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Research Article

Homocysteine Levels in Patients with Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome in Ibadan

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Abstract

Introduction: Homocysteine is produced from the conversion of methionine to cysteine. Conditions resulting in hyper homocysteinemia leads to an increased risk of both arterial and venous thromboembolisms by about 2 fold. 20% of HIV infected patients with objective evidence of venous thromboembolism are found to be thrombophilic with higher homocysteine levels. We enquired into homocysteine levels prior to the development of a clinical evidence of a venous thrombus in both HAART naïve and those on HAART of HIV /AIDS population. We evaluated the association between homocysteine, CD4 lymphocyte count and ART use in order to identify possible risk factors for hyper homocysteinemia in HIV population.

Method: Employing a cross sectional design; we compared mean plasma levels of homocysteine, full blood count parameters and CD4+ lymphocytes counts in HIV positive patients and HIV negative controls. One hundred and twenty patients with HIV infection attending the APIN clinic at the University College Hospital Ibadan and St Mary's Catholic Hospital Eleta Ibadan and one hundred and twenty-six HIV negative healthy controls were compared in the study.

Results: Fifty-nine point one percent of the HIV positive patient had hyperhomocysteinemia i.e. homocysteine levels of >18µmol/l. The mean plasma homocysteine levels were significantly higher at 24.4µmol/l (SD=13.8) (CI -2 to -8; p=0.002) in the HIV positive group compared with 19.5µmol/L (SD=10.6) in the control group. The use of Anti-retroviral drugs was not associated with higher homocysteine level in the seropositive group and neither were factors like age, gender or the use of combined oral contraceptive pills. There was no correlation between CD4 cell count and homocysteine levels (r= -0.01; p=0.9).

Conclusion: Homocysteine levels are elevated in HIV positive patients and hyperhomocysteinemia was found in a significant number of HIV positive patients. None of the patients investigated had features of thromboembolism or outright deep venous thrombosis. Neither CD4 cell counts nor traditional risk factors were associated with the higher homocysteine levels**.**

Background:

McCully observed arterial lesions in children with inborn errors of methionine metabolism and homocysteinemia. He proposes a causal relationship between homocysteine and vascular disease. (1) Homocysteine (HCY) is produced from methionine which when converted to S-adenosylmethionine (AdoMet) acts as a universal methyl donor. During methylation reactions, AdoMet is converted to S adenosylhomocysteine (AdoHcy), which is hydrolyzed to adenosine and homocysteine (Hcy) (2). The Hcy formed is reconverted back to methionine or into cysteine in trans sulfuration and remethylation reactions or alternatively metabolized to the cyclic thioester Hcy-thiolactone (3). The Hcy-thiolactone pathway becomes predominant when remethylation or trans sulfuration reactions are disturbed by genetic alterations of enzymes such as cystathionine β-synthase, methionine synthase, or methylenetetrahydrofolate reductase or by inadequate supply of folate, vitamin B-12, or vitamin B-6. The HCY-thiolactone forms amide bonds with ε-amino groups of protein lysine

residues. (4). It is the high chemical reactivity of homocysteine-thiolactone that induces endothelial dysfunction. (5, 6) It reacts with plasma coagulation proteins and alter their function *in vivo*, modifying factor V and inhibiting activated protein C cleavage (7,8) The sulfhydryl group of the homocysteine thiolactone further mediates disulphide bond formation between fibrinogen and albumin. This cross linking alters polymerization of fibrin strands essential in the formation of stable fibrin clots. (9-11) Hyperhomocysteinemia is relatively common in HIV /AIDS patients and it has been found to directly increase venous thrombosis in HIV /AIDS. Hyperhomocysteinemia is an independent risk factor for both venous and arterial thrombosis in patients between the ages of 20 and 70 years. It is also an independent risk factor for cardiovascular disease (12,13). Patients who were commenced on HAART especially those on protease inhibitors have been found with a higher proportion of hyperhomocysteinemia compared with the HAART naïve. Advancing age is also associated with rising plasma homocysteine levels. With this background information, we set out to investigate the homocysteine levels amongst our HIV positive cohort and to discover if any an association between homocysteine levels, use of HAART and traditional risk factors of hyperhomocysteinemia such as age, gender and smoking history in our HIV group.

Materials and Methods

Study setting and study population: One hundred and twenty diagnosed HIV positive patients were recruited and selected consecutively into the study from the AIDS Prevention Initiative Nigeria (APIN) Clinic at the University College Hospital, Ibadan, Nigeria and St Mary's Catholic Hospital Eleta, Ibadan, Nigeria. Asymptomatic patients with a confirmed diagnosis of HIV infection were included in the study group. Pregnant female and patients with current clinical evidence and or Doppler ultrasound diagnosis- at any time- of deep venous thrombosis were excluded from the study. Control samples were obtained from 126 healthy adult HIV-negative blood donors (both replacement and volunteer) at the blood bank of the University College Hospital, Ibadan. They were recruited by consecutive sampling method.

Study design: The study was cross sectional in design and the subjects were recruited consecutively.

Sample size estimation

The sample size estimation by the formula

$$\mathbf{N}^{87} = \frac{2(Z\alpha + Z\beta)^2\,\sigma^2}{(\mu_1 - \mu_2)^2}$$ therefore

$$N = \frac{2(1.96+0.84)^2 1.7^2}{(0.72)}$$

$$=92.8$$

Where

$Z\alpha$= 1.96(standard normal deviate corresponding to 5% level of significance)

$Z\beta$= 0.84 (standard normal deviate corresponding to 80% power)

σ = standard deviation of Homocysteine=1.7µmol/l (14)

μ_1-μ_2= (minimum difference in mean to be detected)

Conservatively, one hundred and twenty subjects were recruited for the study.

Sample collection and analysis: Ten milliliters of venous blood was collected, using standard phlebotomy techniques, from each subject and control. Five milliliters was transferred to a dipotassium EDTA bottle and was used for both full blood count and CD4+ lymphocytes count. Both tests were performed within 2 hours of sample collection. The remaining five milliliters of blood was transferred to a third bottle containing dipotassium EDTA. The sample was centrifuged and the plasma obtained kept at -20ºC until analysis. The peripheral full blood count obtained from the anticoagulated samples of patients were determined using Cell-Dyne 1200 Haematology Analyzer (Abbott Diagnostics, North Chicago, IL, USA). The CD4+ T cell lymphocyte count was determined by immunofluorescence using the Cyflow cytometer analyzer (Partec©, Germany). The plasma homocysteine level was determined by a sandwich enzyme linked immunosorbent assay (ELIZA) method using EIA HOMOCYSTEINE FHCY100/FHCY200 (Axis –Shield Diagnostics Ltd, the Technology Park Dundee, United Kingdom). It was read by the Multiskan Ex microplate reader from Labsystems.

Statistical analysis: Student t- test was used to compare the mean of plasma homocysteine levels between the two groups. Correlation testing was carried out using the Pearson's correlation test on plasma homocysteine levels and full blood count parameters. Hyperhomocysteinemia is defined as plasma homocysteine levels greater than 18μmol/L. ANOVA was used to determine the association between age, use of hormone contraceptive, cigarette smoking, duration of illness and homocysteine levels in the HIV positive group.

Results

A total of two hundred and forty-six participants were studied consisting of one hundred and twenty HIV positive subjects and one hundred and twenty-six healthy controls. Out of a total of 120 adult HIV positive individuals (study group), 55.8% were males while 44.2% were females. Females' age range between 25 and 65 years and males range between 22 and 65 years. The mean age was 32 ± 7 years. Out of a total of 126 adult HIV negative blood donors (control group), 39.7% were females and 60.3% were males. Mean age was 37 ± 9 years. The average years of living with HIV/AIDS among the study group was 4 years (range 6 months-7 years). Sixty-two of the one hundred and twenty participants in the study group were on HAART, 40 of whom were on NNRTI and the remaining twenty-two on Protease inhibitors. All of the participants had HIV 1. Presence of risk factors for hyper homocysteinemia differed in both groups of participants for example, the number of people that smoke cigarettes are higher in the HIV seronegative group but the use of Oral contraceptives was higher in the HIV positive group (table 1). Hematological parameters also differed significantly in both groups. Table 2 contains the hematological parameters of both groups. The platelet counts and MCV were found to be significantly higher in the HIV positive group compared with the seronegative group (p =0.00 respectively). The haemoglobin concentration and haematocrit were significantly lower (p= 0.00 respectively) in the HIV positive group.

The plasma homocysteine level in HIV positive was 24.4 μmol/L (13.8) and the plasma homocysteine level for HIV negative was 19.5μmol/L (10.6) (CI -2 to -8 p=0.002). The results are presented on table 3. The frequency and percentage of hyperhomocysteinemia (plasma levels > 18 μmol/L) in both study and control groups was 61(48%) and 71(59.2%) respectively. (table 4) The homocysteine levels were higher in those on HAART compared with HAART naive. (table 5)

There was no association found between traditional risk factors (age, sex, use of hormonal contraceptives, cigarette smoking) and plasma homocysteine levels amongst the HIV positive group. The results were presented in Table 6. There was no correlation between plasma homocysteine and CD4+ T cell count (r = -0.01 p=0.9). The mean CD4 count of HIV positive patients with hyperhomocysteinemia is 453 cells / μl (SD= 234). (table 7) Approval was obtained from the University of Ibadan Research and Ethics Committee as well as the Eleta hospital ethics committee

Table 1: Clinical characteristics of the HIV positive and HIV negative groups

Variable	HIV positive Count(%) N=120	HIV negative Count (%) N= 126	χ^2 /fisher's exact test	P value
History of use of hormonal contraceptive	1(0.8)	0(0)	0.5	0.5
Positive history of chronic renal failure	0(0)	1(0.8)	1.0	0.5
Smoking of cigarette	3(2.5)	6(4.7)	0.5	0.3

Table 2: Blood counts and red cell indices of HIV positive and HIV negative groups

Blood Indices	Study n = 120 mean(±SD)	Control n = 126 mean(±SD)	T statistics	P value
WBC ($\times 10^9$/L)	5.3(2.3)	5.2(1.5)	-0.35	0.73
Lymphocyte ($\times 10^9$/L)	2.1(1.0)	2.2(0.7)	0.19	0.86
Monocyte	0.6(0.3)	0.5(0.3)	-1.63	0.11

(×10^9/L)				
Neutrophil (×10^9/L)	2.5(1.9)	2.5(1.0)	-0.24	0.81
Hgb(g/L)	114.4(18.2)	123.4(16.1)	4.11	0.00*
PCV (%)	35.8(5.5)	38.5(4.5)	4.38	0.00*
MCV (fL)	96(10.8)	87(6.7)	-7.90	0.00*
MCH (pg.)	30.6(4.0)	28.0(3.0)	-6.04	0.00*
MCHC (g/L)	319(10.7)	319(11.9)	-0.29	0.77
Platelet (×10^9/L)	232(119)	199(50)	-2.84	0.00*

*statistically significant

Table 3: Homocysteine levels (µmol/L) in the HIV positive and HIV negative groups.

	HIV positive mean(sd)		HIV negative mean(sd)	Test	CI	P value
Homocysteine (µmol/L)	24.4 (13.8)		19.5 (10.6)	-2.4	-2-8	0.002*
	n = 120		n = 126			

Table 4: Hyperhomocysteinemia in the HIV positive and HIV negative groups

Homocysteine	HIV-neg	HIV-pos	Total	χ^2	P value
>18µmol/L	65	71	136	1.3	0.1
<18µmol/L	61	49	110		
Total	126	120			

Table 5: Hyperhomocysteinemia in the HIV positive on highly active antiretroviral therapy

	Homocysteine							
	>18 µmol/l	<18 µmol/l	Total	χ^2	p-val	Mean (sd	p-valu	
HAART-naïve	27	31	58	7.3	0.07	22.5(15.5	0.00	
HAART	44	18	62			26.4(12.8		
Total	71	49						

Table 6: Association between age, use of hormone contraceptive, cigarette smoking, duration of illness and homocysteine levels in the HIV positive group

Variables	n(%)	Mean levels(sd)	P value
Sex			0.5
Male	67(55.8)	23.3(13.8)	
Female	53(44.2)	25.6(14.2)	
Use of hormonal contraceptives			0.2
Yes	1(0.8)	14.4	
No	119(99.2)	24.6(14.2)	
History of smoking cigarettes (now or in past 20 years)			0.6
Yes	3(2.5)	30.2(27.7)	
No	117(97.5)	24.3(14.6)	
Duration of illness			
Less than 1-3 years	87(73)	23(14.9)	0.6
4-7 years	33(27)	24(11.8)	
Age			
<40 years	82(68.4)	22(13)	
>40 years	38(31.6)	28(14)	0.4

Table 7: Association between homocysteine levels and CD4 cell counts in HIV positive group

	> 18 µmol/l	<18µmol/l	R	P
CD4 cell count Mean (sd)	453(234)	439.1(219)	-0.01	0.9

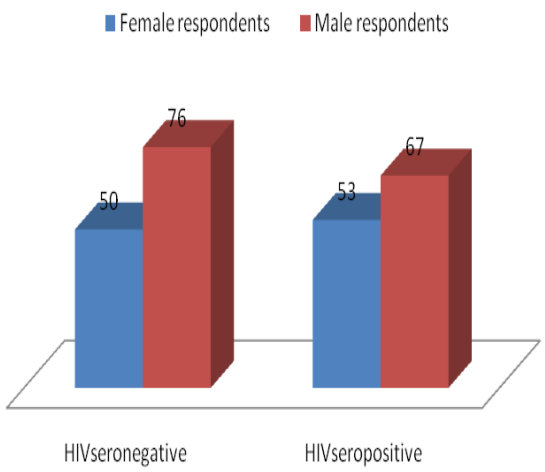

Fig 1: The number of male and female respondents in both HIV positive and HIV negative group

Discussion:

Hypercoagulabilty has been reported in several studies of HIV/AIDS patients since the year 2000.(14) These include studies by Kiser et al (15) and Jong et al (16) both in 2010 and Musselwhite et al (17) in 2011. They carried out studies conducted to identify factors responsible for the hypercoagulable state and homocysteine is a major finding in many of these studies. We studied homocysteine in our population of HIV positives and looked for risk factors as a step in identifying risk factors in our patient population. Plasma homocysteine levels were significantly higher in the HIV seropositive population than seronegative controls in this study; 24.4 µmol/L (13.8) vs 19.5µmol/L (10.6). The prevalence of hyperhomocysteinemia amongst our HIV population is 59.1%. This was similar to a study done by Abdollahi and Shoar in 2012 who assayed homocysteine in HIV living in Iran(18). They reported a prevalence of 91% hyperhomocysteinaemia in a cohort of 58 HIV positive Iranian individuals. They postulated that hyperhomocysteinemia in the HIV positive group was as a result of the effects of HIV replication on host metabolism. In their study, higher homocysteine levels found were associated with older individuals. This study did not however, find any association between age and plasma homocysteine levels. It is important to note, however, that the mean age of his respondents was higher in that study compared with the ages in this study and this could account for the differences found. CD4+ T cell counts are the laboratory parameters used widely to classify and stage HIV/AIDS by the CDC (19). In the current study, there was no statistical correlation between the CD4+ T cell count and homocysteine. But we found that the CD 4 cell count among the patient with hyperhomocysteinemia was only slightly higher than that found with normal homocysteine levels. Abdollahi and Shoar (18) could not find any association between CD 4 cell counts and homocysteine levels either. They surmised that the stage of HIV infection does not appear to be correlated with the plasma homocysteine level. This can have potential implications for intervention.

Farbod et al in 2010 (20), another investigator of homocysteine in HIV infected patients, on the other hand, did not find any significant difference in the plasma homocysteine levels in a cohort of 249 women infected with HIV at the Bronx Women's Interagency HIV study site in the USA when he compared them with HIV negative controls. That study instead reported strong positive associations between older ages, a lower CD4+ T cell and higher plasma homocysteine levels irrespective of their HIV status. We found instead the mirror opposite in our study. The Bronx study differed from our study in that their entire population was made up of targeted group-middle aged females while this study was carried out amongst the young adults of both sexes. The CD4+ T cell counts in both studies were comparable. the patient populations of our study and the Bronx study have completely different population characteristics which accounts for the different outcome. Demince et al (22) performed a meta-analysis on 16 studies and found that the HIV group that were on HAART had a higher mean homocysteine level than the HIV HAART naïve group. they noted that the difference in plasma homocysteine levels between patients on anti-retroviral and those that were HAART naive was 4.13µmol/l. In our study, the patients on HAART had a higher homocysteine level compared with the HAART naive. This may be explained by the presence of patients who are on protease inhibitors which have been known to cause elevation in homocysteine levels. We found no significant association between homocysteine and traditional risk factors for hyperhomocysteinemia (age, smoking of

cigarettes, use of oral contraceptives) but this was in keeping with knowledge on homocysteine levels in HIV. HIV is an inflammatory condition, characterized by the perturbation of many metabolic pathways in the body. Thus traditional risk factors will exert may have less influence on pathogenesis of hyperhomocysteinemia in that particular population.

Conclusion:

Plasma homocysteine level is high amongst the HIV seropositive cohort and the majority of them had hyperhomocysteinemia.

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