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Original Research Article

Effect of CD4 Counts on Coagulation Parameters among HIV Positive Patients in Federal Medical Centre, Owerri, Nigeria

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Abstract

The correlation of values of CD4 count, platelet, PT, APTT, fibrinogen and factor viii was 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. PT, APTT, Fibrinogen, Factor VIII, Platelet and CD4 count were analyzed using standard techniques. Among the HIV positive subjects, platelet count did not differ significantly (p>0.05) between those with CD4 count <200 cells/µl and those with CD4 count ≥ 200 cells/µl. However, PT and APTT showed significant changes and respectively, in HIV Positive subjects with CD4 count <200 cells/µl.

Keywords

Factor VIII concentration
Fibrinogen
Platelet
Prothrombin
Thromboplastin

Introduction

Coagulation 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 clothing 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 (Omoregie 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). Prothrombintime (PT) an activated partial thromboplastin time (APTT) are screening tests for the extrinsic and intrinsic clothing 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, endothlieal 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 CD_4 count and factor VII concentration. Platelet count is a diagnostic test that determines the number of platelets in the patient's blood. Platelet 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 11) 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). 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). The aim of the present study was to determine the values of some coagulation factors on different values of CD4 count.

Materials and methods

The sample size for this study was calculated based on Ijeoma 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, Nigeria were screened. Fifty HIV negative subjects were also screened and they served as controls.

Sample collection

Informed consented subjects were sampled. About 7mls of 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. The remaining 2.5mls was added into ethylene diamine tetra acetic acid (EDTA) bottle and mixed immediately by reverse uniform inversion for platelet and CD4 counts. Platelet count was performed using sysmex automated haematology analyzer KY21N model manufactured by sysmex corporation Kobe, Japan, while CD4 count was performed using cyflow counter 1 manufactured by paretic Gmbh, Germany.

Prothrombin time (Pt) (Quick one stage method, 1935)

The method measures the time it takes blood to form fibrin clot in the presence of an optimal concentration of tissue extract (thromboplastin) and measures the extrinsic haemostatic pathway. The PT reagent was reconstituted by adding 5ml of its diluents mixed by inversion and allowed to stand at room temperature for 30 min. The reconstituted PT reagent was pre-warmed at 37°C for 10 min. 50µl of the sample was added into the test cuvette and incubated for 2 min at 37°C. Then 100µl of the prewarmed PT reagent was rapidly added and the time of clotting in second recorded.

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 30 min. 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 3 min at 37°C. 50µl of calcium chloride was rapidly added and the time of clotting in seconds was recorded.

Fibrinogen assay (Clauss Method, 1957)

In the presence of high concentration of thrombin, diluted plasma will give a required time of clot formation which is inversely proportional to the fibrinogen concentration. The sample was diluted with 450µl of imidazole buffer to 50µl of sample to give a 1:10 dilution. 200µl of pre-diluted sample was added to the test cuvette, the sample was incubated for 5 min at 37°C. Then 100µl of bovine thrombin was added. The time of clot was recorded in seconds, while the concentration was recorded in mg/dl.

Platelet (Direct Current Detection Method)

By automation using Sysmex automated haematology analyzer KY21N model manufactured by Sysmex Corporation Kobe, Japan. The aspirated blood sample is measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer chamber, which has a minute hole- the aperture which also contains the electrodes in which direct current flows. Blood cells suspended in the diluents sample pass through the aperture, causing direct current resistance to change between the electrodes and the blood cell size is detected as electric pulses, and the histogram determined by the pulse sizes.

The sample in EDTA bottle was places in the spiral mixer and allowed to mix very well. Whole blood mode was activated in the LCD screen, the sample number (code) was inputted *via* the key board and then the enter key. Then the sample was mixed very well again, the cap was removed and inserted into the probe, on that condition, the start switch was pressed. The LCD screen displays analyzing, the sample was removed and recapped. The unit executes automatic analysis and displays the result on the screen.

CD4 count

By automation using Cyflow counter 1 manufactured by PartecGmbh Germany. The sample flows through a capillary into the flow cuvette. Here, the sheath fluid takes it with it. Because of the specific flow cuvette geometry the sheath and sample current are speeded up. You get a very narrow, laminar flowing sample stream. This means, the sample stream does not get

mixed with the sheath stream. The cells or particles labeled with fluorescent colouring pass the measuring area one after the other. The cells or particles are individually illuminated by the excitation light and the fluorescent light is measured and analyzed.

The machine was washed twice with 960µl cleansing solution, rinsed with 960µl of rinse solution. The control was run using count check bead to get an acceptable peak. Then 20µl of monoclonal antibody was added to 20µl of blood, mixed and incubated in the dark for 15 min. Then, 800µl of buffer was added and slotted the tube in the machine. Results displayed on the LCD screen were recorded.

Factor VIII assay (One Stage Assay of Factor VIII)

The one stage assay for factor VIII is based on the APTT. It consists of comparing the ability of dilutions of the patient's plasma and of standard plasma to correct the APTT of plasma known to lack factor viii but containing all other factors required for normal coagulation.

The kaolin, phospholipid and $CaCl_2$ were placed at 37°C and the patient's standard and substrate plasma in the ice bath. A 1 in 10 dilution of the test and standard plasma in buffered saline was made in plastic tubes in the ice bath. Using 0.2 ml volume, doubling dilutions in buffered saline was made to obtain 1 in 20 and 1 in 40 dilutions. 0.1ml of the three dilutions (1 in 10, 1 in 20 and 1 in 40) in glass tubes was made. To each dilution 0.1ml of freshly reconstituted substrate plasma was added and warmed at 37°C. The phospholipid/kaolin mixture of 0.2ml was added and the contents were mixed in the tubes. At 10 min, $CaCl_2$ was added and time was noted, and the clotting time was recorded. The clotting time of the test and the standard was plotted against the concentration of factor VIII. The normal range is 50-200 IU/L.

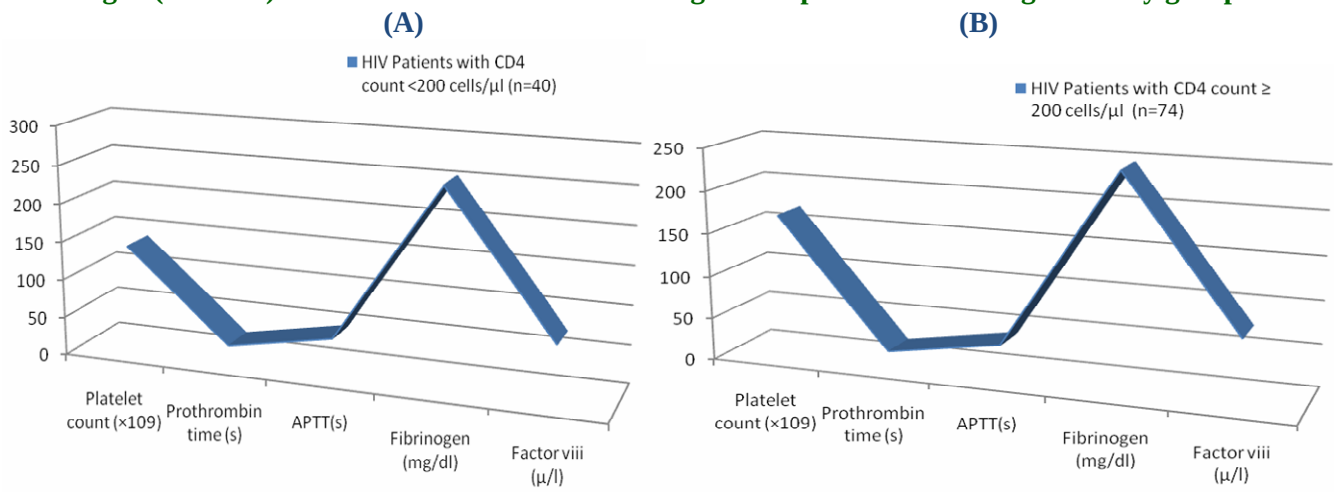
Statistical analysis

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

With a CD4 count of 200 cells / µl as baseline, the platelet counts of HIV patients with CD4 < 200 cells/µl were lower ,but did not differ significantly (p>0.05) from those with CD4 count ≥ 200 cells/µl. However, PT and APTT were significantly higher (p<0.05) respectively in HIV positive subjects with CD4 count < 200 cells/ µl. whereas, fibrinogen and factor viii concentration showed no significant difference (p>0.05) and the results are summarized in Fig. 1.

Fig. 1 (A and B): The effect of CD4 counts on coagulation parameters among the study groups.



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., 1990). The CD4 count is used as a measure of immune status and disease progression, and values < 200 cells/µl make the patients vulnerable to opportunistic infections and other AIDS- defining conditions. Thus, on the basis of CD4 count, the studied coagulation parameters may also indicate disease progression. However, the result obtained in this study, showed no significant difference (p>0.05) in the platelet count of HIV- positive patients whose CD4 Count were <200 cells /µl and those with CD4 count ≥ 200 cells /µl, which also agrees with the works of Omorege et al. (2009).

It has been reported that patients with AIDS have decreased platelet production whereas patients with early onset HIV infection are more likely to have increased peripheral destruction of platelet by anti platelet antibodies. Both Mechanisms result in low platelet count in HIV-positive patients with CD4 Count < 200 cells/µl and those with ≥ 200 cells/µl and may explain the findings in this study. The PT (p< 0.05) and APTT (p<0.05) of HIV -positive patients with CD4 count <200 cells/µl were significantly higher than those of HIV patients with CD4 Count ≥ 200 cells/µl. The possible explanation is that as the HIV infection progressed, which is characterized by reduction in CD4 count, endothelia activation and possibly liver damage may increase, resulting in consumption of blood clotting factors or abnormal production of liver dependent clotting factors, resulting in increased PT and APTT (van Gorp et al., 1999).

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 the present study indicate an increase in PT and APTT in HIV Positive individuals with a decrease in CD4 and platelet counts; 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 counts; between APTT, and PT and APTT; while no correlation was observed with fibrinogen and factor VIII concentrations.

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