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# Elevated D-dimer levels in HIV Positive indigenous black Zambians: A risk factor for thrombosis and other cardiovascular associated complications

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## Abstract

Activated coagulation is a well-known feature of HIV infection and evidence has accrued indicating that it contributes to an increased risk of death. The aim of the study was to determine whether D-dimer levels, as a marker of predisposition to thrombosis, were higher in HIV positive individuals than in HIV negative individuals. A prospective cross-sectional study was carried out at the University Teaching Hospital in Lusaka, Zambia. D-dimers, triglycerides and cholesterol were assessed in HIV positive on ART, HIV positive ART- naïve and HIV negative control participants. Our results showed that HIV ART naïve participants had higher D-dimer concentrations (794.71 ± 318.07 ng/ml) than their counterparts on ART (514.39 ± 187.19 ng/ml) and the HIV negative control participants (375.08 ± 165.95 ng/ml) p =0.004 and p = 0.001 respectively. Triglyceride levels correlated to D-dimer levels in HIV positive ART naïve participants with statistical significance (r = 0.332; p < 0.020). This study showed that the levels of D-dimers were higher in HIV positive ART naïve individuals. This in part explains why they may be more at risk of thrombosis and cardiovascular associated complications which contribute to a high mortality rate in this group of individuals.

**Keywords**: D-dimer, cholesterol, triglycerides, thrombosi, HIV, cardiovascular diseases

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## Introduction

Activated coagulation is a well-known feature of HIV infection [1, 2] and evidence has accrued indicating that it contribute to an increased risk of death [3, 4]. This condition in HIV infected persons is thought to be a consequence of viral replication or persistence, high levels of bacterial lipopolysaccharide (LPS), bacterial DNA and their associated immune activation [5]. High levels of the thrombotic marker D-dimer is strongly associated with a higher mortality risk in patients with HIV infection [6]. It has actually been reported that this marker of coagulation remains an important predictor of death even at higher CD4+ cell counts [7].

D-dimers, the fibrinogen degradation products of cross-linked fibrin, have emerged as the most useful of the procoagulant activity and ongoing fibrinolysis markers. During thrombus formation, fibrinogen is converted to fibrin monomers that are extensively cross-linked into a polymer network. This cross-linking of fibrin takes place in the region of the polymer termed the "D-domain." Adjacent D-domains are covalently linked and constitute a fibrin specific feature of a thrombus, not found in fibrinogen or non-cross-linked fibrin degradation products [8]. One of the terminal products of fibrinolysis is the covalently linked D-Domain called the D-Dimer fibrin fragment. Monoclonal antibodies to D-Dimer have been developed that can differentiate fibrin specific clot from non-cross-linked fibrin as well as fibrinogen. As opposed to other markers that only detect products of acute coagulation, D-Dimer assays expand the diagnostic window [9]. A strong association between HIV replication and raised D-dimer levels has been

 

demonstrated. Correlations of D-dimer with HIV viremia and markers of endothelial dysfunction and microbial translocation [10, 6, 5] have also been reported. This favors the hypothesis that HIV replication and microbial translocation are among the main determinants of the hypercoagulable state seen in HIV-infected persons. HIV positive persons have increased levels of microbial products in their plasma [11]. Certain microbial toll like receptor ligands such as Lipopolysaccharides from these microbes can increase surface expression of the procoagulant tissue factor (TF also known as thromboplastin) on circulating monocytes [12]. This Tissue Factor expression on monocytes promotes coagulation [13]. This has been proved by the presence of dramatically higher frequencies of monocytes expressing TF in fresh blood samples from HIV-infected persons than in samples from uninfected controls [5]. Finally, the in vivo biologic activity of monocyte TF expression is suggested by a correlation with plasma levels of D-dimers. These findings suggest that a variety of microbial products, including those derived from HIV itself, and perhaps bacterial products translocated from the damaged gut in chronic HIV infection, may contribute to a heightened risk for clotting and cardiovascular disease in HIV infection by increasing cell surface expression of the procoagulant TF [5].

## Materials and Methods

*Selection of participants and specimen collection* - Blood was collected with informed consent from HIV positive individuals reporting for ART management at the Adult Infectious Diseases Centre (AIDC) at the University Teaching Hospital (UTH), Lusaka, Zambia. HIV negative participants were recruited from the Voluntary and Counselling and Testing (VCT) centre after testing negative for HIV and consenting to participate in the study. Blood samples were collected from research participants via venipuncture using the Evacuated Tube System (ETS) following the Clinical Laboratory Standards Institute (CLSI) order of draw. Ethical clearance was granted by ERES CONVERGE I.R.B No. 00005948, F.W.A No. 00011697and Ref. No. 2014-May-002

***Specimen Preparation and Storage*** – In the laboratory, blood specimens in the sodium citrate tubes were centrifuged at 3000 revolutions per minute in order to separate plasma from the blood cellular component. Plain tubes were centrifuged at 1500 rpm for 15 minutes to separate serum from the blood cellular components. Only serum and plasma were meticulously collected from the vacutainers using pasture pipettes and transferred to 2ml cryovials with sealable screw caps, which were stored in a freezer at -80$^{o}$C until the specimens were required for analysis.

***D-dimer estimation*** – Plasma samples were analyzed for d-dimer concentrations using the ichroma$^{TM}$ D-Dimer along with the ichroma$^{TM}$ Reader (manufactured by Boditech - South Korea and supplied by Onyx Technologies – Lusaka, Zambia) which is a fluorescence immunoassay that quantifies the total D-Dimer concentration in plasma.

***Cholesterol and triglycerides estimation-*** Serum samples were analysed on the Pentra 400 Chemistry Analyser according to the manufacturer's recommendation and assay procedures for the automated analyser. All test protocols were calibrated and controls ran before samples could be assayed.

| Variable | HIV+ ART | HIV+ ART Naive | HIV- Control | P-Value |
|---|---|---|---|---|
| D-Dimer | 514.39 ± 187.19 | 794.71 ± 318.07 | - | 0.004 |
| | 514.39 ± 187.19 | - | 375.08 ± 165.95 | 0.865 |
| | - | 794.71 ± 318.07 | 375.08 ± 165.95 | 0.001 |

**Table 1**: D-Dimer in HIV+ ART naive was significantly higher than in the HIV+ on ART and in the HIV- negative control groups. Significant at the 0.05 level.

## Results

In total 150 participants were recruited, 50 HIV positive on ART, 50 HIV positive ART naïve and 50 HIV negative. The study found that HIV positive ART naive participants had statistically significant higher d-dimer concentration (794.71 ± 318.07 ng/ml) than HIV negative control participants (375.08 ± 165.95 ng/ml) *p*= 0.001 (Figure - 1). HIV positive ART naive participants also had a statistically significant higher d-dimer concentration compared to HIV positive participants on treatment (514.39 ± 187.19 ng/ml) *p*= 0.004 (Table. 1). However, there was no statistical difference in d-dimer concentration between the HIV negative control group and the HIV treatment group p = 0.865 (Figure - 1).

There was no correlation between CD4 and D-dimer concentration in both the HIV positive non treatment and HIV positive treatment groups with ($r^2$



 

= 0.004, p= 0.744) and ($r^2$ = 0.00, p= 0.904) respectively. However there was a correlation between D- dimer and triglycerides in the HIV+ ART naïve participants $r^2$ = 0.110, p = 0.020 (Figure - 2).

| Variable | HIV+ ART | HIV+ ART Naive | HIV- Control | P- Value |
|---|---|---|---|---|
| Cholesterol | 4.87 ± 0.36 | 3.78 ± 0.34 | - | < 0.0001 |
| | 4.87 ± 0.36 | - | 4.27 ± 0.32 | 0.022 |
| | - | 3.78 ± 0.34 | 4.27 ± 0.32 | 0.131 |
| Triglycerides | 1.24 ± 0.21 | 1.17 ± 0.23 | - | 0.438 |
| | 1.24 ± 0.21 | - | 1.01 ± 0.21 | 0.037 |
| | - | 1.17 ± 0.23 | 1.01 ± 0.21 | 0.421 |

**Table 2**: Multiple comparisons of p-values for cholesterol and Triglycerides Concentrations between HIV+ on ART, HIV+ ART naïve and HIV- control groups significant at the 0.05 level. Cholesterol concentration in the HIV+ on ART was significantly higher than in both the HIV+ ART naïve and the HIV- control groups. Triglycerides concentration in the HIV+ on ART was significantly higher than in the HIV- control group

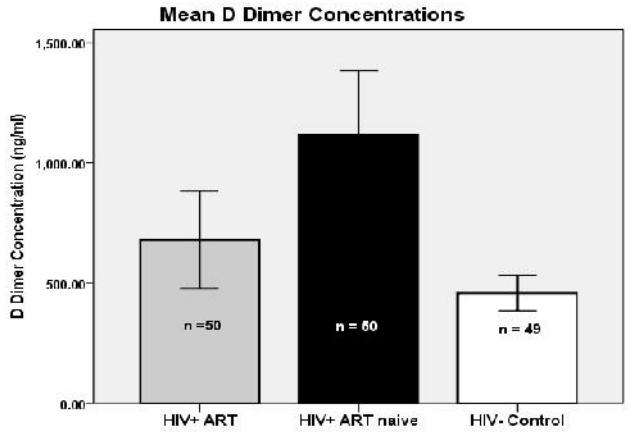


**Figure 1**: Mean d-dimer concentration for HIV+ ART naive (794.71 ± 318.07 ng/ml) was higher than for the HIV+ on ART (514.39 ± 187.19 ng/ml) and HIV- control participants (375.08 ± 165.95 ng/ml).

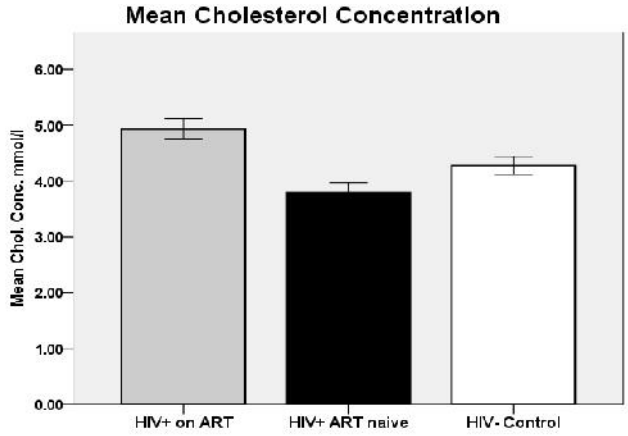


**Figure - 3**: Mean Cholesterol concentrations for HIV+ on ART (4.87 ± 0.36) were higher than HIV+ ART naive (3.78 ± 0.34) and HIV- control participants (4.27 ± 0.32).

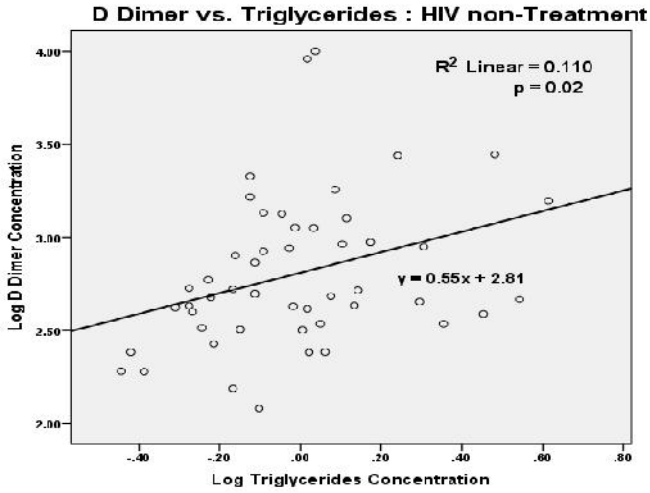


**Figure - 2**: There was a significant correlation between high triglyceride and D-Dimer concentrations in the HIV+ ART naïve group.

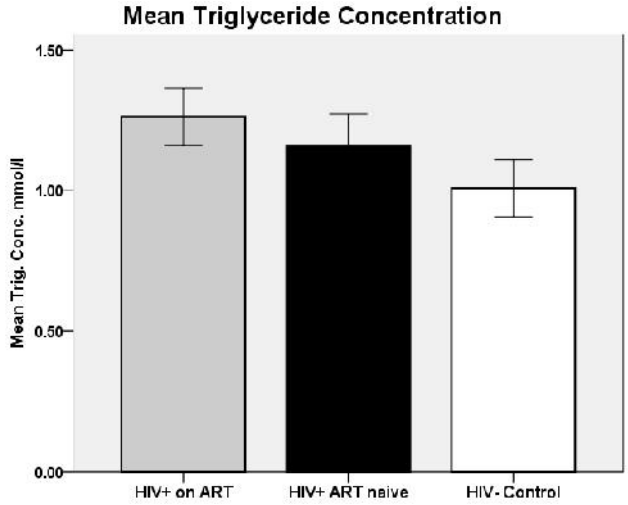


**Figure 4:** Mean Triglycerides concentrations for HIV+ on ART (1.24 ± 0.21) were higher than in HIV+ ART naive (1.17 ± 0.23) and HIV- control participants (1.01 ± 0.21).

## Discussion

Normal haemostasis comprises a series of regulated processes that maintain blood in a fluid, clot-free state in normal vessels while rapidly forming a localized haemostatic plug at the site of vascular injury [14]. The pathologic counterpart of haemostasis is thrombosis, the formation of blood clot (thrombus) within intact vessels. D-dimers, the fibrinolytic degradation products of cross-linked fibrin, have emerged as the most useful of the procoagulant activity and ongoing fibrinolysis markers. Evidence has accumulated confirming the accuracy and cost-effectiveness of incorporating a D-Dimer assay into the diagnostic algorithm of deep vein thrombosis (DVT) and pulmonary embolism (PE).

**D-dimer:** In this study, it was observed that D-dimers measured in HIV positive ART-naive patients prior to ART initiation were significantly raised (794.71 ± 318.07 ng/ml) compared to HIV negative controls (375.08 ± 165.95 ng/ml) p = 0.001 (Fig. 1 and Table 1). Results from other studies have suggested that D-dimer and IL-6 could be useful in identifying ART-naive patients at higher risk of AIDS or death after ART initiation[15]. An elevated D-dimer concentration is thought to be a consequence of viral replication or persistence, high levels of bacterial lipopolysaccharides (LPS), bacterial DNA and their associated immune activation in HIV infected persons [5]. It has also been demonstrated in other studies that plasma samples from HIV-infected patients contain higher levels of bioactive Tissue Factor (TF) the major in vivo activator of coagulation than do samples from HIV negative controls [5]. Increased TF expression in HIV infection is underscored by the correlation between TF expression and D-dimer levels [5]. Thus, high levels of TF probably contribute to an increased coagulation tendency in chronic HIV infection. This could lead to a state of hypercoagulability which predisposes HIV positive individuals to an increased risk of both arterial and venous thrombosis [16].

The study also found that HIV positive ART naive participants had significantly higher mean d-dimer concentration (794.71 ± 318.07 ng/ml) than HIV positive participants on treatment (514.39 ± 187.19 ng/ml) (*p*= 0.004). This probably showed that the commencement of ART improved the immune system leading to reduced viral replication, low levels of bacterial lipopolysaccharides (LPS), bacterial DNA and all mechanisms which lead to an increased expression of Tissue Factor. This agreed with a study by [17]which found that at week 12 after commencement of ART, D-dimer levels were significantly lower compared to pre-ART levels in the same group of individuals. This also agreed with the results obtained from our study which showed no significant difference in D-dimer levels between HIV positive participants on treatment (514.39 ± 187.19 ng/ml) and the HIV negative control participants (375.08 ± 165.95 ng/ml) p = 0.865 (Figure 1 and Table 1). HIV positive on ART still had higher mean D-dimer levels than the HIV negative controls (Table 1) despite the difference not being statistically significant.

Linear regression results showed that triglycerides had some moderate correlation with the elevated levels of D-dimers in the treatment naive HIV positive population as revealed by $r^2 = 0.110$; p = 0.02 (Fig 2). This meant that the concentrations of D-dimers were enhanced by triglyceride levels in combination with other factors which may include viral replication or persistence, high levels of bacterial lipopolysaccharides (LPS) or Tissue factor to induce intravascular thrombosis. However, cholesterol levels were not associated with high D-dimer levels as revealed by linear regression results $r^2 = 0.03$; p= 0.232.

**Cholesterol:** The study revealed that HIV positive participants on treatment had a significantly higher serum concentration of Cholesterol (4.87 ± 0.36 mmol/l) compared to HIV negative control participants (4.27 ± 0.32 mmol/l) with p = 0.022(Fig. 3 and Table 2). However, this did not correspond to high D-dimer levels. The pathogenesis of HAART-related dyslipidaemia is complex and involves various drug induced effects in association with hormonal and immunological influences superimposed upon genetic predisposition [18, 19]. All the HIV positive participants on ART who took part in this research were on first line HIV treatment taking a combination of Nucleoside Reverse – Transcriptase Inhibitors (NRTIs) and Nonnucleoside Reverse – Transcriptase Inhibitors (NNRTIs), both of which could cause increases in cholesterol concentration. Out of the 50 participants on HIV treatment, 39 were on Atripla which contains Efavirenz (EFV) an NNRTI given with NRTIs (Tenofovir (TDF) and Emtricitabine (FTC)). EFV given with NRTIs raised total cholesterol levels as early as 4-8 weeks of therapy [20] and this could be the main cause of the high mean cholesterol



 

concentration obtained. There was no significant difference in mean total cholesterol concentration between the HIV negative control group (4.27 ± 0.32 mmol/l) and the HIV positive treatment naïve (3.78 ± 0.34 mmol/l) p= 0.131 (Fig. 3 and Table 2). However, the treatment naive group had a higher mean total cholesterol concentration.

## Conclusion

This study showed that the levels of d-dimers were higher in HIV positive ART naive individuals. This in part explains why they may be more at risk of thrombosis and cardiovascular associated complications which contribute to a high mortality rate in this group of individuals.

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**Conflicts of interest:** Declare no conflicts of interest

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