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The values of activated partial thromboplastin time (APTT) among HIV positive patients in FMC Owerri

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KEYWORDS

Activated Partial Thromboplastin Time, HIV, HIV Positive subjects

A B S T R A C T

The values of APTT were carried out. One hundred and sixty four subjects were sampled, comprising one hundred and fourteen HIV positive subjects and fifty HIV negative subjects which served as the control. APTT was analyzed using standard techniques. APTT was significantly higher ($P < 0.05$) in HIV positive subjects (42.86 ± 7.10) when compared with the HIV negative subjects (37.90 ± 3.98).

Introduction

Human Immunodeficiency Virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired Immunodeficiency Syndrome (AIDS) (Weiss, 1993). This is a condition in humans in which progressive failure of the immune system allows life threatening opportunistic infection and cancers to thrive.

Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate or breast milk of the infected person to HIV free person. Within these body fluids, HIV is present as both free virus particles and virus within infected immune cells.

The four major routes of transmission are unsafe sex, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth (perinatal transmission) (Fox and Cottler-Fox, 1992).

Viruses such as HIV cannot grow or reproduce on their own, the need to infect the cells of a living organism in order to replicate. The human immune system usually detects and kills viruses fairly quickly, but HIV attacks the immune system itself, the very thing that would normally get

rid of the virus (Ascher and Sheppard, 1990).

HIV is a causative organism of autoimmune deficiency syndrome which was recognized as a new disease syndrome in the early 1980's in the USA with the unusual occurrence of pneumocystis carinii pneumonia and Kaposi's sarcoma in previously healthy young men (Greene, 1991). This retrovirus was isolated from a young homosexual man with lymphadenopathy. The virus was identified and classified in the family Retroviridae genus lentiviranae (Baker *et al.*, 2007).

Under the electron microscope, the viruses were revealed as a cylindrical core with nucleic acid cloned and sequenced. The cylindrical core is 80-130nm in diameter, it has a unique three layered structure, and innermost is the genome nucleocapsid complex. This complex is enclosed within a capsid which is surrounded by a host cell membrane derived envelope, from which viral envelope glycoprotein 'spikes' project. HIV infects a wide variety of tissues in humans including the marrow, lymph node, brain, skin and bowel (Baker *et al.*, 2007). This retrovirus differs from other retroviruses such as human T lymphotropic virus (HTLV) 1 and 2. The virus was eventually named Human Immunodeficiency Virus (Cohan *et al.*, 1986).

It is transmitted mostly sexually in blood or blood products and pre-natally. The most at risk of acquiring HIV infection are homosexuals, injecting drug misusers and those with bisexual orientation. Others include individuals receiving unscreened blood or blood products, infants born of infected women. There are various strains of HIV and are designated by a code with geographically informative letters and

sequential numbers placed either in brackets, or as a number, or as a subscript. Example HIV_{-sf33} and HIV -2 (Pantaleo and Fauci, 1995).

If there is a laboratory evidence of HIV infection, certain indicator diseases that require presumptive and definitive diagnosis are diagnostic of AIDS, AIDS is an illness characterized by one or more indicator diseases (Safrit and Koup, 1995).

Acute HIV is usually characterized by fever, malaise, lymphadenopathy and rash. These conditions are subclinical. A chronic infection of AIDS that follows is asymptomatic in early stages. If an individual is infected with this virus, the virus acts so quick destroying the immune system making the individual prone to little infections. HIV is present all over the world and the long term consequences of this pandemic will affect every country one way or another over time. This is an evolving pandemic threatening global public health and health care provision, as well as political and economic stability (Kuby, 1997).

Coagulation

This is a complex physiological process by which blood forms clot. It is important part of haemostasis (the cessation of blood loss from damaged vessel), wherein a damaged blood vessel wall is covered by a platelet and fibrin-containing clot to stop bleeding and begin repair of the damaged vessel) (Shapiro, 2003).

Coagulation results from interactions among vessel wall, platelet and coagulation factors. When an injury occurs that results in bleeding, the coagulation system is activated and plugs the hole in the bleeding vessel while still keeping blood flowing through the vessels by preventing the clot from

getting too large. The end result is the formation of insoluble fibrin threads that link together at the site of injury, along with aggregated cell fragments called platelets to form a stable blood clot. The clot prevents additional blood loss and remains in place until the injured areas have healed. The clot is eventually removed as the injured site is healed. In normal healthy individuals, this balance between clot formation and removal ensures that bleeding does not become excessive and that clots are removed once they are no longer needed (Shapiro, 2003).

A number of coagulation abnormalities have been described in human immunodeficiency virus (HIV) disease. High levels of plasma von Willebrand factor have been reported in HIV disease and might be indicative of activated endothelium. Endothelium is involved in important homeostatic mechanisms of non-thrombotic vascular surfaces, vascular tone regulation and immunomodulation (Karpatkin *et al.*, 2002). Injured endothelium leads to localized inflammatory response of which the direct consequence is the occurrence of occlusive thrombosis events mediated between leucocyte recruitment and platelet adhesion and aggregation, blood clotting activation and fibrinolysis derangement. HIV infection has been associated with endothelial dysfunction. Since HIV infection is associated with endothelial dysfunction it may therefore result in activation and consumption of coagulation factors and ultimately coagulation defect (Omoriege *et al.*, 2009).

In HIV infection, the liver is affected. The liver is the major organ responsible for the synthesis of most coagulation factors and infection of the liver by HIV can lead to abnormal production of coagulation factors. The CD4⁺ count is used to measure immune

status and HIV disease progression (Tolstrup *et al.*, 2004).

Prothrombin time (PT) and an activated partial thromboplastin time (APTT) are screening tests for the extrinsic and intrinsic clotting systems respectively. They detect deficiency or inhibition of clotting factors in either system, and are the first tests in screening for coagulation disorders. As HIV infection progresses, endothelial dysfunction and liver damage will increase and this may result in severe clotting impairment.

In reference to the abnormality of coagulation in HIV positive individuals, the coagulation disorders will be investigated, by considering platelet count, prothrombin time, activated partial thromboplastin time, and blood fibrinogen concentration, as well as CD₄ count and factor VII concentration.

Platelet count is a diagnostic test that determines the number of platelets in the patient's blood. Platelets which are also called thrombocytes, are small disk-shaped blood cells produced in the bone marrow and involved in the process of blood clotting. There are normally between 150,000-450,000 platelets in each microlitre of blood. Low platelet count or abnormally shaped platelets are associated with bleeding disorders (Henry, 2001).

Prothrombin time (PT), is one of the coagulation factors produced by the liver. One of the final steps of the cascade is the conversion of Prothrombin (factor II) to thrombin. The Prothrombin time test evaluates the integrated function of the coagulation factors that comprises the extrinsic and common pathways.

The international Normalized Ratio (INR) is used to standardize PT result gotten (Horsti *et al.*, 2005).

Activated partial thromboplastin time (APTT), is a screening test that is done to help evaluate a person's ability to form blood clot. It assesses the amount as well as the function of coagulation factors XII, IX, VII, X, V, II and I which are part of haemostasis (Pagana and Pagana, 2006).

Fibrinogen (factor 1) is a soluble plasma glycoprotein synthesized by the liver and is converted by thrombin into fibrin during blood coagulation.

Fibrinogen deficiency (hypo-fibrinogenemia) or disturbed function of fibrinogen can lead to either bleeding or thromboembolic complications (Acharga & Dimichele, 2008).

CD4 count is the number of CD4 cells per microlitre of blood. It is used to stage the patient's disease, determine the risks of opportunistic illness, assess prognosis and guide decisions about when to start antiretroviral treatment (CDC, 2009).

To determine the value of APTT among HIV positive subjects in FMC Owerri.

Materials and methods

The sample size for this study was calculated based on Ifeoma *et al.*, (2010) 8.1% prevalence of HIV in Owerri in 2010.

$$n = \frac{1.96^2 \times 0.08 (1.00-0.08)}{(0.05)^2} = 114 \text{ samples}$$

Informed consent

Participant information sheet (PIS) was given to the prospective participants. After reading and understanding the PIS, questions were asked and proper explanations given. They consented to

participate in the study by signing the informed consent form.

Eligibility criteria

Informed consented subjects (both HIV/AIDS positive patients and HIV negative controls).

Subjects

One hundred and fourteen HIV positive subjects aged 18-65 years attending Heart to Heart clinic of Federal Medical Centre, Owerri were screened. Fifty HIV negative subjects were also screened and they served as controls.

Sample collection

Informed consented subjects were sampled. Blood was collected from all the subjects, 4.5mls of which was added into trisodium citrate container containing 0.5mls of trisodium citrate for coagulation studies (PT, APTT, Fibrinogen concentrate and factor viii assay).

The sample was spun at 3000rpm for 10 minutes, and then the clear plasma was collected into a clean dry plastic container. The test was performed using Rayto semi auto coagulation analyzer, RT-2204C model manufactured by Rayto life and analytical sciences co. Ltd.

Activated partial thromboplastin time (APTT) (Modified Kaolin Method)

It measures the capacity of the blood to form fibrin clot, which indicates the overall efficiency of the intrinsic pathway. In the presence of Kaolin when pre-incubated, factor XIIa is formed and cleaved to factor XI to XIa, with the presence of calcium coagulation takes place.

The APTT reagent was reconstituted with 4mls of distilled water, mixed by inversion and allowed to stand at room temperature for 30mins. 50µl of the sample was added into a test cuvette. Then 50µl of the reconstituted APTT reagent was added to the sample, the mixture was incubated for 3mins at 37⁰C. 50µl of calcium chloride was rapidly added and the time of clotting in seconds was recorded.

Statistical analysis

The data obtained were subjected to some statistical analysis such as the mean (X), standard deviation (SD), standard error of mean (SEM), student’s t-test and Pearson moment of correlation using statistical package for social sciences (SPSS) version 17. The results were expressed in mean ± standard error of mean.

Results and Discussion

The mean value of APTT among the study subjects are shown in table below. APTT was significantly lower in HIV seronegative

subjects (control). (P< 0.05) when compared with the HIV positive subjects.

HIV infection is associated with endothelial dysfunction and liver damage. Both endothelial dysfunction and liver damage can result in coagulation defects. It is therefore expected that as the HIV infection progresses, the coagulation abnormalities will increase, (Linder *et al.*, 1970)

The APTT in HIV patients was significantly higher than the values obtained in HIV Negative controls (P < 0.05) which also agrees with the works of Omoregie *et al.* (2009), who reported a significant increase PT and APTT among HIV positive patients. HIV infection is associated with endothelial damage, which can result in activation of coagulation pathways and consumption of clotting factors (Jenkins *et al.*, 1991). Also, Lupus anticoagulant (LA), anticardiolopin antibodies (CL) and Liver damage are seen in HIV infected patients. All these (Damaged endothelium, LA. aCL and Liver damage) can affect APTT Values observed among HIV patients (Ehmam *et al.*, 1997).

Table.1 The mean values of APTT among the study group

Parameter	HIV Negative subjects (n = 50)	HIV Positive subjects (n =114)	P Value
APTT (s)	37.90 ± 3.98	42.86 ± 7.10	P < 0.05

NB: Figures are in mean ± standard deviation; n= number tested.

Conclusion

Coagulation abnormalities have been described in HIV disease. HIV infection has been associated with endothelial dysfunction which may result in activation and consumption of coagulation factors and ultimately coagulation defect.

The findings of this study indicates an increase in PT and APTT in HIV Positive

individuals with a decrease in CD4 and platelet count whereas fibrinogen and factor VIII concentrations showed no significant changes .However, there were positive correlations between CD4 count and platelet, CD4 Count and PT, CD4 count and APTT, and PT and APTT, while no correlation was observed with fibrinogen and factor viii concentrations.

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