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EVALUATION OF COAGULATION PROFILE PATIENTS WITH PULMONARY TUBERCULOSIS AND HUMAN IMMUNODEFICIENCY VIRUS IN OWO, ONDO STATE, NIGERIA

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ABSTRACT

HIV continues to be a major global public health issue, HIV infects vital cells in the human immune system, such as helper T cells (specifically CD4+T cells), macrophages, and dendritic cells. Loss of CD4 cells leads to the development of serious illnesses, or 'opportunistic infections'. Active infection of tuberculosis (TB) occurs more often in people with HIV/AIDS as opportunistic infection. The aim of this research was to compare Prothrombin time test (PT) and Partial thromboplastin time with kaolin in HIV and Tuberculosis patients. Two hundred participants at FMC Owo were enrolled into this study. There was a significant increase ($p<0.05$) in the PT and PTTK of HIV positive and TB positive subjects including Co-infected HIV and TB subjects when compared with apparently healthy individuals, There was no significant difference ($p>0.05$) in the prothrombin time of HIV positive subjects when compared with TB positive subjects. This study revealed that HIV and TB affects haemostasis by prolongation of Prothrombin time test (PT) and Partial thromboplastin test with kaolin (PTTK). Treatment strategies targeting haemostasis and endothelial activation may be of interest for evaluation in patients with HIV, TB and HIV-tuberculosis co-infection who continue to have an unacceptably high mortality rate.
Keywords*: HIV, tuberculosis, prothrombin time, activated partial thromboplastin time, haemostasis, HIV-TB co-infection*

INTRODUCTION

HIV continues to be a major global public health issue, having claimed more than 32 million lives so far. However, with increasing access to effective HIV prevention, diagnosis, treatment and care,

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including for opportunistic infections, HIV infection has become a manageable chronic health condition, enabling people living with HIV to live long and healthy lives **(**WHO, 2019). The virus spread through certain body fluid to attack the body immune cells that move around the body to fight infections. HIV infects vital cells in the human immune system, such as helper T cells (specifically CD4+T cells), macrophages, and dendritic cells. HIV cannot replicate on its own, so in order to make new copies of itself, it must infect cells of the human immune system. CD4 cells are white blood cells that play a central role in responding to infections in the body. Over time, CD4 cells are killed by HIV and the body's ability to recognise and fight some types of infection begins to decline. When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections, leading to the development of AIDS (Brencley, *et al*., 2012).

Tuberculosis (TB) is an infectious disease usually caused by *Mycobacterium tuberculosis* (MTB) bacteria. Tuberculosis generally affects the lungs, but can also affect other parts of the body. Most infections do not have symptoms, in which case it is known as latent tuberculosis. About 10% of latent infections progress to active disease, which if left untreated, kills about half of those affected. Tuberculosis is spread through the air when people who have active TB in their lungs cough, spit, speak, or sneeze, People with latent TB do not spread the disease. Active infection occurs more often in people with HIV/AIDS as opportunistic infection (WHO, 2010).

The Prothrombin time is a measure of the integrity of the extrinsic and final common pathways of coagulation cascade (Bibas *et al*., 2011). A prothrombin time test can be used to check for bleeding problems. A PT test may also be called an INR test. INR (international normalized ratio) stands for a way of standardizing the results of prothrombin time tests, no matter the testing method. Prothrombin time is an important test because it checks to see if five different blood clotting factors (factors I II, V, VII, and X) are present therefore measures the quality of the extrinsic pathway (as well as the common pathway) of coagulation (Bibas *et al*., 2011).

The Partial thromboplastin time with kaolin (PTTK) or activated partial thromboplastin time (aPTT or APTT) is a blood test that characterizes coagulation of the blood. A historical name for this measure is the kaolin-cephalin clotting time (KCCT), reflecting kaolin and cephalin as materials historically used in the test as surface activator. Apart from detecting abnormalities. Blood clotting, partial thromboplastin time is also used to monitor the treatment effect of heparin, a widely prescribed drug that reduces blood's tendency to clot, It examines the intrinsic pathway of coagulation (factors VIII, IX, XI, XII) (Erikkson *et al*., 2014). The partial thromboplastin time test provides a convenient and sensitive screening procedure for deficiencies of thromboplastic

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factors, especially factors VIII and IX the test is carried out after preincubating the plasma for 10 minutes with kaolin, and Inosithin is used as a platelet substitute. With the test modified in this way the clotting time is prolonged, though the range of differences among normal subjects is unaltered, and plasmas with factor VIII concentrations below about 30%, *i.e.*, in undiluted plasma, would be unlikely to be regarded as normal (Erikkson *et al*., 2014).The partial thromboplastin time with kaolin may be similarly modified as a screening test for factor IX deficiency.

MATERIALS AND METHOD

STUDY AREA

SUBJECTS

Subjects for this study were recruited from HAART laboratory FMC which serves as a tertiary health institution in Owo ondo state. Ondo state lies at 710'Ń and 505'É and occupies a land area of 15,500km^2 while Owo lies at latitude 710'59, 988'E with altitude of 306m. The Study is to be carried out in Southwestern part of Nigeria.it has an estimated population of 276,593 (National Population Commission, 2006). It is located within low rain forest zone of Nigeria and has two seasons. Dry and Wet. The dry season lasts from mid-October to march while rainy season lasts from April to September.

SAMPLE SIZE: The work comprised of 200 subjects consisting of 50 HIV positive subjects and 50 subjects who were HIV negative, 50 subjects who were tuberculosis Patients and 50 subject HIV-Tuberculosis patients.

SAMPLE COLLECTION

Venous Blood

Blood samples was obtained from each subject by applying tourniquet around the arm above elbow. The ante-cubital fossa will be disinfected with a 70% alcohol soaked swab. 5ml of venous blood will be withdrawn with 5ml syringe and 21G needle, 4.5ml of blood will be dispensed into 5ml sterile universal bottle containing 0.5ml of 3.2% trisodium citrate solution in a ratio of blood – citrate 9:1 (v/v) as an anticoagulant and thoroughly mixed by inverting the container several times gently. Plasma was subsequently prepared by centrifugation of the blood at 200g for 15minutes at room temperature (approximately 4000 revolutions per minute) to obtain platelet poor plasma (PPP).

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INCLSION CRITERIA

Patients diagnosed with HIV positive and patients diagnosed with tuberculosis within adult hood (18years – 65years) were recruited into the study.

EXCLUSION

Those with known history of blood coagulation/ bleeding disorders and patients with any condition other than HIV and Tuberculosis were excluded.

METHOD OF ASSAY

PROTHROMBINE TIME PROCEDURE (PT)

Procedure

Into a clean glass tube, 100ul of brain thromboplatin was added, it was incubated for 2minutes at 37^0C, 100ul of plasma was dispensed into it, 100ul of prewarmed calcium chloride was also added and stop watch was started. The Tube was gently titled at 2 seconds intervals (returning to the water bath between titling) and the time for the formation of a clot was recorded in seconds. Control test was equally run along with the plasma for each batch of test.The test was carried out in duplicate for both subject's sample and a normal control, and the mean value was obtained

PARTIAL THROMBOPLASTIN TIME WITH KAOLIN

PROCEDURE

Into a cleaned glass tube, 200ul of kaolin/platelet substitute was dispensed which was placed in a water bath kept at 37C, 100ul of plasma was added, after one minute, 100ul of 0.025M calcium chloride ($CaCl_2$) was added. The contents will be added gently mixed and stopwatch was started immediately. Control test was equally run along with the test plasma for each batch of the test.

STATISTICALANALYSIS

The statistical analysis was carried out using statistical package for social science (SPSS version 23.0 software). All values will be expressed as mean± standard error of the mean. Results from the specimen was compared. Statistical significance was set at $p<0.05$. In all cases p value of equal or greater than 0.05 was taken as statistically non-significant. The data was presented on tables.

RESULT

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Table 1: Mean ± SD of PT and PTTK of HIV positive subjects and the control

Parameters	Test	Control	P-value
PT(s)	16.79±2.18	14.36±0.79	0.035
PTTK(s)	44.23±4.41	35.20±4.87	0.000

Legend: PT= Prothrombin Time Test, PTTK= Partial Thromboplastin Time with Kaolin, HIV= Human Immunodeficiency Virus

PT AND PTTK OF HIV POSITIVE SUBJECTS AND THE CONTROL

This study was conducted to assess the prothrombin time (PT) and partial thromboplastin time with kaolin among HIV positive individuals. The mean ± SD values of prothrombin HIV subjects and the control are 16.79±2.18 sec and 14.36±0.79 sec respectively. There was a significant increase ($p<0.05$) in the prothrombin time of HIV positive subjects when compared with apparently healthy individuals. The mean ± SD values of partial thromboplastin time (PTTK) of HIV positive subjects and the control are 44.23±4.41 sec and 35.20±0.79 sec respectively. There was a significant increase (<0.05) in partial thromboplastin time among HIV positive subjects compared to apparently healthy individuals.

Table 2: Mean ± SD of PT and PTTK of TB positive subjects and control

Parameters	Test	Control	P-value
PT (s)	17.00±1.79	14.36±0.79	0.000
PTTK(s)	44.23±3.51	35.20±4.87	0.000

Legend: PT= Prothrombin Time Test, PTTK= Partial Thromboplastin Time with Kaolin, TB= Tuberculosis

PT AND PTTK OF TB POSITIVE SUBJECTS AND CONTROL

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This study was also conducted to assess the prothrombin time and partial thromboplastin time with kaolin among TB positive individuals. The mean ± SD values of Prothrombin time of TB subjects and the control are 17.00±1.79 sec and 14.36±0.79 sec respectively. There was a significant increase ($p<0.05$) in the prothrombin time of TB positive subjects when compared with apparently healthy individuals. The mean ± SD values of partial thromboplastin time (PTTK) of TB positive subjects and the control are 44.23±3.51 sec and 35.20±4.87 sec respectively. There was a significant increase (<0.05) in partial thromboplastin time among TB positive subjects compared to apparently healthy individuals.

Table 3: Mean ± SD of PT and PTTK of Co-infection of HIV and TB and the control

Parameters	Test	Control	P-value
PT	17.08±1.81	14.36±0.79	0.002
PTTK	45.25±4.66	35.20±4.87	0.000

Legend: PT= Prothrombin Time Test, PTTK= Partial Thromboplastin Time with Kaolin, HIV= Human Immunodeficiency Virus, TB= Tuberculosis

PT AND PTTK OF COINFECTION OF HIV AND TB AND THE CONTROL

This study was also conducted to assess the prothrombin time and partial thromboplastin time with kaolin among co-infected HIV and TB individuals. The mean±SD values of Prothrombin time of co-infected HIV and TB subjects and the control are 17.08±1.81 sec and 14.36±0.79 sec respectively. There was a significant increase ($p<0.05$) in the prothrombin time of co-infected HIV and TB subjects when compared with apparently healthy individuals. The mean ± SD values of partial thromboplastin time (PTTK) of coinfected HIV and TB subjects and the control are 45.25±4.66 sec and 35.20±4.87 sec respectively. There was a significant increase (<0.05) in partial thromboplastin time of co-infected HIV and TB subjects compared to apparently healthy individuals.

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Table 4: Mean ± SD of Comparison of PT and PTTK between HIV and TB positive subjects

Parameters	HIV	TB	P-value
PT	15.79±2.18	17.09±1.97	0.055
PTTK	44.22±4.12	44.23±3.51	0.994

When post hoc of HIV positive and coinfection, TB and coinfection were done there is no significant difference were recorded.

Legend: PT= Prothrombin Time Test, PTTK= Partial Thromboplastin Time with Kaolin, HIV= Human Immunodeficiency Virus, TB= Tuberculosis

PT AND PTTK BETWEEN HIV AND TB POSITIVE SUBJECTS

The mean ± SD values of Prothrombin time of HIV positive subjects and TB positive subjects are 15.59±2.18 sec and 17.09±1.97 sec respectively. There was no significant difference ($p>0.05$) in the prothrombin time of HIV positive subjects when compared with TB positive subjects. The mean ± SD values of partial thromboplastin time (PTTK) of HIV positive subjects and TB positive subjects are 44.22±4.12 and 44.23±3.51 sec respectively. There was no significant difference (>0.05) in partial thromboplastin time of HIV positive subjects when compared with TB positive subjects.

DISCUSSION

Haematological disorders are known complications of viral and bacterial infections and influence all the blood cell lineages leading to anaemia, leucopaenia and thrombocytopenia (Kirchhoff and Silvestri, 2008). This study was carried out to investigate the prothrombin time (PT) and partial thromboplastin time with kaolin (PTTK) among Human immunodeficiency virus (HIV) patients, pulmonary tuberculosis (PTB) patients and patients with both infections (HIV and PTB co-infection). From this present study, The PT and PTTK were significantly higher in HIV-positive subjects compared to the values obtained in the HIV-negative subjects (controls). This was in agreement with the works of Omoregie *et al*., (2009), Okoroiwu *et al*., (2014), Obeagu and Obeagu, (2015), Ifeanyichukwu *et al*., (2016) and Masresha *et al*., (2018) who in their various studies reported prolonged PT and PTTK in HIV infected individuals. HIV infection has been associated with endothelial dysfunction and liver disease. 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

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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 predisposing for hypercoagulable state such as the presence of antiphospholipid antibodies and lupus anticoagulant, and deficiencies of protein C and protein S which cause activation and consumption of coagulation factors might be the possible reason for the occurrence prolonged PT and PTTK in HIV-infected individuals (Park *et al*., 2009; Crum-Cianflone *et al*., 2010; Rajendran *et al*., 2013).

The result of this study also shown that the Pulmonary Tuberculosis (PTB) patients had both deranged extrinsic and intrinsic pathways of coagulation as indicated by significant ($p<0.05$) prolonged PT and PTTK than normal healthy individuals. The result is consistent with the findings of (Saidu *et al*., 2019). It was reported that various cytokines including tumor necrosis factor-alpha and interleukin 6 (IL-6) emerging from the TB granulomatous lesions were thought to influence the prolonged procoagulant biomarkers. The prolonged PT and PPTK was also believed to be due to phospholipid-dependent coagulation marker, known to be prolonged by antiphospholipid antibodies such as lupus anticoagulant (Jiang *et al*., 2010).

Patients with HIV-tuberculosis have been reported to display a hypercoagulable state that is associated with mortality (Saskia *et al*., 2016). This present study showed an increase in PT and PTTK values in patients with HIV/TB co-infection when compare with patients with HIV infection only but it was not significant ($p>0.05$). This result partially agreed with the work of (Janssen *et al*.,2017) who reported a significant increase in PT and PTTK values in in patients with HIV/TB co-infection when compare with patients with HIV infection only. They reported that the changes are likely to be driven by tuberculosis rather than advanced HIV only, as suggested by the more marked alterations of these markers observed in patients with HIV-tuberculosis, compared with HIV-infected controls.

CONCLUSION

This study revealed that HIV affects haemostasis due to endothelial dysfunction and liver disease, Tuberculosis also affects both the extrinsic and intrinsic pathway leading to prolongation of Prothrombin time test (PT) and Partial thromboplastin test with kaolin (PTTK).

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