statistical Package for Social Sciences (SPSS) version 11 and the difference in means was determined using two-tailed students t-test. Results were expressed as mean ± standard deviation (± SD).

Results

The results in table 1 show that statistically significant increases ($P<0.05$) were recorded in serum total protein (98.32 ± 14.8 vs 71.95 ± 16.3), globulin (57.68 ± 8.2 vs 57.7 ± 11.3), with decreases in albumin (36.92 ± 5.2 vs 46.6 ± 10.4) and $CD4^+$ count (1323.84 ± 45.8 vs 1611.75 ± 360.4) whereas non-significant differences were recorded in the serum iron, ferritin and total iron-binding capacity (TIBC) of the test subjects compared with the controls.

There was also significant increases in the $CD4^+$ cell count of the male test subjects compared with the female test subjects (656.3 ± 130.9 vs 546.7 ± 107.2) as shown in table 2. Further comparison of the male and female test subjects with their respective controls showed significant increases ($P<0.05$) in total protein (Males: 98.0 ± 8.12 vs 70.4 ± 17.4, Females: 85.65 ± 10.1 vs 70.2 ± 5.7) and globulin (Males: 57.7 ± 11.8 vs 24.45 ± 6.0, Females: 57.7 ± 11.3 vs 27.4 ± 5.7) whereas significant decreases were recorded in the $CD4^+$ count (Males: 656.3 ± 130.9 vs 1610.4 ± 371.3, Females: 546.7 ± 107.2 vs 1541.8 ± 341.4) of the male and female test subjects compared with their respective controls (table 3 and 4).

Table 1: Mean ± SD of the studied parameters in Asymptomatic HIV patients (test subjects) and Control Subjects.

Parameter	Test subjects (n= 50)	Control subjects (n= 60)	P-Value
Total Protein (g/L)	98.32 ± 14.8	71.95 ± 16.3	P<0.05 *
Albumin (g/L)	36.92 ± 5.2	46.6 ± 10.4	P<0.05 *
Globulin (g/L)	57.68 ± 8.2	26.35 ± 5.9	P<0.05 *
Iron (µg/dl)	127.76 ± 18.1	117.72 ± 26.3	P>0.05
Ferritin (µg/dl)	140.38 ± 19.9	132.81 ± 29.7	P>0.05
TIBC (µg/dl)	331.43 ± 46.9	331.76 ± 74.2	P>0.05
CD4 (Cells/ ml)	1323.84 ± 45.8	1611.75 ± 360.4	P<0.05 *
Viral load (Copies/ml)	62,889.41 ± 12837.2		

***Table 2*:** Mean ± SD of the studied parameters in Asymptomatic HIV patients (male and female subjects).

Parameter	Male test subjects (n=25)	Female Test subjects (n= 25)	P-Value
Total Protein (g/L)	98.0 ± 8.12	85.65 ± 10.1	P>0.05
Albumin (g/L)	35.0 ± 4.9	37.4 ± 7.3	P>0.05
Globulin (g/L)	57.7 ± 11.8	57.7 ± 11.3	P>0.05
Iron (µg/dl)	124.7 ± 25.4	130.6 ± 25.6	P>0.05
Ferritin (µg/dl)	140.2 ± 28.6	140.5 ± 27.6	P>0.05
TIBC (µg/dl)	334.3 ± 66.2	337.0 ± 66.3	P>0.05
CD4 (Cells/ ml)	656.3 ± 130.9	546.7 ± 107.2	P<0.05 *

Table 3: Mean ± SD of the studied parameters in Asymptomatic male HIV patients and male control subjects.

Parameter	Male test subjects (n=25)	Male control subjects (n=30)	P-Value
Total Protein (g/L)	98.0 ± 8.12	70.4 ± 17.4	P<0.05 *
Albumin (g/L)	35.0 ± 4.9	48.6 ± 11.5	P>0.05
Globulin (g/L)	57.7 ± 11.8	24.45 ± 6.0	P<0.05 *
Iron (µg/dl)	124.7 ± 25.4	119.7 ± 25.4	P>0.05
Ferritin (µg/dl)	140.2 ± 28.6	130.8 ± 26.5	P>0.05
TIBC (µg/dl)	334.3 ± 66.2	332.6 ± 73.4	P>0.05
CD4 (Cells/ ml)	656.3 ± 130.9	1610.4 ± 371.3	P<0.05 *

Table 4: Mean ± SD of the studied parameters in Asymptomatic female HIV patients and female control subjects.

Parameter	Female test subjects (n=25)	Female control subjects (n=30)	P-Value
Total Protein (g/L)	85.65 ± 10.1	70.2 ± 5.7	P<0.05 *
Albumin (g/L)	37.4 ± 7.3	41.4 ± 17.4	P>0.05
Globulin (g/L)	57.7 ± 11.3	27.4 ± 5.7	P<0.05 *
Iron (µg/dl)	130.6 ± 25.6	121.6 ± 27.4	P>0.05
Ferritin (µg/dl)	140.5 ± 27.6	133.8 ± 29.6	P>0.05
TIBC (µg/dl)	337.0 ± 66.3	330.7 ± 69.3	P>0.05
CD4 (Cells/ ml)	546.7 ± 107.2	1541.8 ± 341.4	P<0.05 *

Discussion

From the results obtained in the study, there was statistically significant increases (P<0.05) in the serum total protein and globulin whereas significant decreases were recorded in albumin and $CD4^+$ cell count in the test samples of asymptomatic HIV sero-positive patients when compared with the controls. Also, non-significant increases were recorded in serum iron and ferritin, while TIBC showed non-significant decreases.

However, the statistically significant decreases (P<0.05) in the $CD4^+$ count of the test samples when compared

with the controls, may not be unrelated to the protein status or fluctuation in the body total protein in such patients, as reported by Weinberg *et al* [10].

A comparison of the male and female test subjects in table 2, showed significant increases in the $CD4^+$ count of the males, whereas the rest of the studied parameters recorded non-significant differences. The reason for this sex-dependent variation is not yet clear. Further comparison of the male and female test subjects with their respective controls recorded significant increases ($P<0.05$) in total protein and globulin fractions whereas highly significant decreases were recorded in the $CD4^+$ cell count of the male and female test subjects compared with their respective controls (Tables 3 and 4). The other parameters also showed non-significant differences.

This is also not in agreement with the work done by Palella *et al* [2] on serum albumin level in association with survival in HIV-1 infected women where it was observed that albumin level may be a useful additional marker of HIV-1 disease progression, particularly among asymptomatic women with little or no evidence of immune-suppression. These results are however in agreement with the study by Figge *et al* [11], who reported that increase in globulin levels could be the normal response to infections.

The value obtained from the viral load estimation (62,889.41 ± 12,837.2) was significantly increased, compared with the value stated by Irwin [12], as the viral load at which antiretroviral therapy should be initiated. According to that study, the antiretroviral therapy should be started when $CD4^+$ count falls below 500 or to wait until it is below 350 when the viral load is above 20,000 copies per ml. In this study, although the $CD4^+$ count of the test subjects had not fallen below the stipulated marks, the recommended viral load levels were seen to have been increased by up to three-folds.

The viral load obtained in the asymptomatic sero-positive patients studied has shown that even in the absence of obvious symptoms of HIV, there is need for the viral load to be estimated, as this will possibly help to indicate when to start medication.

Conclusion

The study revealed significant rises in total protein and globulin, with significant decreases in albumin and CD_4^+ counts in the test subjects compared to the controls. Whereas CD_4^+ counts were increased in males compared to females, total protein, globulin and CD_4^+ counts varied significantly in the male and female test subjects compared with their respective controls. The hypoalbuminaemia may not be of predictive value to asymptomatic HIV sero-positive patients. However, the present study has shown that some biochemical parameters such as serum total protein and globulin concentrations, in addition to $CD4^+$ count and viral load, not only help to determine the severity or otherwise of the disease in asymptomatic patients, but could also be predictive on when to initiate HAART therapy.

References

1. Gottlieb MS, Schroff R, Schanker HM *et al*. Pneumocystic carinii pneumonia and mucosal candidiasis in previously healthy homosexual men. N Eng J Med 1981; 305: 1425-1431.
2. Hymes KB, Greene JB, Marcus A et al. Kaposi's sarcoma in homosexual men-a report of eight cases. Lancet 1981; 2: 598-600.
3. Ocheni S, Aken'Ova YA. Association between HIV/AIDS and Malignancies in a Nigerian tertiary institution. West African Journal of Medicine. 2004; 23(2): 151-155.
4. Tsoukas CM, Bernard NF. Markers predicting progression of human immunodeficiency virus-related disease. Clin Microbiol Rev. 1994; 7: 14–28.
5. Calabrese LH, Proffitt MR, Gupta MK, et al. Serum B_2-microglobulin and interferon in homosexual males: Relationship to clinical findings and serologic status to the Human T Lymphotropic Virus (HTLV-III). AIDS Res. 1984; 1: 423-438.
6. Gardner LI. Jr., Brundage JF, McNeil JG, et al. Predictors of HIV-1 Disease Progression in Early- and Late-Stage Patients: The U.S. Army Natural History Cohort. Journal of Acquired Immune Deficiency Syndromes. 1992; 5: 782-793.
7. Tietz NW. Fundamentals of Clinical Chemistry; W.B. Saunders publishers: Philadelphia 1987; pp 114-198.
8. Fryland M, Chaillet P, Zachariah R, Barnaba A, Bonte L, Andereassen R, Charrondiere S, Teck R, Didakus O. The Partec CyFlow Counter could provide an option for CD4 T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Trans R Soc Tropic Med Hyg 2006; 100: 980-985.
9. Stevens WS, Scott LM, Crowe SM. Quantifying HIV for monitoring antiretroviral therapy in resource poor settings. J Infect Dis 2010; 201(Supplement 1) S16-S26.
10. Weinberg GA. Iron overload as a mechanism for the lowered survival in AIDS patients receiving Dapsone-iron protoxalate for secondary prophylaxis of pneumocystis carinii pneumonia; J Infect Dis 1996; 174 (1): 241-242.
11. Figge J, Rossing TH and Fencl V (1991): The role of serum protein in acid-base equilibria. J Lab Clin Med; 453-461.
12. Irwin M. Low CD4+ T Lymphocyte Counts: A Variety of Causes and Their Implications to a Multifactorial Model of AIDS. Virusmyth Homepage, 2001. http://www.virusmyth.com/aids/hiv/milowcd4.htm. Accessed 12th September, 2012

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