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Pathology Section

Study of Basic Coagulation Parameters among HIV Patients in Correlation to CD4 Counts and ART Status

THULASI RAMAN R[1], MANIMARAN D[2], PRAVEEN RACHAKATLA[3], BHARATHI K[4], TAMEEM AFROZ[5], RADHA SAGAR[6]

ABSTRACT

Introduction: HIV infection is known to cause coagulation abnormalities by various mechanism, especially during its late course.

Aim: The objective of this study is to analyse platelet count, prothrombin time and activated partial thromboplastin time among HIV infected patients and to analyse these parameters with respect to their CD4 count and ART status.

Materials and Methods: A case control study was conducted with 120 HIV infected patients and 40 normal individuals. The blood samples were collected after obtaining consent from the subjects. The blood samples were processed for platelet count, prothrombin time and activated partial thromboplastin time and CD4 count. The results were tabulated and analysed with statistical package.

Results: The platelet count was significantly decreased in HIV infected patients compared to controls. Though HIV patients with CD4 count less than 200cells/mm^3 showed a decreased platelet count compared to those with CD4 count greater than 200cells/mm^3, it was not statistically significant. Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) was significantly prolonged in HIV patients, but only aPTT showed significant inverse correlation with CD4 count. None of the parameters showed statistical significance on comparing HIV patients on ART with those not on ART.

Conclusion: Basic coagulation tests like platelet count, PT and especially aPTT can be used as prospective screening test to assess severity in HIV patients in resource limited settings where CD4 count is not available.

Keywords: Activated partial thromboplastin time, Anti-retroviral agents, Blood coagulation, Prothrombin time

INTRODUCTION

Human Immunodeficiency Virus (HIV) infection is a global burden and rapidly spreading. It causes significant morbidity and mortality by various mechanisms and one among them is coagulation abnormalities. This is a quite serious complication especially in late stage of HIV infection. There are uncertainties in pathogenesis of coagulation abnormalities in HIV patients. The cause for the defect may be due to the host, drug and viral factors. Host factors include age, IV drug abuse, CD4 count, presence of opportunistic infections, associated malignancies, acquired hypercoagulable state and endothelial dysfunction. Anti-retroviral drugs especially protease inhibitor are also proposed to cause endothelial dysfunction by their effects on metabolism of lipid and glucose [1]. The viral load is also another important determinant [2]. These coagulation abnormalities get worse in the later course of HIV infection with associated immunosuppression as measured by the CD4 cell counts and with the presence of concurrent infectious or neoplastic diseases [3].

Hepatic damage is caused by virus itself or by the anti-retroviral (ART) drugs that may also contribute to coagulation defects in HIV patients. Platelets play an important role in haemostasis, by forming the primary haemostatic plug following endothelial injury. Platelets decrease in HIV infection due to autoimmune destruction, direct infection of megakaryocytes by virus. Platelets also decrease due to consumption coagulopathies occurring in Acquired Immune Deficiency Syndrome (AIDS). The basic tests to assess the intrinsic and extrinsic pathways of coagulation are Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) respectively. Hence we used the above basic parameters along with platelet count to assess the coagulation abnormalities in HIV infected individuals.

AIM

To study platelet count, prothrombin time and activated partial thromboplastin time among HIV infected individuals and to analyse these parameters in correlation with the CD4 counts. Similarly these parameters are compared between treatment naïve HIV infected patients and those on Antiretroviral Therapy (ART).

MATERIALS AND METHODS

Study Population Setup

The study was done over a period of 60 days in May- June 2014. The study was carried out on 120 HIV positive cases including the patients on ART & those not on ART. The HIV positive cases were recruited regularly from anti-retroviral clinic, civil hospital, Karimnagar, India. Cases were subjected to inclusion and exclusion criteria. Exclusion criteria include the subjects not willing to participate, patients with bleeding disorder and patients on anticoagulant therapy.

A control of 40 cases with similar age & sex distribution was setup. The control group were apparently normal individuals with no disease complaints. Thus, total number of study subjects were 160, which includes 120 cases and 40 controls.

Consent was obtained from each case. The required data was collected using a structured questionnaire.

Sample Collection

(1) Platelet count

- A 2ml of venous blood was collected under aseptic precautions in a vacutainer containing ethylene diaminetetra acetic acid (EDTA). Sample was analysed for platelet count via automated cell counter (ABX-MICROS 60), which is subjected to strict regular internal and external quality assurance [4].

(2) PT and aPTT

- A 2 ml of venous blood was collected in 3.8% tri sodium citrate vacutainer in a propotion of 1:9 and processed in Helena automated coagulometer, subjected to strict regular internal and external quality assurance [4].

(3) CD4 counts

- A 2ml of blood collected in another vacutainer containing Ethylenediaminetetraacetic Acid (EDTA) and processed on flow cytometry [4].

Methods of Analysis

A) Analysis of coagulation parameters of HIV infected cases with Non infected controls.

- Platelet count, PT and aPTT was comparatively studied between the treatment naïve HIV cases and controls [4].

B) Analysis of coagulation parameters in ART and NON-ART HIV positive cases with respect to CD4 count.

- A further analysis was done in the present study by comparing above parameters in ART and Non-ART group. The patients are separated into ART and Non-ART group by their ART status during the time of sample collection. Hence few cases which were put on ART recently may slightly bias the data [4] [Table/Fig-1].

C) Analysis of coagulation parameters in HIV positive cases with respect to CD4 count.

- The most important biomarkers of disease stage and progression in patients with HIV infection are CD4 count and HIV RNA concentration. Hence the CD4 count was taken in this study as a marker for disease severity. The HIV positive cases were divided into two groups using a CD4 count of 200cells/mm^3 as cut off. The parameters were compared between these two groups [4].

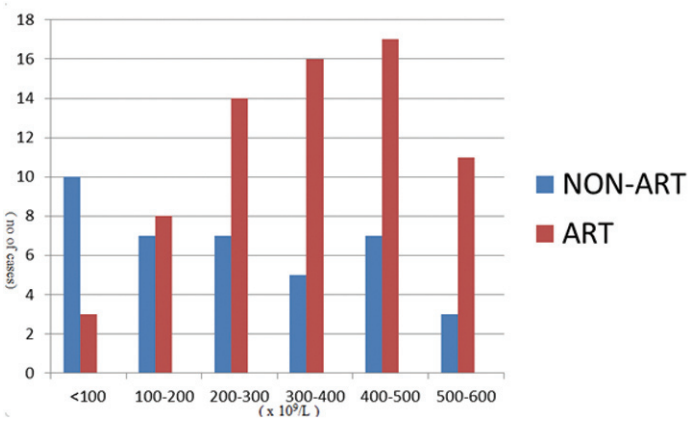
STATISTICAL ANALYSIS

The statistical analysis is done using SPSS version 17.0 Statistical package. The p-value is calculated using unpaired t-test.

RESULTS

The HIV infected patients, when compared to controls showed statistically significant decrease in platelet count. Similarly HIV patients also showed significant prolongation in PT and aPTT in contrast to HIV negative controls [Table/Fig-2].

aPTT showed a significant prolongation in HIV patients with CD4 count less than 200cells/mm^3 compared to patients with CD4 greater than 200cells/mm^3 (p<0.001). Thus there was an inverse correlation between aPTT and CD4 count. Though PT was prolonged in HIV patients with CD4 less than 200cells/mm^3 compared to patients with CD4 greater than 200cells/mm^3, it was not statistically significant. Similarly platelet count was relatively decreased in patients with CD4 less than 200 cells/mm^3, but was again statistically insignificant [Table/Fig-3]. Platelet count, PT and aPTT showed no correlation between the cases on ART and the cases not on ART [Table/Fig-4].



[Table/Fig-1]: Distribution of cases according to CD4 count in Art and Non-Art group.

PARAMETER	CONTROLS (n=40)		TREATEMENT NAÏVE HIV INFECTED CASES (n=44)		p - value
	MEAN	S.D	MEAN	S.D	
PLATELET COUNT (x 10^9/L)	273	63.17	231	79.04	0.0026
PT (in sec)	16.47	1.10	18.23	4.55	0.0168
aPTT (in sec)	30.59	1.66	41.7	5.04	0.0001

[Table/Fig-2]: Comparison of platelet count, prothrombin time and activated partial thromboplastin time between controls and treatement naïve HIV infected cases.

DISCUSSION

HIV patients show endothelial dysfunction which is evident by increased von willebrand factor levels [5]. Gp120 causes endothelial dysfunction by reducing eNOs expression [6]. The endothelial dysfunction affects the balance between thrombotic and antithrombotic functions, leading to activation and consumption of coagulation factors, resulting in coagulation abnormalities. Various hypercoagulable states predisposing to consumption coagulopathy have also been reported in AIDS patients. Anti cardiolipin antibodies and lupus anticoagulant have been demonstrated in HIV patients [7]. Deficiencies of protein C, protein S, heparin cofactor II and antithrombins were also noted [8]. Hepatic damage by virus and Highly Active Anti-Retroviral Therapy (HAART) may lead to decreased production of coagulation factors causing coagulation abnormalities.

The study was designed to ascertain the basic coagulation defects in HIV infected individuals with routine parameters like platelet count, prothrombin time and aPTT with respect to their CD4 counts. The study group (n = 160) enrolled 120 cases and 40 controls. Among the 120 HIV infected cases, 62 cases were males and 58 were females. Among these patients, 76 were on ART and 44 were not on ART. The mean age of the HIV infected cases was 35. The demographic status of the present study is concordant with other studies like the one done by Karstaedt et al., in HIV infected cases with respect to age and sex ratio [9].

In our study, we found that PT and APTT were significantly increased in treatement naïve HIV individuals when compared to controls. This finding is concordant with Omoregie et al., who also reported increased PT and APTT in HIV patients [4]. Similarly, Eefje Jong et al., also reported prolonged PT and APTT in 6% and 2% of their study subjects respectively [10]. The cause for increased PT and APTT is due to endothelial activation, predisposing to thrombosis and consumption of coagulation factors. There is increased incidence of thrombosis in HIV patients due to hypercoagulable states like protein C, protein S, antithrombin III deficiency and presence of antiphospholipid antibodies. Myung S Park et al., also reported an elevated PT and aPTT in patients with hypercoagulable state [11].

PARAMETER	CD4 < 200cells/ cu.mm (n=28)		CD4 > 200cells/cu.mm (n=92)		p - value
	MEAN	S.D	MEAN	S.D	
PLATELET COUNT (x 10^9/L)	222	71.82	248	71.49	0.0993
PT (in sec)	19.16	5.47	17.69	4.06	0.1250
aPTT (in sec)	44.80	5.66	40.98	4.76	0.0006

[Table/Fig-3]: Comparison of platelet count, prothrombin time and activated partial thromboplastin time betweenhiv cases with CD4 < 200 and HIV cases with CD4 >200.

PARAMETER	NON ART CASES (N=44)		ON ART CASES (N=76)		p - value
	MEAN	S.D	MEAN	S.D	
PLATELET COUNT (x 10^9/L)	231	79.97	248	66.04	0.2063
PT (in sec)	18.23	4.55	17.92	3.77	0.6893
aPTT (in sec)	41.7	5.04	41.98	4.62	0.7553

[Table/Fig-4]: Comparison of platelet count, prothrombin time and activated partial thromboplastin time between HIV cases not on art and cases on art.

Anti cardiolipin antibodies and lupus anticoagulant noted in some HIV patients can also cause raised aPTT. Circulating coagulation inhibitors are also reported among HIV patients. Zeichner S et al., reported the presence of an acquired inhibitor for factor VIII in a patient with co-existing HIV and HCV [12]. The HAART drugs themselves and its damaging effect on liver can also lead to coagulation abnormalities.

We also found a significant decrease in platelet count in HIV patients. Omoregie et al., also observed a similar reduction in platelet count in HIV cases compared to controls [4]. The cause for decreased platelet count is due to increased platelet destruction by deposition of circulating immune complexes on platelets. Presence of specific antiplatelet antibodies and direct infection of megakaryocytes by HIV is also being hypothesized [13].

A CD4 count of less than 200 cells/mm^3 is one of the qualifications for the diagnosis of AIDS. Life threatening opportunistic infections commonly occur at this stage. We used a CD4 count cut-off of 200cells/mm^3 to divide the cases by disease severity. On comparing the platelet count, PT and aPTT in HIV positive cases with CD4 count < 200cells/mm^3 and those with count > 200cells/mm^3, we found that only aPTT is significantly higher in HIV cases with CD4 count less than 200cells/mm^3. The probable reason is due to aberration in intrinsic pathway and also the increased incidence of lupus anticoagulant and anticardiolipin antibodies in HIV infected cases with CD4 count less than 200cells/mm^3. Though PT is higher in HIV cases with CD4 count less than 200cells/mm^3 it was statistically insignificant. However, Omoregie et al., reported a statistically significant higher PT and aPTT in patients with CD4 count < 200cells/mm^3 compared to those with CD4 count > 200cells/mm^3. Similarly Platelet count is lower in HIV cases with CD4 count less than 200cells/mm^3 compared to cases with CD4 greater than 200cells/mm^3. But this did not stand statistically significant which is concordant with the above study.

De Andrade CM et al., reported that ART reduces the aPTT in HIV patients [14]. Similarly Ifeanyichukwu M et al., found significantly increased aPTT in HIV cases not on ART compared with HIV cases on ART [15]. They also reported a significantly higher PT in HIV cases on ART compared with HIV cases not on ART. They opined Lupus anticoagulant is likely cause of observed prolonged APTT in HIV positive not on ART. In supporting this, Awodu OA et al., reported decreased incidence of lupus anticoagulant in HIV patients on HAART compared to treatment naïve patients [16]. To substantiate this and to ascertain the role of ART in coagulation, we compared the parameters between treatement naïve HIV cases and those on ART. The parameters showed no statistical significance between the two groups. Thus HAART or liver damage by HAART is not the ideal cause for prolonged PT and aPTT in HIV positive cases. The other causes like endothelial dysfuction and hypercoagulable states predisposing to thrombosis leads to consumption of coagulation factors causing prolonged PT and aPTT.

LIMITATION

We used basic parameters like platelet count, PT, aPTT and CD4 count to study the coagulation defects in HIV patients, in relation to disease severity. This being a limitation of the study, more extensive works up in detecting the exact cause for prolonged PT and aPTT should be done. A panel of tests including lupus anticoagulant, anti cardiolipin antibodies, protein C, protein S, anti-thrombin III, factor assay, thromboelastography, platelet function tests, homocysteine levels, D dimer, etc. with viral load is desirable to substantiate the above results and to find the cause.

CONCLUSION

HIV infected individuals can be screened for coagulation defects with these basic parameters and if any abnormality found should be evaluated further. Since the coagulation defects become more severe as the disease advances, the basic coagulation tests like platelet count, PT and especially aPTT can be used as prospective screening test to assess severity in HIV patients in resource limited settings where CD4 count is not available.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, Shri Sathya Sai Medical College and Research Institute, Ammapettai, Chengalpet Taluk, Kancheepuram District, Tamil Nadu, India.
2. Professor, Department of Pathology, Tagore Medical College and Hospital, Vandalur-Kelambakkam Road, Rathinamangalam, Tamil Nadu, India.
3. Senior Resident, Department of Pathology, Kakatiya Medical College, Warangal, Telangana, India.
4. Associate Professor, Department of Pathology, Shri Sathya Sai Medical College and Research Institute, Ammapettai, Chengalpet Taluk, Kancheepuram District, Tamil Nadu, India.
5. Senior Consultant, Department of Pathology, Aware Global Hospitals, Hyderabad, Telangana, India.
6. Head of Department and Senior Consultant, Department of Pathology, Aware Global Hospitals, Hyderabad, Telangana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Thulasi Raman R,
Old No 37, New No 77, New Street, Mannady, Chennai- 600001, India.
E-mail: drthulas31@gmail.com

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