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Functional Antithrombin Levels in HIV/AIDS Patients at Ibadan, Nigeria

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Abstract: HIV infected patients have an increased incidence of venous thromboembolism and an important risk factor is antithrombin deficiency. Currently it is estimated that 10% of HIV/AIDS patients who developed venous thromboembolism had antithrombin deficiency with functional antithrombin levels less than 70%. The purpose of this study is to measure functional antithrombin levels in HIV patients prior to development of venous thromboembolic disorder. One hundred and twenty HIV positive and one hundred and twenty-six HIV negative apparently healthy blood donors were studied. Socio-demographic, medical history and clinical characteristics were obtained from the patients. Blood was analyzed for CD4+ lymphocytes count, full blood count and functional antithrombin levels. We also investigated the relationship between functional levels of antithrombin, CD4 + lymphocytes count and some haematological parameters. The functional antithrombin levels were significantly reduced in the people living with HIV/AIDS (72.6%) when compared with the controls (93.7%). There was no correlation between CD4 + lymphocytes count (by proxy the stage of the disease) and functional antithrombin levels in HIV positive patients ($r = 0.02$, $p = 0.9$). Patients with HIV infection have a mild deficiency of functional antithrombin that may lead to hypercoagulability.

Keywords: Functional, Antithrombin, HIV

1. Introduction

HIV infection is a pro thrombotic condition with a 2-10-fold increase in thrombotic episodes compared with a normal population of the same age and has a frequency of 0.19-7.63% per year. [1, 2] With the tendency to thrombosis, individuals with HIV/AIDS as distinct population could benefit from primary thrombo-prophylaxis. Work done in defining the exact mechanism of their thrombophilia discovered that antithrombin deficiencies were present in HIV/AIDS with venous thromboembolism. [3] We considered the possibility of a pre thrombotic antithrombin deficiency in this population. Antithrombin is a natural anticoagulant, a 58 kilo Dalton serine protease inhibitor produced by the liver. It is a α2-globulin protein molecule. Antithrombin inactivates thrombin, factor Xa, factor IXa, factor XIa, factor XIIa and is the major inhibitor of blood coagulation. [4, 5] When a serine protease like thrombin attempts to cleave antithrombin at its reactive site (arginine 393, serine 394) an ester bond forms between the active serine site of the thrombin and arginine site of the antithrombin molecule forming a stable complex that is removed from circulation. [6] The formation of the antithrombin-thrombin complex is accelerated by heparin. [7] Deficiencies in natural antithrombin levels (40–60% activity), may be congenital or acquired such as in liver disease and increases the risk of venous thromboembolism by up to 16 fold. [8, 9] It remains one of the few deficiencies to date, whose treatment appears to impact the outcome and mortality of VTE. [10]

Antithrombin deficiency has been discovered in HIV patients who have developed deep venous thrombosis and pulmonary thromboembolism since the year 2000. [11-13] Plasma levels of antithrombin was incidentally found correlates with the severity of the virus associated immunosuppression. [10]

2. Materials and Methods

The study carried out at the University College Hospital and Eleta Catholic Hospital. Approval was sought and received from the Research and Ethics Committee of both hospitals. One hundred and twenty adults with HIV infection attending the APIN clinic and one hundred and twenty-one apparently healthy blood donors from the Oyo State Blood Bank as well as volunteer medical and nursing staff from the University College Hospital were recruited. Interviewer administered questionnaires were filled. A written informed consent was obtained from each participant. Both HAART-naïve and those on HAART aged 16 years and above were included. Their sociodemographic data, past medical history, family and social history was documented. Clinical information relating to deep venous thrombosis was taken and documented and patients who answered in the affirmative for past or present DVT were excluded. Age and sex matched controls from apparently healthy blood donors and medical staff served as control. Nine milliliters of venous blood was collected, using standard phlebotomy techniques, from each subject and control. Four point 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 4.5 milliliters of blood was transferred to bottle containing 0.5 milliliters of sodium citrate bottle (this is to ensure blood/anticoagulant ratio for coagulation study: 1 part of anticoagulant to 9 parts of blood = 0.5ml of Na Citrate to 4.5ml of blood), centrifuged and plasma obtained kept at -20°C until analysis for plasma antithrombin.

The full blood count and CD4 + T lymphocyte count obtained from anticoagulated samples of patients were determined using Cell-Dyne 1200 haematology analyzer (Abbott Diagnostics, North Chicago, IL, USA) and Cyflow Cytometer Analyzer (Partec©, Germany) respectively. Functional antithrombin III assays were performed using an Amidolytic (chromogenic) Assay, BIOPHEN Antithrombin 5: Ref A221105 (Aniara, USA). The Spectrumlab 23A spectophotometer was used to read the results. Antithrombin percentage activity of less than 60% was considered deficient.

3. Results

A total of two hundred and forty-six participants were studied. The ages of the subjects and controls range between 25 and 55 and 15 to 55 years respectively. The mean age of the subjects was 32 ± 7 years and the mean age of the HIV negative controls was 37± 9 years. The clinical characteristics of the HIV positive subjects and control are in table 1. Eight (6%) of the HIV positive subjects had past history of jaundice while none (0%) of the control had. The hematological parameters are as shown on table 2. The mean platelet count was significantly higher (t = 2.84; p = 0.00) in HIV positive subjects (232 x 10^9) compared to that of the control subjects (119 x 10^9). Similarly, the mean corpuscular volume was significantly higher (t = 7.90; p = 0.00) in HIV positive subjects (96.0fL) compared to the control subjects (87fL). However, the mean haematocrit (35.8%) and the mean haemoglobin concentration (114g/L) values in HIV subjects were significantly lower (t = 4.38, p = 0.00 and t = 4.11, p = 0.00 respectively) compared to those of the controls (38.5% and 123g/L respectively) The CD4+ lymphocytes count was significantly lower (t = 21.5; p = 0.00) in the HIV positive 447.6 cells/µl (227.6) group compared with the HIV negative 905.3 cells/µl (423.4) healthy blood donors.

Table 3 compares the level of functional antithrombin between HIV positive and negative groups. Functional antithrombin levels were significantly lower amongst the HIV/AIDS patients compared with controls (72.6% versus 93.7%; t = 5.5; p = 0.000 CI = 15-29). Forty-two (35%) of the HIV positive subjects had less than 60% enzyme activity compared with 13 (10.2%) of the HIV negative participants (χ^2 = 21.8, p=0.00) as seen in table 4. There was no correlation between CD4+ lymphocytes count and functional antithrombin levels in HIV positive patients (r = 0.02, p = 0.9). Of the 120 HIV positive participants, 58 were treatment naïve while 62 were on treatment. 31% of the treatment naïve patients had antithrombin level lower than 60% whereas 38.7% of those on treatments had antithrombin level below 60% (χ^2 =0.8; p = 0.3) as shown on table 6. The figure shows that median antithrombin level in the control group was 95.9% activity (range, 79.5-114), and the median in the HIV positive group was 77.3% activity (range, 51.23-95.25).

Table 1. *Clinical characteristics of HIV positive and Negative group.*

Variable	HIV positive Count (%) N = 120	HIV negative Count (%) N = 126	χ^2 /fisher's exact test	P value
Positive history of chronic renal failure	0 (0)	1 (0.8)	1.0	0.5
Positive history smoking of cigarette	3 (2.5)	6 (4.7)	0.5	0.3
Positive History of casual sexual encounters	17 (14.2)	16 (12.6)	1.3	0.7
Positive history of jaundice	8 (6)	0 (0)	0.03	0.03*

<0.05 *significant.
>0.05 not significant.

Table 2. *Haematologic Parameters of the HIV positive and HIV negative groups.*

Parameters	HIV pos n = 120 Mean (± SD)	HIV neg n = 126 Mean (± SD)	t-value	p-value
WBC ($\times 10^9$/L)	5.3 (2.3)	5.2 (1.5)	-0.35	0.73
Lymphocyte count ($\times 10^9$/L)	2.1 (1.0)	2.2 (0.7)	0.19	0.86
Monocyte ($\times 10^9$/L)	0.6 (0.3)	0.5 (0.3)	-1.63	0.11
Neutrophil ($\times 10^9$/L)	2.5 (1.9)	2.5 (1.0)	-0.24	0.81
Haemoglobin (g/L)	114.4 (18.2)	123.4 (16.1)	4.11	0.00*
Haematocrit (%)	35.8 (5.5)	38.5 (4.5)	4.38	0.00*
MCV (fL)	96 (10.8)	87 (6.7)	-7.90	0.00*
MCHC (g/L)	319 (10.7)	319 (11.9)	-0.29	0.77
Platelet ($\times 10^9$/L)	232 (119)	199 (50)	-2.84	0.00*
CD4 cell count	447.6 (227.6)	905 (423.4)	21.5	0.00*

Table 3. *Antithrombin (% activity) in the HIV positive and HIV negative groups.*

	HIV positive (mean ± SD) n = 120	HIV negative (mean ± SD) n = 126	T value	CI	p-value
Antithrombin (% activity)	72.6 ± 31.4	93.7 ± 24.9	5.5	15-29	0.000*

Table 4. *HIV positive and HIV negative groups with antithrombin deficiency.*

Antithrombin (% activity)	HIV negative	HIV positive	Total	χ^2	P value
< 60%	13 (10.3%)	42 (35%)	55	21.8	0.000*

Table 5. *Association between jaundice or chronic liver disease and antithrombin levels in the HIV positive group.*

	History of jaundice N (%)	No history of jaundice N (%)	P value
	8 (6)	112 (94)	0.4
Antithrombin activity % mean (SD)	82 (16)	72 (33)	

Table 6. *Association between plasma antithrombin activity (%) levels in the HIV positive and use of highly active antiretroviral therapy.*

	Antithrombin (%)				
	< 60%	> 60%	Total	χ^2	p-value
ART-naïve	18	40	58	0.8	0.3
ART-experience (NNRTI+NRTI)	24	38	62		
Total	42	78	120		

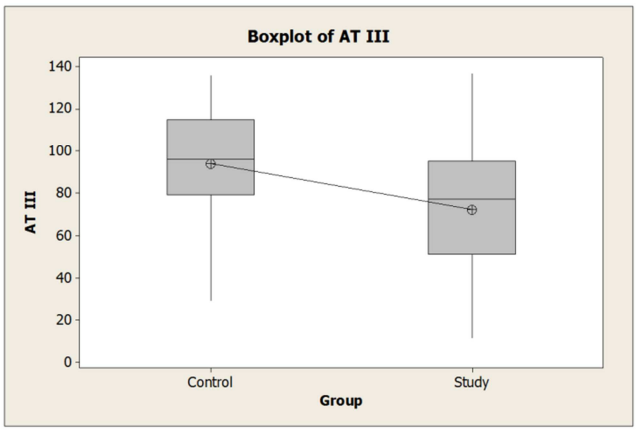

Figure 1. *Antithrombin % activity in both the HIV seronegative and HIV positive groups.*

This box plot for plasma levels of functional antithrombin shows that the mean antithrombin level noted by the encircled cross was 95.9% in the controls and 77.3% in the HIV positive. The interquartile range for the controls was 80% to 110% activity while the interquartile range for the seropositive group was 55% to 90% activity.

4. Discussion

This cross sectional study was performed to explore functional antithrombin levels amongst patients living with HIV/AIDS at Ibadan. Infection with the human immunodeficiency virus results in a thrombophilic state but etiology, risk factor and clinical course are only recently being elucidated and defined. Antithrombin deficiency has been established as a risk factor for venous thromboembolism. Recently, studies have discovered that even mild reductions of antithrombin (70-80% of normal) have a higher risk of developing venous thrombo embolism compared with those with normal antithrombin levels (80-120%). Few studies have searched for mild or moderate deficiencies in HIV AIDS patients without clinical features of VTE. In this study, we compared the functional antithrombin levels of HIV/AIDS patients with healthy blood bank donors. We discovered that functional antithrombin levels were significantly lower (72.9%) in the HIV positive population compared with the seronegative population (93.7%), when controlled for venous thromboembolism. Saif et al in 2001 carried out a retrospective review of the medical records of 131

HIV/AIDS patients in the United States. He found that 7% of their patients developed thrombosis (deep venous and or pulmonary embolism) and 10% of those with confirmed thrombosis had an acquired antithrombin deficiency. [12] In a small group of HIV-seropositive patients with venous thrombo-embolism carried out by Sule et al in 2013 in Singapore, they found 20% of those with confirmed deep venous thrombosis had an acquired antithrombin deficiency. [13] The prevalence of antithrombin deficiency in the general population is less than one percent, but in both studies, antithrombin deficiency is more in the HIV population compared with normal individuals. However, there are a number of differences between those two studies and this. Our study excluded patients with venous thromboembolism while Saif [12] and Sule et al [13] carried out studies on patients with radiologically confirmed VTE. The study conducted by Sule et al [13] consisted of males and the data may not be directly translatable to our study that was carried out on both male and females. Thirty-five percent of our HIV/AIDS patients had antithrombin levels of less than 60% which was four times greater than HIV negative controls. HIV infection itself is not directly associated with antithrombin deficiency [14], liver disease that are frequent co-morbidities in HIV however, do cause an acquired deficiency. Cao et al [15] and Huffert et al [16] have confirmed that HIV replicates in Kupffer liver cells which leads to reduced protein synthesis but they did not directly reference antithrombin synthesis. We searched for a correlation between a history or physical findings of jaundice and antithrombin levels and found none.

The use of HAART especially protease inhibitors have been associated with thrombotic events in HIV/AIDS. The mechanism has been suggested to be interference with hepatic metabolism (especially cytochrome P450 metabolism), as well as a possible decrease in synthesis of anticoagulants like protein S. [17] Our findings show that the use of highly active antiretroviral drugs was not associated with the level of functional antithrombin. The patients in our study used a combination of NNRT and NRTI and none were on PI. Also important to note is that this study centered on the anticoagulant antithrombin.

Jacobson et al [18] found out that lower CD4+ lymphocytes were associated with and contributed to the hypercoagulability in HIV/AID subjects. Their group noted that those patients with a CD4+ cell count of less than 200 cells/ μL were in poor health. The majority of them developed opportunistic infections which was strongly correlated with hypercoagulability. In our study we investigated the correlation between CD4+ lymphocytes count and antithrombin level. There was no correlation between the two parameters but it is important to note that the mean CD4+ cell count in our study was greater 500 cells/ μL. Antithrombin deficiency was prevalent in the population of HIV/AIDS studied though none of the patients had any clinical evidence of VTE. The use of NNRTI/NRTI as well as CD4+ cell count had no association with the level of functional antithrombin levels.

5. Conclusion

The plasma levels of functional antithrombin are lower in HIV positive individual compared with their sero negative counterpart. This mild deficiency in this population predisposes them to hyper-coagulable state. Therapy aimed at correcting this mild deficiency could reduce the risk of thrombosis in people living with HIV. The CD 4 count used as a surrogate for immunosupression was not associated with functional anti-thrombin levels. Neither was the type of Highly Active Antiretroviral therapy found to be correlated with functional anti thrombin levels. Therefore, the levels of functional anti-thrombin do not depend on the degree of immune suppression or therapy and may be an independent contributor to overall morbidity in people living with HIV.

Strenghts and Weaknesses of this study

Work done on this topic is relatively scarce. Searching through literature reveals that while an increase incidence of thrombotic episode has been documented by research, the underlying pathologic mechanisms are yet to be elucidated. Anti thrombin deficiency as a cause of this hyper coagulability can be countered. This study is however limited by its cross sectional design and no inferences on temporal relationships could be made. The number of subjects in the study was limited to two hundred and forty-six participants due to cost implications. Further experimental studies will be needed to conclusively link anti thrombin levels with episodes of thrombosis in people with HIV.

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