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SOME INFLAMMATORY AND CARDIOVASCULAR MARKER LEVELS IN HUMAN IMMUNO – DEFICIENCY VIRUS SEROPOSITIVE INDIVIDUALS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY WITH HYPERTENSION IN NNEWI

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ABSTRACT

Background: Human Immunodeficiency Virus (HIV) is one of the world's most serious health and development challenge. Cardiovascular complications are more common in human immunodeficiency virus infected individuals than in age matched uninfected individuals. **Aim**: This study was designed to evaluate some inflammatory and cardiovascular markers in HIV seropositive individuals on HAART with hypertension in Nnewi as a marker of inflammatory response and fibrinolytic activity in these subjects. **Materials and methods:** A total of 150 subjects were recruited and grouped using systematic sampling technique into 3 groups of 50 subjects each. Group1 were HIV seropositive individuals on HAART with hypertension, group2 were HIV seropositive individuals on HAART without hypertension. Control group were recruited among Nnamdi Azikiwe University Teaching Hospital HIV negative staff and students without hypertension. Interleukin – 6, D-dimer and CRP were estimated using enzyme-linked immunosorbent assay method, BMI was calculated using the formular: BMI (kg/m^2) = Weight (kg) divided by Height (m^2). **Results and conclusion:** The study revealed significant higher levels of CRP (p=0.03) in HIV seropositive individuals on HAART with hypertension than in HIV seropositive individuals on HAART without hypertension, while IL-6, D-dimer and values of BMI showed similar mean values. There were significant higher levels in CRP (p=0.00) and D-dimer (p=0.02) with a significant lower levels in BMI values (p=0.00) in HIV seropositive individuals on HAART with hypertension than in HIV negative controls without hypertension, while IL-6 showed similar mean values. These levels might indicate increased inflammatory response and fibrinolytic activity in HIV infection.

KEYWORDS: Human Immunodeficiency Virus; Cardiovascular disease; IL-6; D-dimer; CRP; BMI.

1. INTRODUCTION

Human Immunodeficiency Virus is one of the world's most serious health and development challenge. There were approximately 36.7 million people worldwide living with HIV/AIDS at the end of 2015, of these, 18.2 million were children (less than 15years old). As of June 2016, 18.2 million people living with HIV were accessing antiretroviral therapy (ART) globally, up from 15.8million in June 2015, 7.5 million in 2010 and less than 0ne million in 2000.[1] With effective antiretroviral therapy, there has been a significant increase in long term survival rate and life expectancy in HIV-infected adults.[2] Even with this success, there is increased risk of non-AIDS related co-morbidities such as cardiovascular disease.[3] Hypertension is a major risk factor of cardiovascular disease development with prevalence of hypertension being higher in HIV-infected individuals even with the viral suppression achieved with ART.[4] Some studies on HIV infection reported higher levels of inflammatory and cardiovascular biomarkers such as CRP, IL-6, and D-dimer.[5,6,7,8] The persistent inflammation associated with HIV infection could be due to immune dysregulation, occult viral replication and coagulopathy.[9] Studies measuring levels of these inflammatory and cardiovascular biomarkers in HIV seropositive individuals on HAART with hypertension are limited in Nigeria. Therefore, this study was designed to determine the levels of some inflammatory and cardiovascular markers in HIV seropositive individuals on HAART with hypertension. This will add to the existing information on risk of cardiovascular disease in HIV-infected individuals on HAART.

2. MATERIALS AND METHODS

This was a cross-sectional study conducted at Nnamdi Azikiwe University Teaching Hospital in Nnewi, Anambra State, Nigeria to evaluate some inflammatory and cardiovascular marker levels in human immuno – deficiency virus seropositive individuals on highly active antiretroviral therapy with hypertension. A total of 150 subjects (aged between 18 and 75 years) were systematically studied. Among these were 50 HIV seropositive individuals on HAART with hypertension, 50 HIV seropositive individuals on HAART without hypertension and 50 apparently healthy individuals (screened for HIV and blood pressure measured). Ethical approval was obtained from Nnamdi Azikiwe University Teaching Hospital Ethics Committee (NAUTHEC). Informed consent was obtained from all subjects before the commencement of the study.

2.1. Inclusion criteria

Already diagnosed HIV positive patients on HAART with or without hypertension between the age of 18 and 75 with CD4+ T cell levels > 200cells/mm^3 were recruited for this study.

2.2. Exclusion criteria

Pregnant subjects, subjects within the age bracket of 10 - 17 years, subjects with other chronic diseases aside HIV were all excluded from this study.

Anthropometric measurements, blood pressure were measured and CD4 + T cell count noted. Venous blood sample, (5ml) volume was collected from each subject using 5.0ml sterile disposable syringe and dispensed into 5ml plain sample containers all labelled with the subject's name, age and sex. The blood in the plain containers was spun for 5minutes at 3000 rpm after allowing the blood to clot for 30minutes and the serum was separated from the red cells using a dry clean Pasteur pipette into a dry clean plain specimen container. The serum samples were used to measure IL-6, D-dimer and CRP using enzyme-linked immunosorbent assay methods as described by Engela.[10]

The data from this study were subjected to statistical analysis using a computer program known as Statistical Package for Social Science (SPSS), version 22. The results of the test groups were compared within groups using ANOVA and between groups using student's t-test at 95% level of confidence.

3. RESULTS

In table 1, when HIV seropositive individuals on HAART with hypertension were compared with HIV seropositive individuals on HAART without hypertension, IL-6 and D-dimer serum levels and values of body BMI showed similar mean values whereas CRP showed significantly higher levels in HIV seropositive individuals on HAART with hypertension. When HIV seropositive individuals on HAART with hypertension were compared with HIV negative controls without hypertension, CRP and D-dimer serum levels showed significantly higher values in HIV seropositive individuals on HAART with hypertension, IL-6 showed similar mean values while BMI values were higher in HIV negative controls without hypertension.

Table 2 shows that in HIV seropositive individuals on HAART with hypertension and in control group, levels of IL-6 showed strong positive significant relationship with D-dimer. But in HIV seropositive individuals on HAART without hypertension, IL-6 show insignificant positive correlation with D-dimer.

Table 1: Mean ± SD serum level of IL-6 (pg/ml), serum level of CRP (mg/dl), serum level of D-dimer (ng/ml) and value of BMI (kg/m^2) among HIV seropositive individuals on HAART with hypertension, HIV seropositive individuals on HAART without hypertension and subjects without HIV or hypertension (Controls) compared using ANOVA and Post hoc.

	IL-6 Pg/ml	CRP mg/dl	D-dimer ng/ml	BMI kg/m^2
1. HIV positive on HAART with HBP	5.30±1.61	0.89±0.13	118.1±31	22.3±1.0
2. HIV positive on HAART	5.02±1.01	0.81±0.02	117.6±27.1	23.3±5.0
3. Subjects without HIV or HBP controls	4.77±0.86	0.006±0.001	103.2±21.9	25.7±4.3
F(P-value)	2.43(0.09)	613.9(0.00)	4.95(0.01)	10.7(0.00)
1vs2 P value	**0.56**	**0.03[a]**	**1.0**	**0.30**
1vs3 P value	**0.11**	**0.00 [a]**	**0.02[a]**	**0.00 [a]**

KEY: [a] = significant at p≤0.05

F(P)= Mean ± SD of parameters compared among HIV seropositive individuals on HAART with hypertension, HIV seropositive individuals on HAART without hypertension and control subjects using ANOVA.

1 vs 2 P value = Mean±SD of parameters compared between HIV positive on HAART with HBP[1] and HIV positive on HAART[2] using t test

1 vs 3 P value = Mean±SD of parameters compared between HIV positive on HAART with HBP[1] and Subjects without HIV or hypertension[3] (Control) using t test

Table 2: Correlation between serum level of IL-6 (pg/ml), CRP (mg/dl), D-dimer (ng/ml) and value of BMI (kg/m^2) in HIV seropositive individuals on HAART with hypertension, HIV seropositive individuals on HAART without hypertension and subjects without HIV or hypertension.

	HIV positive on HAART with HBP		HIV positive on HAART without HBP		Control subjects	
Variables	**(r)**	**P-value**	**(r)**	**P-value**	**(r)**	**P-value**
IL-6 vs CRP	-0.06	0.71	-0.22	0.13	0.12	0.39
IL-6 vs D-dimer	**0.72**	**0.00**[a]	0.007	0.96	**0.69**	**0.00**[a]
IL-6 vs BMI	-0.05	0.74	-0.24	0.10	0.15	0.30
CRP vs D-dimer	-0.10	0.50	0.13	0.37	**0.28**	**0.05**[a]
CRP vs BMI	-0.08	0.60	0.20	0.16	-0.27	0.06
D-dimer vs BMI	-0.08	0.60	-0.01	0.93	0.21	0.14

Key: r= Pearson correlation coefficient, [a] = significant at $p \leq 0.05$

4. DISCUSSION

This study revealed significant higher value in CRP mean serum level among HIV seropositive individuals on HAART with hypertension when compared with HIV seropositive individuals on HAART without hypertension. Since CRP is an inflammatory marker and hypertension being a risk factor for cardiovascular disease development, this observation may suggest that inflammation plays a role in the pathogenesis of cardiovascular disease in HIV-infected population. This finding is in line with studies that reported that HIV-infected adults with hypertension have higher risk of cardiovascular events and all- cause mortality than HIV-infected adults with normal blood pressure or HIV un-infected adults with hypertension.[11,12] D-dimer serum level showed similar mean values in HIV seropositive individuals on HAART with hypertension when compared with HIV seropositive individuals on HAART without hypertension. This may suggest that cardiovascular risk in HIV-infection is independent of hypertension as a traditional risk factor of cardiovascular disease development. Body mass index (BMI) showed similar values in HIV seropositive individuals on HAART with hypertension compared with HIV seropositive individuals on HAART without hypertension. However, BMI was elevated in control subjects who are HIV –uninfected without hypertension when they were compared with HIV-infected individuals on HAART with hypertension. This suggests that HIV infection and its pathogenesis was associated with lower BMI which is evident with the weight loss seen in HIV infection reflecting HIV-related wasting syndrome. This finding is in line with the work which reported that BMI was similar in HAART treated and HAART naïve patients but elevated in HIV negative controls compared to HAART treated patients.[13] Another work reported that HIV infection was associated with a lower BMI and a lower prevalence of obesity compared with HIV negative subjects.[14]

We recorded similar mean values in IL-6 levels in HIV seropositive individuals on HAART with hypertension when compared with HIV seropositive individuals on HAART without hypertension. This is believed to be because initiation of HAART improved the immune system which caused a reduction in the inflammatory process that occurs with HIV infection. Also, when HIV seropositive individuals on HAART with hypertension were compared with HIV negative controls without hypertension, IL-6 showed similar mean values. Our findings are in line with those of some authors who reported that IL-6 levels dropped by 32% over the first 24 weeks on HAART.[15] Another work also reported that at week 12 after commencement of HAART, IL-6 levels were significantly lower compared to pre-HAART levels in the same group of individuals thereby leading to reduced risk of associated opportunistic infection and tumour development. This IL-6 pattern we observed in our study may also be attributed to the fact that IL-6 has both pro-inflammatory and anti-inflammatory effects through the classical signaling pathway or through the trans-signaling pathway.[16]

C-reactive protein and D-dimer showed higher values in controls compared to HIV seropositive individuals on HAART with hypertension. This observation may suggest that inflammation plays a greater role in the pathogenesis of cardiovascular disease in HIV–infected population than in the general population, since it is widely known that inflammation is an important contributing factor of the pathophysiology of coronary heart disease. Our findings are in line with report that markers of inflammation are elevated in HIV-infected patients, and elevations in markers such as CRP and D-dimer have been associated with increased risk of cardiovascular disease.[17] The higher CRP levels observed in our study among HIV-infected individuals may also indicate the presence of opportunistic infections. Serum CRP levels in HIV-infected persons increase in the presence of opportunistic infections.[18] Evidence from some studies indicated that both HIV-infected.[5,19] and HIV- uninfected[20,21] with higher D-dimer levels are at a significant risk of death. Significantly higher D-dimer levels have been seen in HIV-infected patients with ongoing viral replication and lower CD4+ cells.[5,22,23] A study reported that HIV-infected individuals receiving antiretroviral therapy had significantly lower D-dimer levels than those off antiretroviral therapy at baseline.[24]

We found D-dimer to be strongly and positively correlated with IL-6 in HIV seropositive individuals on

HAART with hypertension. Inflammatory responses promote fibrin formation and lysis, resulting in elevated D-dimer levels.[25] This agrees with the work of Borges who reported that IL-6 and CRP were independently and positively correlated with D-dimer.[24]

5. CONCLUSIONS

It is evident from the study that HIV infection with or without hypertension can significantly increase the levels of CRP and D-dimer which are indicative of an increased inflammatory response and fibrinolytic activity. It is thus recommended that these biomarkers should be monitored in the treatment of HIV and HIV related conditions.

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