Original Research Article

Evaluation of first-line coagulation tests in Highly Active Antiretroviral Therapy (HAART) Naïve and Treatment Group

ABSTRACT

Background: Coagulation disorders are common in patients with Human Immunodeficiency virus (HIV). Coagulation abnormalities occur as a result of HIV-related thrombocytopenia, induced hepatotoxicity due to highly active antiretroviral therapy (HAART) that impairs liver function and diminishes the function and synthesis of coagulation factors. The aim of this study was to evaluate prothrombin time (PT), activated partial thromboplastin time (APTT), platelet count, mean platelet volume, plateletcrit and platelet distribution width in HAART-naïve HIV infected patients, HAART treated and HIV-seronegative controls.

Place and Duration of Study: Department of Haematology and antiretroviraltherapies (ART) clinic both of Enugu State University of Science and Technology Teaching Hospital, between March and June 2023.

Methodology: A total of 150 study participants, consisting of 50 HAART-naïve HIV-infected subjects, 50 HIV-infected subjects who were taking HAART, and 50 HIV-seronegative apparently healthy subjects, were included. Coagulation tests such as PT, APTT were determined by manual procedures. Platelet counts (PC), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW) were analyzed by Mindray/BC-5150 automated analyzer. The data were analyzed using SPSS version 21. Analysis of variance (ANOVA) and Pearson correlation analysis were used. P-Value < 0.05 was considered as statistically significant.

Results: Mild thrombocytopenia (100-<150 x 10^9/L) was found in 48% of HIV-infected subjects who were taking HAART, 76% mild thrombocytopenia and 24% moderate thrombocytopenia (50-<100 x10^9/L) was found in HAART-naïve HIV-infected subjects, but no thrombocytopenia was found in apparently healthy HIV-seronegative control. Prothrombin time and APTT were significantly higher, whereas PC and PDW was significantly lower in HIV-infected subjects (both who were taking HAART and HAART-naïve) than HIV-seronegative subjects ($p<0.05$). Prothrombin time and APTT were significantly higher, and PC was significantly lower in HAART-naïve HIV-infected subjects than HIV-infected subjects who were taking HAART. In Pearson correlation analysis, PT and APTT has shown a significant negative correlation with a PC in those taking HAART and HAART-naïve HIV-infected subjects, whereas significant positive correlation was found in HIV-seronegative subjects.

Conclusion:Prothrombin time and APTT significantly increased, whereas platelet count and PDW significantly decreased in HIV-infected subjects who were taking HAART and HAART-naïve. Basic coagulation parameters need to be monitored regularly in HIV-infected subjects in Enugu.

Keywords: Prothrombin, time, thromboplastin, platelet, HIV, HAART

1. Introduction

Human immunodeficiency virus (HIV) is a virus that attacks the body's immune system. If Human immunodeficiency virus is not treated, it can lead to acquired immunodeficiency syndrome (AIDS). Nigeria

has 1.9 million people living with HIV, 51,000 AIDS-related deaths and 1.7 million people on antiretroviral treatment, making them the second highest number in the world [1]. Adult HIV prevalence in Nigeria was approximated to 1.4% among people aged 15–49 years in the 2018 Nigeria AIDS indicator and Impact Survey (NAIIS). Nigeria AIDS indicator and Impact Survey 2018 HIV prevalence in south eastern Nigeria comprising Enugu, Ebonyi, Anambra and Abia was 1.8, 0.8, 2.2, 2.0 respectively [2]. The prevalence rate of HIV in Nigeria differs by region. In the South-South region, the prevalence was 5.5% which is the highest compared to other regions, while in the South-East region the prevalence of HIV was 1.8% [3]. A study done by Awofala and Ogundele suggests that these differences in prevalence rates may be because of factors such as levels of education, religion, socioeconomic status and cultural diversity [4]. Human immunodeficiency virus infection is an ailment with erratic manifestations such as opportunistic infections, autoimmune disorders and hematological abnormalities [5]. Hemostatic disorders occur very often in patients with human immunodeficiency virus. During HIV infection, hemostatic abnormalities arise as a result of acquired deficiency of anticoagulant proteins such as protein C and protein S [6, 7], heparin cofactor II and increased concentrations of coagulation and fibrinolytic markers [8-10]. Human immunodeficiency virus infection and highly active antiretroviral therapy mar liver function by causing hepatotoxicity that reduces the function and synthesis of coagulation factors [11]. Persistent immune activation and inflammatory condition in both HAART treated and untreated human immunodeficiency virus infections can lead to abnormal hemostatic changes. Immune-mediated knocking down of platelets by antibodies, opportunistic infections, decreased megakaryocytes, hypersplenism, malignancy and myelosuppressive effects of human immunodeficiency drugs may cause thrombocytopenia and changes in the hemostatic system of patients with human immunodeficiency virus [12, 13]. Some studies in HIV infection had reported changes in vascular function and endothelial cell dysfunction due to chronic inflammation [14, 15]. Defaced endothelial cells can begin a coagulation cascade and raise the levels of von Willebrand factor (vWf), which may increase platelet adhesion and clot formation [10]. Plasma levels of PT, APTT are elevated among HAART treated and untreated HIV-positive patients compared to HIV-seronegative controls [16]. Increased APTT, due to the production of a lupus anticoagulant and anticardiolipin antibodies, have been reported in patients with human immunodeficiency virus [17]. This study aimed to evaluate first-line coagulation tests in HAART-naïve HIV infected patients, HAART treated and HIV-seronegative controls.

2. Materials and Methods

2.1 Study Area: A case-control cross sectional study was conducted at the Enugu State University Teaching Hospital (ESUTH) ART (antiretroviral therapies) clinic from March to June 2023.

2.2 Study Population

HIV infected subjects who were taking HAART and HAART-naïve at the Enugu State University Teaching HospitalART clinic during the study period were included in the study for the case groups. A total of 150 study participants (50 HAART-naïve HIV-infected subjects, 50 HIV-infected subjects who were taking HAART, and 50 HIV-seronegative apparently healthy control subjects) were enrolled in the study. A simple random sampling technique was used to select study participants.

2.3 Inclusion and exclusion criteria

Serologically confirmed HIV-infected subjects who were HAART-naïve and were taking HAART for at least six months were included in the study. Subjects who were on anticoagulant therapy, pregnant women, subjects who were having hypertension and diabetes mellitus were excluded from the study.

Laboratory Analysis

Fourteen milliliters of venous blood was collected, five milliliters in EDTA anticoagulated tube for platelet count and platelet indices. Nine milliliters of the venous blood were transferred into plastic tube containing 1 ml of aqueous tri-sodium citrate anticoagulant for basic coagulation parameters analysis. The blood was

mixed well with anticoagulant. Without delay, the venous blood was centrifuged at 1200g for 15 minutes. Immediately after centrifuging, the plasma was removed from the plastic tube, vial and stopper. Prothrombin time was determined by Quick one stage method [18]. The APTT was determined by manual method, while platelet count and platelet indices were analyzed by Mindray/BC-5150 automated analyzer.

Statistical analysis

The data obtained were subjected to some statistical analysis such as the mean, standard deviation (SD), Analysis of variance (ANOVA) and Pearson moment of correlation using statistical package for social sciences (SPSS) version 21.

Result

The mean value of prothrombin time, activated partial thromboplastin time, platelet count, MPV, PCT and PDW among the study subjects are shown in table 1. The mean values of PT, APTT, PC, MPV, PCT and PDW of HIV-infected adults (both HAART and HAART-naïve groups) were significantly higher than the control group (P< 0.05). The platelet count and PDW were highest in apparently healthy control subjects and lowest in HAART-naïve HIV-infected subjects.

Table 1: Comparison of PT, APTT, platelet count and platelet indices in HAART, HAART-naïve groups and control

PT (sec)	APTT (sec)	PC (10^9/L)	MPV (fl)	PCT (%)	PDW (%)
TG (N=50)	31.06 ± 4.59	39.66 ± 1.77	148.86 ± 19.75	6.55 ± 0.33	0.09 ± 0.02	22.66 ± 2.20
HAN (N=50)	44.66 ± 3.85	50.92 ± 4.80	103.46 ± 7.39	5.15 ± 0.29	0.05 ± 0.01	20.03 ± 0.71
C (N=50)	11.96 ± 1.50	27.40± 5.60	262.60 ±39.87	8.12 ± 0.65	0.21 ± 0.05	32.34 ± 3.15
F(p) value	1058(<0.001)	360.56 (<0.001)	495.45 (<0.001)	0.23 (0.10)	0.25 (0.08)	412.90 (<0.001)
TG VS HAN	<0.001	<0.001	<0.001	1.000.17	0.91	
TG VS C	<0.001	<0.001	<0.001	0.42	0.11	<0.001
HAN VS C	<0.001	<0.001	<0.001	0.25	0.09	<0.001

Abbreviations: HAN=HAART-naïve, TG=treatment group, C=control

According to table 2, Pearson moment of correlation analysis showed that PC, MPV, PCT and PDW had been significantly and negatively correlated with PT and APTT in HIV subjects on HAART.

Table 2: Correlation of platelet indices with PT and APTT in HIV subjects on HAART

Parameters	PT		APTT	
	r	p-value	r	p-value
PC	-0.858	0.000**	- 0.687	0.000**
MPV	-0.547	0.000**	-0.598	0.000**
PCT	-0.661	0.000**	-0.697	0.000**
PDW	-0.564	0.000**	-0.620	0.000**

According to table 3 Pearson moment of correlation analysis showed that PC, MPV, PCT and PDW had been significantly and negatively correlated with PT and APTT in HAART-naïve HIV-infected subjects.

Table 3: Correlation of platelet indices with PT and APTT in HAART-naïve HIV-infected subjects

Parameters	PT		APTT	
	r	p-value	r	p-value
PC	-0.697	0.000**	- 0.653	0.000**
MPV	-0.638	0.000**	-0.762	0.000**
PCT	-0.586	0.000**	-0.704	0.000**
PDW	-0.353	0.012**	-0.060	0.679**

According to table 4, Pearson moment of correlation analysis showed that PC, MPV, PCT and PDW had been significantly and positively correlated with PT and APTT in apparently healthy control subjects.

Table 4: Correlation of platelet indices with PT and APTT in apparently healthy control subjects

Parameters	PT		APTT	
	r	p-value	r	p-value
PC	0.906	0.000**	0.930	0.000**
MPV	0.944	0.000**	0.905	0.000**
PCT	0.949	0.000**	0.957	0.000**
PDW	0.666	0.000**	0.733	0.000**

Mild thrombocytopenia was found in 48% of HIV-infected subjects who were taking HAART and 76% of HAART-naïve HIV-infected subjects, moderate thrombocytopenia was found in 24% of HAART-naïve HIV-infected subjects. But no thrombocytopenia was found in apparently healthy HIV-seronegative control group

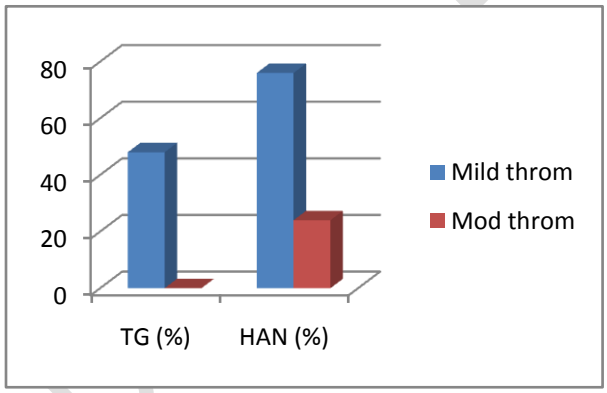

Fig1: Thrombocytopenia in HAART treated group and HAART naïve HIV infected subjects.

Abbreviation: TG=Treatment group, HAN=HAART naïve, Mild throm= mild thrombocytopenia, Mod throm= moderate thrombocytopenia

One hundred percent of subjects taking HAART and HAART naïve HIV infected subjects had prolonged prothrombin time (11-16 sec) [19]. 28% of HAART naïve HIV infected subjects had prolonged activated partial thromboplastin time aPTT (36-50 sec) [19], while normal PT and aPTT was found in apparently healthy control subjects.

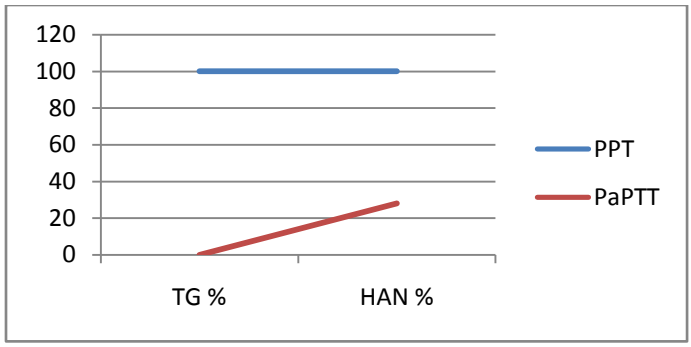

Fig 2: Showed percentages of prolonged PT and aPTT
Abbreviations: PPT= prolonged prothrombin time, Pa PTT= prolonged activated partial thromboplastin time

Discussion

This study was conducted to determine some basic coagulation parameters in HIV patients on HAART and those that have not started taking highly active antiretroviral treatment. Some common medical tests used to assess the function of coagulation system are prothrombin time, activated patial thromboplastin time, platelet count, platelet function test, fibrinogen test and thrombodynamics test. Studies had shown that coagulation abnormalities are common in HIV infection. Highly active antiretroviral treatment reduced the mortality of HIV but raised coagulopaties.HIV infection and HAART reduces liver function by generating hepatotoxicity that impairs the function and synthesis of coagulation factors [11]. Opportunistic infections, reduced megakaryocytes, immune-mediated destruction of platelets by antibodies and thrombocytopenia due to toxic and myelosuppressive effects of HIV drugs have been reported in HIV infection [20].The findings of this study showed that the prothrombin time was significantly higher in HIV-infected subjects on HAART and HAART naïve than in HIV- seronegative control subjects. The aPTT value was also significantly higher in HIV-infected subjects (both on HAART and HAART naïve) than control subjects. The finding was in line with study done by Abdollahi *et al*. [21], Ifeanyichukwu*etal*. [22] and Seyoun*etal*. [23] who reported that PT and aPTTwere significantly higher in HIV-infected adults than HIV-seronegative adults. The normal adult range of platelet count is 280 ±130 X 10^9/l. A platelet count of more than 450 X 10^9/l is a condition called thrombocytosis; while platelet count less than 150 X 10^9/l is known as thrombocytopenia. In this study the values of the platelet count were lower in HAART- naïve HIV subjects than HAART treated HIV infected subjects. Again, the platelet count of both HAART- naïve HIV subjects and HAART treated HIV infected subjects were lower compared to HIV-seronegative subjects. Mild thrombocytopenia was found in 48% of HAART treated HIV infected patients, while moderate thrombocytopenia was found in 24% of HAART-naïve HIV infected patients. However, our finding was nearly similar with study done in India [24], Rwanda [25] and Ethiopia [26] where they found 18%, 13.5% and 5.9% of thrombocytopenia in people living with HIV/AIDS (PLWHA). The correlation analysis showed strong negative significant correlation between PT, aPTT and platelet indices in HAART-naïve HIV-infected subjects and HAART treated HIV infected subjects, while strong positive significant correlation were observed in HIV –seronegative subjects. The result of this study differs from study done in Owerri Nigeria where positive correlations were found in HIV positivesubjects [27].

Conclusion:

Prothrombin time and aPTT was significantly higher in HAART-naïve HIV-infected subjects and HAART treated HIV infected subjects compared with HIV- seronegative control subjects, whereas platelet count and PDW was significantly higher in HIV- seronegative control compared to HAART-naïve and HAART treated group. In correlation analysis, PT and aPTT negatively correlalated with platelet indices in HAART-naïve and HAART treated group, while in HIV–seronegative subjects PT and aPTT positively correlated with platelet indices. Therefore, in addition to complete blood cell count, coagulation tests like PT, aPTT need to be included in routine tests for managements of HIV infected patients in resources-poor setting, where CD4 count are not done routinely.

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