

EVALUATION OF ACTIVATED PARTIAL THROMBOPLASTIN TIME AND PROTHROMBIN TIME IN HIV AND TB PATIENTS IN OWERRI METROPOLIS

ABSTRACT

Background: Human immunodeficiency Virus (HIV) and Tuberculosis (TB) are the leading infectious diseases with high morbidity and mortality in the developing countries; it has been known to be associated with some coagulation abnormalities especially as the disease progresses.

Aim: The study aimed at evaluating the effect of HIV- Tuberculosis co- infection on some haemostatic parameters (APTT & PT). It was carried out at Federal Medical Centre Owerri, Imo state.

Materials and methods: Sixty (60) subjects were sampled comprising fifteen (15) HIV positive subjects, fifteen (15) TB positive subjects, fifteen (15) HIV-TB co-infected subjects and fifteen (15) HIV and TB negative subjects (Control). Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) were analyzed using standard techniques.

Results: The results showed that HIV positive subjects showed a significant values of PT (15.45 ± 1.44 sec, $P < 0.05$) when compared with control subjects ($12.45 + 1.23$) and a non-statistically significant values of Activated Partial Thromboplastin Time (APTT) (33.33 ± 6.27 sec, $P > 0.05$) when compared with the control subjects ($29.05 + 2.19$). TB subjects showed statistical significant values of PT (15.84 ± 0.98 sec, $P < 0.05$) when compared with control subjects ($12.45 + 1.23$) and non- statistical significant values of APTT (33.55 ± 5.26 sec $P > 0.05$) when compared with the control subjects ($29.05 + 2.19$ sec). Both the PT and APTT of HIV- tuberculosis Co-infected subjects showed significant values (17.03 ± 1.46 sec; 37.59 ± 4.69 sec, $P < 0.05$) compared with the control subjects ($12.45 + 1.23$ sec; $29.05 + 2.19$). One way analysis of variance showed no significant difference of a PT of TB patient ($15.84 + 0.98$) compared to PT of HIV patients ($15.45 + 1.44$ sec). PT of HIV- TB co-infected subjects higher values ($P < 0.05$) when compared with HIV patients but not significantly ($P > 0.05$) higher than compared with TB patients. Also there is a significant increase in APTT of TB patients ($P < 0.05$) when compared with HIV patients. APTT of HIV- TB Co-infected subjects were not significantly elevated when compared with HIV and TB patients ($P > 0.05$).

Conclusion: HIV infections have been shown to affect both the Activated partial thromboplastin time and prothrombin time. There is significant alteration in coagulation parameters (particularly PT and APTT) on tuberculosis, lesions as a result of the mycobacterial infection can induce pro- coagulant tissue factor expression which can lead to coagulation defects.

Keywords: activated partial thromboplastin time, prothrombin time, HIV, TB

INTRODUCTION

The human immunodeficiency virus (HIV) is a lentivirus (a subgroup of retrovirus) that causes HIV infection and over time, acquired immunodeficiency syndrome [1]. AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV

subtype [2]. Infection with HIV occurs by transfer of blood, pre-ejaculate, semen, vaginal fluids, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

One-third of the world's population is thought to be infected with **Tuberculosis (TB)**. New infections occur in about 1% of the population each year [3]. In 2014, there were 9.6 million cases of active TB which resulted in 1.5 million deaths. More than 95% of deaths occurred in developing countries. The number of new cases each year has decreased since 2000. About 80% of people in many Asian and African countries test positive while 5-10% of people in the United States population tests positive by the tuberculin test [4]. Tuberculosis has been present in humans since ancient times [5].

Pulmonary TB causes bleeding disorders. **Haematologic** abnormalities have been described in association with mycobacteria infections for almost 100 years. Also activation of coagulation and **fibrinolytic** pathways in response to various bacterial infections are critical component elicited by both pathways [6]. Experimental studies also confirmed impairment in coagulation parameters in patients with alcoholic pulmonary TB.

MATERIALS AND METHODS

Study Area

This research was conducted at HIV and TB clinics of Federal Medical Centre, Owerri with co-ordinate location of latitude 5°28 '59 "N and 7° OF 49' E longitude and 159metres above sea level.

Study Population

This study was carried out on both male and female **already established** to be HIV and TB patients attending Federal Medical Centre, Owerri, Imo State Nigeria for treatment and check-up. People of the same age group and sex was used as control who prior to this research have not been diagnosed of HIV and TB.

Sample Size and Sampling Techniques

A total sample size of 60 was collected 45 test subjects (15 patients with HIV only, 15 patients with TB only and 15 TB patients with HIV {Co-infection}) and 15 control subjects (Non HIV and TB). The test population comprised of individuals who are within the age bracket of 20 and 50 **years**. While the control group was made up of individuals who have been tested and confirmed to be non-HIV and non-TB as at the time this research was carried out who are within the afore mentioned age bracket.

Subjects were recruited by random sampling techniques.

Subject Recruitment

All consenting HIV and TB patients were recruited for the study in their clinic during their clinic days through the help of the nurses and physicians after a well and elaborate explanation of the importance of the study, as well as the harmless nature of the procedure for the sample collection.

Inclusion Criteria

The criteria for selecting test subjects for this research included: willingness to participate, subject could be male or female, must be up to 20 years and not more than **50 years** of age, must have been diagnosed of HIV and TB according to WHO standard.

Exclusion Criteria

Criteria for excluding test subjects includes: people with established coagulation problems, those not diagnosed of HIV and TB according to WHO standard, those below 20 years of age and above 50years of age, those with other complications of HIV and TB.

Sample Collection, Preparation and Storage

Nine (9) ml of various bloods **were** collected from every participant into 1 ml of 3.8% sodium citrate anticoagulant well mixed and centrifuged at 1200g for 15mins to obtain platelet poor plasma. The plasma was then separated to a plain container, labeled properly and the test

performed immediately. Some of the test sample that could not be analysed immediately was stored at 4-8°C and the test performed within 1 hour of storage. Questionnaire was issued to the participants to obtain other vital information about them.

METHODOLOGY

Activated Partial Thromboplastin Time Estimation

Method: Kaolin platelet substitute mixture

Procedure

In a small glass tube, 0.2ml of well mixed Kaolin/platelet substitute was pipetted 0.1ml of the separated plasma was added, mixed and incubated at 37°C for exactly 2 minutes. The tube was tilted at intervals.

0.1ml of 0.025 mol/L calcium chloride was added, mixed and the **stopwatch** started.

The tube was held in water bath and the mixture tilted back and forth looking out for clot formation. The **stopwatch** was stopped immediately clot formation was observed and the time recorded.

Prothrombin Time Estimation

Method: Diagen Rabbit Brain capillary reagent

Procedure

1. 0.25ml of the thromboplastin/calcium reagent was pipetted into a small glass tube and placed in a 37°C water bath for 2 minutes.
2. 50µl (0.05ml) of plasma was added using delivery pipette, mixed and the stop watch started.
3. The tube was held in the water bath and the mixture tilted back and forth looking out for clot formation. The stop-watch is stopped when clot begins to form.
4. The control sample was also run with the test sample using the same procedure

Calculation

The result gotten in seconds was converted into INR (International Normalised Ratio) as follows:

STATISTICAL ANALYSIS

The results are presented as descriptive statistics (mean ± standard deviation). Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 20 (SPSS, Inc., IBM Company, Chicago, IL, USA). The comparisons among the experimental groups were analyzed by one-way analysis of variance (ANOVA) followed by least significance differences (LSD) test to evaluate statistical difference between two groups. P value < 0.05 was considered to be statistically significant.

RESULT AND ANALYSIS

Table 1: Mean and Standard deviation of PT and APTT of control and HIV patients.

Parameters	Control (n=15)	HIV patients (n=15)	p-value
PT (sec)	12.45 ± 1.23	15.45 ± 1.44* [^]	P< 0.05
APTT (sec)	29.05 ± 2.19	33.33 ± 6.27	p>0.05

Key: *: Statistically significant when compared with control

PT: Prothrombin time.

APTT: Activated partial thromboplastin time.

N: Sample size

From the table 1 above, PT of HIV patients (15.45±1.44sec) was statistically significantly (p<0.05) higher when compared with the control (12.45 ± 1.23sec).

APTT of HIV patients (33.33±6.27sec) was not statistically higher (p>0.05) than the control (29.05±2.19sec).

Table 2: Mean and Standard deviation of PT APTT of TB patients and control

Parameters	Control (n=15)	TB patients (n=15)	p-value
PT (sec)	12.45 ± 1.23	15.84±0.98* [^]	P< 0.05
APTT (sec)	29.05 ± 2.19	33.55±5.26	p>0.05

Key *: Statistically significant (P< 0.05) when compared with the control.

Comparison between the PT mean of TB patient (15.84±0.98sec) and control (12.45 ±1.28sec) shows a statistical significantly (p<0.05) higher value in TB patient. The APTT value of TB patient was not significantly (p>0.05) higher in TB (33.55±1.23sec) patients when compared with the control subjects. (29.05±2.19sec).

Table 3: Comparison of mean and standard deviation of PT and APTT of HIV & TB co-infected subjects and control.

Parameters	Control (n=15)	HIV/ TB patients (n=15)	p-value
PT (sec)	12.45 ± 1.23	17.03 ± 1.44*	P< 0.05
APTT (sec)	29.05 ± 2.19	37.59 ± 4.69*	P< 0.05

Key *: statistically significantly higher when compared with the control.

From table 3 above, the PT of HIV & Tb co- infected subjects (17.03 ± 1.46sec) was significantly higher (P< 0.05) than the control (12.45 ± 1.23sec). APTT of HIV & TB co-infected patients (37.59 ± 4.69sec) was significantly higher than the control (29.05 ± 2.19sec).

Table 4: Mean and standard deviation of prothrombin time (PT) and a activated partial thromoplastin time (APTT) of HIV, TB and co-infected

	HIV Group 2	TB Group 3	HIV/TB Group 4
PT (sec)	15.45 ± 1.44	15.48 ± 0.98`	17.03 ± 1.46
APTT (sec)	33.33 ± 6.27	33.55 ± 5.26	37.59 ± 4.69

Key *: Statistically significant when compared with HIV patient (P< 0.05).

PT: Prothrombin time

APTT: Activated partial thromboplastic time

The table below indicates mean and standard deviation of PT and APTT of HIV, TB and HIV/TB co-infected groups when compared among each other. From the table, one way analysis of variance shows that a PT of TB patient (15.84 ±0.98) was not significantly higher than HIV patients (15.45 ±1.44sec). PT of HIV and TB co- infection was significantly (p<0.05) higher when compared with HIV patients but **significantly** (p<0.05) higher when compared with TB patients.

Comparison of APTT among HIV patient (33.33 ±6.27) TB patient (33.55±5.25) and HIV/TB co-infected patient (37.59 ±4.69) shows an increase in TB patients when compared with HIV patient. APTT level of **HIV** & TB co-infected patient when compared with **HIV** and TB patient shows a non-significant higher value in HIV and TB patients.

DISCUSSION

Both hematological and haemostatic disorders are known complications of HIV infection. HIV infection is associated with endothelial dysfunction and liver damage which can result in coagulation defects. It is therefore expected that as the HIV infection progresses the coagulation abnormalities will increase too [7]. In this study The PT and APTT were significantly higher in HIV-positive subjects (**ART HIV positive and non-ART HIV positive patients**) compared to the values obtained in the HIV-negative subjects (control). (p<0.05) and is in consonance with previous independent studies of Van **Grop** *et al*[8], Omoregie *et al* [9], Obeagu and Obeagu [10]. These studies reported that PT and APTT were significantly higher in HIV-positive patients. Moreover, HIV infection results in liver derangement, immune dysregulation and presence of anticardiolipin antibodies (aCL) and Lupus anticoagulant (LA). These anomalies of clothing factors, liver derangement may account for higher PT and APTT values observed in HIV-positive subjects [11]. However, the differences in 76 duration of **antiretroviral therapy (ART)** and gender did not affect both parameters (PT and APTT) as observed in this study.

Tuberculosis is a chronic granulomatous infection caused by mycobacterium tuberculosis. Various inflammatory cells, cytokines and mediators arc involved in the formation of

granulomatous lesions encountered in tuberculosis [12] of variety of cytokines. In the study, the PT of TB patients was shown to be increased significantly ($p < 0.05$) while the APTT was not significantly increased ($p > 0.05$) when compared with the control subjects. Also immune complexes and many other factors elaborated in various infectious diseases (e.g. Mycobacterial infection) are shown to induce pro-coagulant tissue factor (TF) expression in monocytes/macrophages and the endothelium which under normal healthy state doesn't express TF [13-15]. This could be the reason for the significant increase in PT, since activation of TF leads to the pathway (extrinsic pathway) that involves prothrombin time (PT) estimation.

Lastly, there was also a significant increase in the PT and APTT of HIV and TB- Coinfected subjects ($p < 0.05$) when compared with the control subjects. The exact reason for this is not known because much research has not been done specifically involving APTT and PT. A research have shown Bacterial sepsis in association with activation of procoagulant responses, endothelial activation, and inhibition of fibrinolysis and decreased anticoagulant response [16]. In the most extreme cases, these changes lead to Disseminated intravascular Coagulation (DIC) and micro vascular thrombosis [16]. Much less is known about haemostatic changes during severe HIV-tuberculosis infection [17-19].

CONCLUSION

HIV infections have been shown to affect both the Activated partial thromboplastin time and prothrombin time. There is significant alteration in coagulation parameters (particularly PT and APTT) on tuberculosis, lesions as a result of the mycobacterial infection can induce pro-coagulant tissue factor expression which can lead to coagulation defects. HIV-TB co-infection being a complex state can lead to complex alterations in coagulation pathways and even haemostasis generally in the individual involved.

Ethical Approval

Ethical consideration for this research was obtained from the federal medical centre Owerri, Imo State.

Consent

An informed consent of individual patient was obtained before recruitment. The same was applied to the control group.

References

1. Obeagu EI, Onyenweaku FC, Nwobodo HA, Ochei KC, Ochiabuto OMTB et al. Impact of HIV and Hepatitis Vvirus Coinfection on selected Haematological Markers of the patients in Umuahia, Abia State, Nigeria. *Annals of Clinical and Laboratory Research* 5:2.doi:10.21767/2386-5180.1000175.
2. UNAIDS, WHO . 2007 AIDS epidemic update.2007:10.
3. Smith JA, Daniel R. Following the path of the virus: the exploitation of host DNA repair mechanisms by retroviruses". *ACS Chem Biol*. 2006; 1 (4): 217- 26.
4. Gilbert PB, McKeague IW, Eisen G, Mullins C, Gueye-NDiaye A, Mboup S, Kanki PJ. Comparison of HIV-1 and HIV-2 infectivity from a prospective cohort study in Senegal". *Statistics in Medicine*. 2003; 22 (4):

573-593.

5. Reeves JD, **Doms** RW. Human Immunodeficiency Virus Type 2".Journal of General Virology. 2002; **83** (Pt 6): 1253-65.
6. Bergmann S, **Hammerschmidt** S. Fibrinolysis and host response in bacterial infections. **ThrombHaemos**. 2007; 98: 512-520.
7. Linder M, Muller-Berghaus G, Lasch LIG.Virus infection and blood coagulation".ThrombDiath hemorrhage. 1970; **23(1):1-11**.
8. Van Gorp ECM, Suharti C, ten Cate II, Dolmans WMV, Brangjes DPM. Review: Infectious diseases and coagulation disorders. Journal of Infectious diseases. 1999; 180: 176-186.
9. Omoregie R, Osakue SI, IHEMEJE V, Omokaro EU, OGCFERET H. **Correlation of CD4 count with platelet count, prothrombin time and activated partial thromboplastin time among HIV patients in Benin City, Nigeria. West Indian Med J. 2009;58(5): 437-440**
- 10.Obeagu EI, Obeagu GU (2015) Effect of CD4 Counts on Coagulation Parameters among HIV Positive Patients in Federal Medical Centre, Owerrri, Nigeria. International Journal of Current Research in Biosciences and Plant Biology 2: 45-49.
- 11.Karpatkin S, Nardi M, Green D. Platelet and coagulation defects associated with HIV-1 infection. Thrombosis and Haemostasis.2002; 88: 389-401.
- 12.Bost TW, Riches DW, Schumacher B. **Alveolar** macrophages from patients with beryllium diseases and **sarcoidosis** express increased levels of mRNA for tumor necrosis factor alpha and interleukin-1 beta. Am J Respir cell Molecular Biology. 1994; 10:1506-13.
- 13.Pawlinski R, Mackman N. Cellular sources of tissue factor in endotoxemia and sepsis", Thromb Res **2010;125(1): S70-S73**.
- 14.Taylor FB, Chang A, Ruf W, Morrissey JFI, Hinshaw LB. LethalE.coli septic shock is prevented by blocking tissue factor with monoclonal antibody". Circ Shock. 1991; 33: 127-134.
- 15.Osterud B. Monocytes and hypercoagulable states. Hypercoag Sts. 1995; 1-11
- 16.**Taylor FB Jr, Toh CH, Hoots WK, Wada H, Levi M; Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the**

International Society on Thrombosis and Haemostasis (ISTH).

17. Ezeoru VC, Enweani IB, Ochiabuto O, Nwachukwu AC, Ogbonna US, Obeagu EI. Prevalence of Malaria with Anaemia and HIV Status in Women of Reproductive Age in Onitsha, Nigeria. *Journal of Pharmaceutical Research International*, 2021];33(4):10-9.
18. Ifeanyi OE, N. EB, Uzoma OG, O.M.T.B. O, Ejike Felix C , Stella EI, Chinedum O , K. Evaluation of Some Cytokines, CD4, Hepcidin, Iron Profile and Some Haematological Parameters of Pulmonary Tuberculosis Patients Coinfected with HIV in Southeast of Nigeria. *J Journal of Pharmaceutical Research International*, 2020;32(13):118-30.

UNDER PEER REVIEW