Biomedical & Pharmacology Journal Vol. **10**(1), 67-73 (2017)

Changes in Endocan Levels and Blood Coagulation in HIV Infection

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http://dx.doi.org/10.13005/bpj/1082

(Received: February 06, 2017; accepted: February 20, 2017)

ABSTRACT

Alteration in endothelial function may precede the development of morphological changes in disorders and may contribute to morbid development and clinical complications. Therefore, this work attempted to evaluate the levels of endocan (endothelial specific molecule-1) and other coagulation parameters and find their prognostic significance with respect to severity of human immuno-deficiency virus (HIV) infection. Sixty HIV infected patients on drugs and antiretroviral (ART) naïve were enrolled in a prospective, cross- sectional study while thirty HIV non reactive, apparently healthy individuals were recruited as control. Endocan was measured using high sensitive Enzyme linked immunosorbent assay. Plasma levels of prothrombin time and activated partial thromboplastin time were determined to check both intrinsic and extrinsic coagulation pathways. CD4+ count and platelet count were also analyzed by standard methods. HIV positive patients who are already on antiretroviral therapy (ART) had significantly increased endocan levels (471.134$\pm$92.84 pg/ml) compared to normal control (208.277$\pm$106.60 pg/ml) ($p<0.05$) while patients that are ART naïve had significantly increased endocan levels when compared to those already on drugs (611.60$\pm$608.77pg/ml) ($p<0.05$). HIV – 1 infected subjects not on drugs had significantly increased platelet count (145.1$\pm$580 cumm) when compared with normal subjects (90.100$\pm$40.00 cumm) ($P< .0001$) however, group on drugs had marginal decrease compared to normal group (85.000$\pm$192cumm). Markers of intrinsic and extrinsic coagulation- APTT and PT were significantly elevated in HIV positive patient when compared with apparently healthy controls. This is significantly associated with severity.

Keywords: Endocan, CD4+count, Antiretroviral therapy, Human Immunodeficiency virus, coagulation, prothrombin time.

INTRODUCTION

HIV infection continues to spread in recent times despite advances in the study of virology and it has come with devastating effects as it affects not only health but also social, economic and quality of life[1]. One of the characteristics of the infection is its ability to cause death due to opportunistic infection. However, the use anti retroviral drugs has limited the frequency of opportunistic infection and thus

increased the life span of those infected [2]. Although the use of antiretroviral drugs to a great extent, reduces viral replication and preserves the CD4+ lymphocyte count, other complications caused by HIV infection has begun to be noticed by researchers[3]. Haemostatic abnormalities have been recognized as a major contributor to survival [4]. The emergence of other major causes of mortality like venous thrombotic event and cardiovascular disease has also been implicated[5,6]. HIV infected patients are identified as having increased risk for arterial and venous thromboembolic events [7]. Preliminary evidence had suggested the culpable involvement of inflammation and chronic immune activation [8-10].

Currently, endothelial dysfunction has been suggested as one mechanism that may be contributory to cardiovascular risk involvement in these patients [11]. There is increasing evidence that high levels of some biomarkers like circulating endothelial cells, D-dimer levels, microparticles tissue factor expression and platelet microparticles may predict cardiovascular disease in these patients [12-14]. Endothelial dysfunction may be considered as a shift from the quiescent state of the endothelium into a more activated state. This state may lead to a pro inflammatory, procoagulant state and changes in availability of nitric oxide[15]. Damage to the endothelium will result in an imbalance between vasoconstriction and vasodilatation which may ultimately exacerbate atherosclerosis. Therefore, endothelial dysfunction maybe an early marker of atherosclerosis and a response to risk factors of cardiovascular disease[16].

Experimental evidence has implicated elevated endocan levels in several diseases especially as it relates to endothelial activation, endothelial dysfunction and neovascularization[17]. Furthermore, endocan is a potential immune-inflammatory marker that may be linked with cardiovascular disease[18].

The continuous use of HAART has now been linked with cardiovascular disease in HIV infection. Some researchers are convinced that cardiovascular and thrombotic events in these individuals may be as a result of long term use of HARRT probably because HAART can modify lipid profile and may lead to atherosclerosis[19]. In contrast, other studies agree that cardiovascular events may be due to the HIV infection itself [20].

There is growing awareness on the abnormal coagulation in HIV infection[21]. Recent studies have demonstrated that changes in coagulation and inflammation may be responsible and predict risk for non-AIDS defining events [22]. Of which cardiovascular disease is one. In this respect, studies on the levels of biomarkers of coagulation and inflammation have been associated with risk of mortality due to cardiovascular disease [23].

In summary, alteration in the endothelial biology and coagulation may explain non-AIDS defining clinical events [18]. It is still debated on the mechanism behind the abnormal coagulation in both those HIV reactive on drugs and those not on drugs. Changes in the endothelium, coagulation and platelet activation in association with CD4+ count may help link severity of infection in HIV infections. The aim of this study is to estimate the level of endothelial specific molecule-1, biomarkers of intrinsic and extrinsic (APTT and PT) pathways especially in association with CD4+count and treatment.

MATERIAL AND METHOD

Study population

Study participants enrolled included HIV positive subjects who were on drugs, those that were not on highly anti- retroviral therapy (HAART) and drug naïve patients attending Enugu State University Teaching Hospital Parklane, Enugu, Nigeria. The normal control consisted of non infected individuals that screened negative for HIV and other viral, bacterial infections.

Ethical considerations

The study was approved by the ethics and review committee of Enugu State University Teaching Hospital. Informed consent was duly provided by each participant as a factor to recruitment.

Study design

The study design was a cross sectional prospective study conducted on outpatient HIV

positive subjects between December 2015 and August 2016. Patients attending Hematology Clinic of Enugu State University Teaching Hospital Parklane, Enugu, were enrolled for the study. Ninety subjects were included into the study consisting of sixty HIV positive patients and thirty apparently healthy HIV negative individuals. HIV positive patients were further grouped into those that are antiretroviral naïve and those on drugs. Peripheral blood samples were collected by venepunture from all participants into sodium citrate; for the determination of prothrombin time and activated partial thromboplastin time, ethylene diamine tetra-acetic acid(EDTA); for the measurement of platelet and CD4+ count and plane tube; serum was expressed for the measurement of endothelial specific molecule -1(endocan). The study was performed according to the guidelines as stipulated by the Helsinki declaration.

Measurements of coagulation parameters: prothrombin time, partial thromboplastin time and platelet count were analyzed based on manual method according to Baker and Sylverton [24] while CD4counts were assayed using Cyflow (Partec, serial number 090439122 2009)

Measurement of Endocan was performed using commercially available high sensitive Enzyme Linked Immunosorbent (ELISA) kits by Cloud Clone Corp Houston TX USA. Intra-/ inter assay variation coefficient were respectively. The assay range of the ESM-1 ELISA kit was 15.6 to1000pg/ml. Intra-assay and the inter-assay variation coefficients were <10% and <12%, respectively.

Data analysis

The data was analyzed using Statistical Package for Social Sciences version 20. The values were reported as mean ± standard deviation. For One way analysis of variance was the statistical technique used for analysis for continuous variable while Pearsons' correlation was used to ascertain relationship.

RESULTS

A total of sixty HIV infected patients; thirty treatment naïve, thirty on drugs and thirty HIV non infected subjects were enrolled into the study. Equal gender distribution was sought among participants. The mean ages of participants were 38.7 ± 14. 9years. The mean values of CD4+ counts of normal subjects, those on drugs and those not on drugs were 838±137, 800±19 and 359±56µml respectively (P=0.022).

Levels of endocan (endothelial specific molecule), APTT (activated partial thromboplastin time), PT (prothrombin time) and platelet count in HIV reactive patients on drugs and drug naïve patients compared with non reactive apparently healthy subjects are shown in table 1.

Patients on treatment with HAART showed significant decrease in the mean levels of endocan (471.134$\pm$92.84) when compared to those not on drugs (611.60$\pm$608.77pg/ml). However, there was a significant increase ($p<0.05$) when mean endocan values of HIV infected treatment naïve patients() were compared with healthy controls (208.277$\pm$106.60pg/ml).

Treatment with HAART decreased progressively the mean levels of platelet counts of HIV patients (p= 0.000) when compared with normal healthy uninfected subjects while those infected but without treatment remained elevated.

Markers of intrinsic and extrinsic coagulation- APTT and PT were measured in all subjects. Those patients on drugs and those not on drugs all had significant elevated levels (in seconds) of platelet count, APTT and PT when compared with apparently healthy controls. Also, there was a statistically significantly decrease when levels of APTT and PT of HIV infected patients on drugs were compared to those that were not on treatment.

Figure 1 shows the levels of PT, APTT and platelet count of in normal and HIV subjects as stratified by their CD4+ count. When HIV patients were stratified according to their CD4+ count and treatment options, CD4+ counts of non reactive were decreased when compared to others.

No significant correlations were observed between endocan and prothrombin time p=0.41,

Table1: levels of endocan, APTT, PT and platelet count in HIV reactive patients on drug and drug naïve compared with non reactive apparently healthy subjects

Test	Reactive Not on Drugs	Reactive on Drugs	Non-Reactive Control
CD4+ µml	800±19	359±156	838±137
ESM1 pg/ml	611.60±608.77	471.134±92.841	208.277±106.60
Platelet cumm	145.1±580	85.000±192	90.100±40.00
Prothrombin Time (seconds)	26.0±14.4	20± 17.5	14±18
Partial Thromboplastin Time (seconds)	43.5±16.9	38.3±24.3	34.5±11.15

r=0.32; endocan and activated partial thromboplastin time p=0.30, r=-0.63; and between endocan and platelet count p=0.20, r=-0.18.

DISCUSSION

Alteration in endothelial function may precede the development of morphological changes in disorders and may contribute to morbid development and clinical complications [25,26]. In this respect, endothelial activation with resultant coagulation anomalies has been implicated in defining non-AIDS clinical events[27,28]. Therefore, this work attempted to evaluate the levels of endocan (endothelial specific molecule-1) and other coagulation parameters (activated partial thromboplastin time, prothrombin time and platelet count) with a view in finding their prognostic significance with respect to severity of HIV infection and treatment.

It this study, we revealed that endocan levels were quite elevated in HIV positive patients that are not on treatment when compared to those on treatment and normal healthy controls. It has been previously indicated that HIV positive patients may be at a higher risk of cardiovascular disease because they are more prone to vascular changes brought about by inflammation 29. Already biomarkers of inflammation have been found in these patients [30]. Immune activation probably due to co- factors such as co-infection with cytomegalovirus and bacteria or as a result of cytokines, elaboration of adhesion molecules and activation of innate/adaptive immunity has been suggested as reason for this situation [31].

The findings also revealed that though HAART may be effective in reducing viral multiplication and increasing CD4+ count, there may still be a low grade persistent inflammation and immune activation. This is because serum endocan levels were still significantly increased in patients already on treatment when compared with normal control found in this study. Viral replication has been suggested as being responsible for endothelial injury. Endocan is a soluble proteoglycan secreted by the vascular endothelium and has been associated with other bioactive molecules with properties such as cellular signaling, adhesion properties, regulation of proliferation and differentiation of cell types [32,33]. Endocan has also shown promising result as a biomarker of inflammation [34]. An increase in endocan levels may also suggest endothelial activation [35,36]. In contrast, other authors argue that added clinical changes may be as a result of ART[37]. Though in this case those not already on drugs had increased endocan levels.

Furthermore, derangement in the endothelium may lead to blood clotting activation[]. Previous study had suggested decreased levels of markers of endothelial activation and coagulation markers in those receiving antiretroviral treatment [38]. The result the current study showed a marked increase in platelet count, activated partial thromboplastin time and prothrombin time of HIV patients not on

treatment when compared to non infected control. Increased procoagulant state has been linked to non AIDS defining characteristics. Increased expression of tissue factor with subsequent generation of fibrin cloth may have initiated coagulation in this group of subjects [39]. Furthermore, this is in agreement with some authors who had formerly reported increased markers of platelet activation [13]. They had argued that platelet activation markers may offer novel ways of looking at disease progression probably because platelet activation plays a role in inflammation and thrombosis. Also, selectin, an important molecule in regulation of haemostasis may stimulate other mediators such as platelet factor 4 that is important for inflammation[40]. Intrinsic and extrinsic coagulation cascade activation may lead to formation of thrombosis probably through the elaboration of tissue factor[41].

CONCLUSION

The findings from this study revealed an increase in an serum endocan levels, platelet count, activated partial thromboplastin and prothrombin time both in ART naïve and ART treated individuals.

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