

Original Research Article

Alterations in Some Coagulation Factors (*F VII and F XII*), *PT, APTT and D-Dimer*) in Newly Infected Tuberculosis Patients from South-Southern Nigeria

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Abstract: Tuberculosis (TB) is a major health concern as well as leading infectious killer disease in man caused by the bacterium *Mycobacterium tuberculosis*. This study was carried out to assess the effect of tuberculosis on some specific coagulation factors (*F VII and F XII*), *PT, APTT and D-Dimer*). It was carried out at the Tuberculosis and Leprosy Hospital Igbogene, the Federal Medical Center (FMCY) and Niger Delta University Teaching Hospital (NDUTH) all in Yenagoa Local Govt Area of Bayelsa state, Nigeria. Ethical approval and participant's informed consent were obtained. Fifty (50) newly diagnosed TB subjects and 50 apparently healthy control subjects participated in this study. Specimens used were Sputum and blood. *PT* was assayed by the Quick one stage method. Fortress diagnostics reagents were used for the *APTT* assay while *D-Dimer* was tested by the sandwich immunodetection method by ICHROMAX. The data gathered from this research were analyzed using SPSS statistical software version 20.0. The results for the haemostatic parameters shows significantly higher ($p < 0.05$) *PT, APTT, and D-Dimer* values, but significantly lower ($p < 0.05$) *F VII* in newly diagnosed TB subjects when compared to controls group. There was no significant difference ($P > 0.05$) in *F XII* in newly diagnosed TB subjects when compared to the controls. The results from this research indicates that Tuberculosis disease greatly altered almost all the, haemostatic, parameters studied, except coagulation factor *XII* that was virtually unaltered.

Keywords: Tuberculosis, Factor VII, Factor XII, *PT, APTT, D-Dimer*.

INTRODUCTION

Tuberculosis (TB) a major health concern and top infectious killer disease is caused in man by the bacterium *Mycobacterium tuberculosis* (Ochei & Kolhatkar, 2006). The disease problem in Nigeria is rated as the topmost in Africa (Federal Ministry of Health, 2019). India is known to have the highest annual occurrence of Tuberculosis (TB) and is estimated at 1.98 million, one fifth of the global incidence (World Health Organization, 2009).

After inhalation, the bacillus enters the body via droplets or dust particles containing it. The infection is categorized as pulmonary or non- pulmonary tuberculosis. In the first, the pulmonary aveoli and surrounding lymph glands are lodged by the bacilli causing lesions characterized by acute inflammatory reactions with accumulation of fluid

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and white blood cells around the aveoli, while the later includes the renal and urogenital tuberculosis, miliary tuberculosis and tuberculosis meningitis with variable symptoms (Ochei & Kolhatkar, 2006).

When tissue damage occurs due to infection, the body reacts in a process known as inflammation. This defense response attempts to remove or at the least limit the spread of the offending agent, and clear necrosed cells and tissues from the affected region (Mohan 2010). TB as a disease is a state of prolonged granulomatous inflammation that arises from infection by a family of organisms collectively called the Mycobacterium tuberculosis complex (Iseman, 2000). It is well reported that inflammation results in activation of the haemostatic system (Verhamme & Hoylaerts, 2009).

The process of Haemostasis is a system in humans that has the ability to stop loss of blood from areas of blood vessel injury through a series of enzymatic reactions. Haemostasis describes a delicate balance between procoagulant as well as anticoagulant mechanisms that involves an intricate series of occurrences. When tissue is injured due to infection, the body reacts in a process known as inflammation (Hoffbrand, 2011).

Coagulation, also known as clotting, is a process whereby blood changes from a liquid to a gel, forming a blood clot. This process of blood coagulation leads to hemostasis, which is the cessation of blood loss from a injured blood vessel and is followed by repair of the vessel. This complex mechanism of coagulation involves activation, adhesion and aggregation of platelets, as well as deposition and maturation of fibrin strands (Furie & Furie, 2005).

The process of Coagulation begins almost instantaneously when damage occurs to the endothelium lining a blood vessel. When blood is exposed to the subendothelial space it initiates two processes: changes in platelets, and the exposure of subendothelial tissue factor to plasma factor VII, which ultimately leads to cross-linked fibrin formation. Platelets immediately form a plug at the site of injury; this is known as primary hemostasis. Secondary hemostasis occurs at the same time: additional coagulation factors other than factor VII respond in a cascade to form fibrin strands, which reinforce the platelet plug (Pallister & Watson, 2010).

The sequences of coagulation cascade of secondary hemostasis has two initial pathways that leads to fibrin formation. These are a series of reactions, of which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are triggered to become active components which then catalyze the next reaction in the cascade, finally resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase *a* appended to indicate an active form. These are the contact activation pathway (also known as the intrinsic pathway), and the tissue factor pathway (also known as the extrinsic pathway), which together leads to the same fundamental reactions that produce fibrin. It was formerly thought that the two pathways of coagulation cascade were of the same importance, but now it is known that the primary pathway for the initiation of blood coagulation is the tissue factor (extrinsic) pathway (David, 2009). Coagulation factor VII also known as Proconvertin, is a beta-globulin and a vitamin K depended clotting factor synthesized in the liver. It is involved in the extrinsic pathway of coagulation and activates tissue thromboplastin (Ochei & Kolhatkar, 2006).

Hageman factor is another name for Coagulation factor XII. It is involved in the intrinsic pathway of coagulation. It is a gamma-globulin produced in the liver and is not vitamin K deopended (Ochei & Kolhatkar, 2006). Disorders of coagulation are disease states which can culminate in problems with hemorrhage, bruising, or thrombosis (Asakura, 2020).

D-dimer is one of the fibrin degradation products (FDP). It is a small protein fragment that is present in the blood after a blood clot is degraded by the process of fibrinolysis. It is so named due to the fact that it contains two D fragments of the fibrin protein joined by a cross-link, hence forming a protein dimer (Ponti *et al.*, 2020). D-dimer levels are used as a prognostic biomarker for the blood disorder, disseminated intravascular coagulation and in the coagulation disorders associated with COVID-19 infection (Akpan *et al.*, 2018).

Considering the importance of the coagulation factors and the reported elongation of the clotting process during TB infection ((Akpan *et al.*, 2018, Awodu, 2007 and Aditya *et al.*, 2017) we thought it wise to evaluate two coagulation factors –factor F VII of the extrinsic system and F XII of the intrinsic system as well as PT and APTT and a fibrin degradation product D-Dimer to find their role in the elongated PT and APTT.

EXPERIMENTAL SECTION

Tuberculosis test

Analysis was by the Ziehl Neelson (AFB) technique as described by Ochei and kolhatkar, (2006) and the Genexpert technique.

Principle of Ziehl Neelson (AFB) technique

TB bacilli when stained picks up the primary stain and appears red under the microscope.

Principle of the GeneXpert

The Gene Xpert MTB/RIF assay is a nucleic acid amplification (NAA) test that uses a disposable cartridge with the GeneXpert Instrument System.

Prothrombin Time (PT)

The one stage prothrombin time method was used as described by Ochei and Kolhatkar, 2006.

Principle of Prothrombin Time (PT) assay

When tissue thromboplastin and calcium ions are added to plasma, the extrinsic clotting factors are activated. This results to the generation of Thrombin and the formation of fibrin clot.

APTT

Principle of APTT test

The capacity of blood to form a fibrin clot by way of the intrinsic haemostatic pathway requires coagulation factors, i, ii, v, viii, ix, x, xi and xii, platelet lipids and calcium. The assay is performed by the addition of a suspension of rabbit brain cephalin with surface activities (by Ochei and Kolhatkar, 2006). The APTT has proven that to be a highly reliable measurement of the intrinsic coagulation mechanism. This test measures deficiency of coagulation factors in the intrinsic and the final common pathway.

D-Dimer

Ichromax D-Dimer assay method was used.

Principles of D-Dimer assay test

The test uses the sandwich immunodetection method such that the detection antibody in buffer binds to D-dimer in the plasma sample and antigen – antibody complexes are captured by antibodies that have been mobilized on the test strip as sample mixture migrates through nitro cellulose mixture. The more D-dimer antigen in the plasma, the more antigen antibody complexes are accumulated on the test strip. Signal intensity of fluorescence on detection antigen reflects amount of antigen captured and is processed by ichromax reader to show the D-dimer concentration in the specimen.

Coagulation tests (Factor VII and F XII)

Coagulation factor VII and XII was assayed by reagents from Glory Science company Ltd

Principle of Factor VII and F XII test

At the final stage of the test set up, a stop solution changes the colour from blue to yellow and the intensity of the colour is measured at 450nm with a spectrophotometer.

RESULT AND DISCUSSION

A total of one hundred (100) subjects divided into two treatment groups: control subjects (50) group 1, newly diagnosed TB subjects (50) group 2 constituted the study.

Figure 1a and 1b gives some demographic features of the Tuberculosis subjects and controls. Figure 1a shows that the control subjects were made up of 21 males and 29 females while the newly diagnosed TB subjects consists of 20 males and 30 females. In figure 1b, it is revealed that out of the 100 participants, males constituted 41 (41%) while the remaining 59 (59%) were females. Their age ranges from 19 years to 50 years for the males and 21 to 59 for the females.

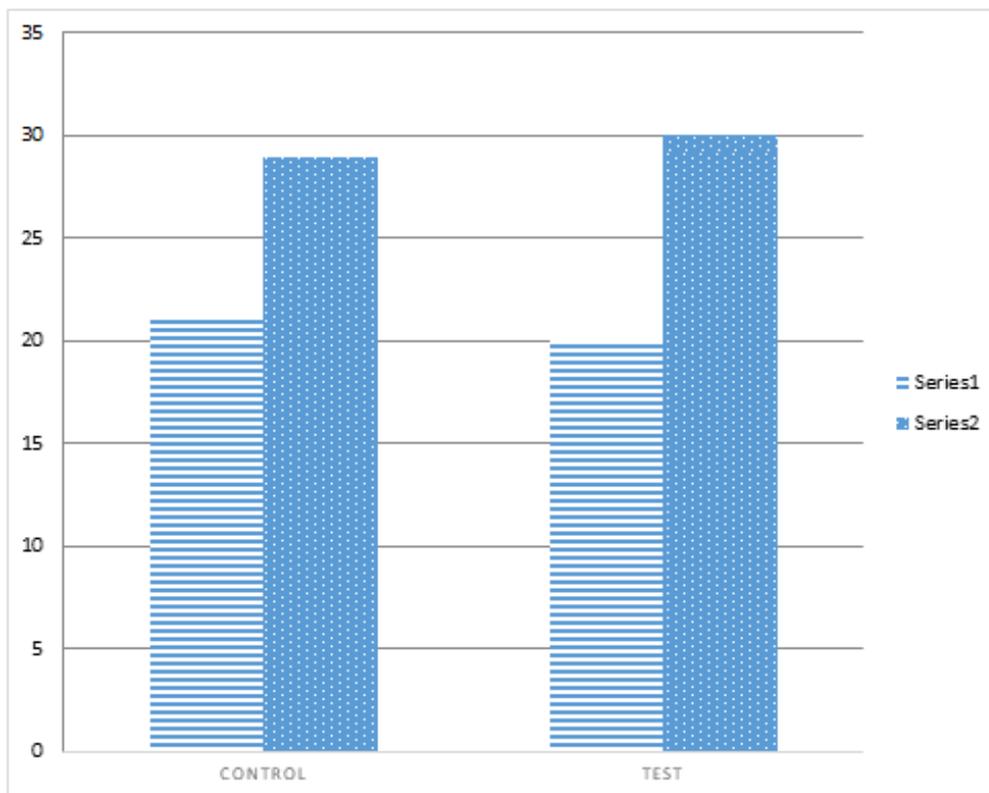


Fig-4.1a: Demographic features of control and test Tuberculosis subjects

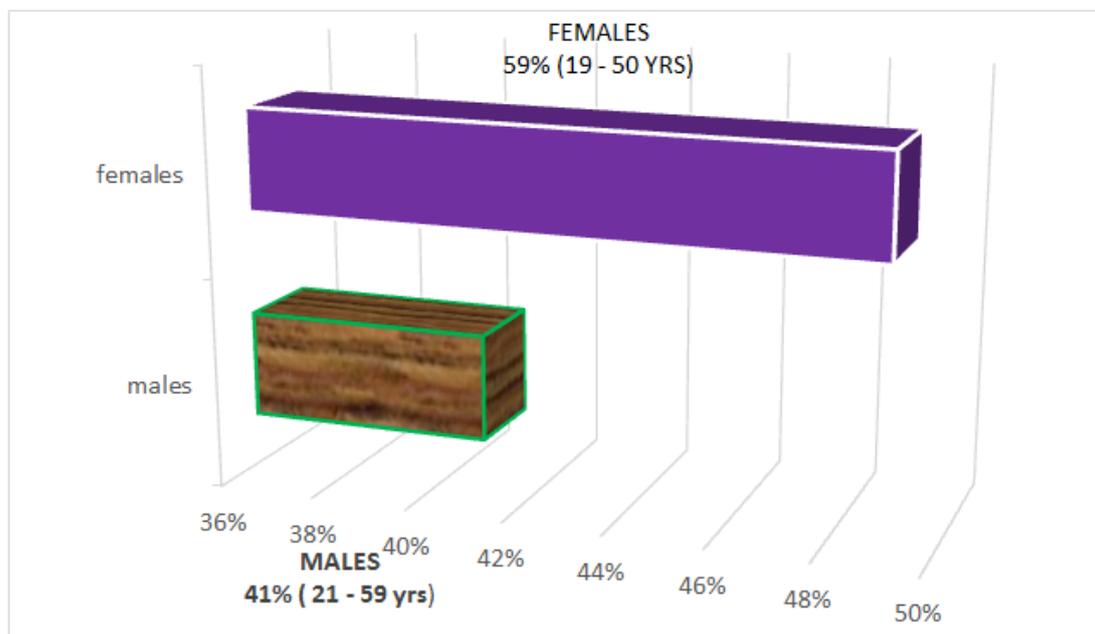


Fig-4.1b: Demographic features of Male and female Tuberculosis subjects

Table 1. Shows the Mean \pm SD of the measured haemostatic parameters of the control and the newly diagnosed TB subjects as well as the results of their comparison using t-test analysis. The PT for the control group was 11.66s while that of the test group was 14.12s. This gave a t- value of -7.544 and a P- value of 0.000 thus showing that there was a statistically significant increase. Comparison of the values for the control and test revealed a significant increased ($P < 0.05$) in value for APTT- $p = 0.00$. D-Dimer on the table revealed a value of 209.82 ± 78.73 mg/dl for control, 418.10 ± 111.75 mg/dl for test group, a t- value of -10.77 and a P value of 0.000.

The control and test Mean ± SD values for coagulation factors VII and XII is also given in table 1. The control values for F VII is 10.2±5.1ng/ml and 7.5±0.34ng/ml for test subjects giving a t`-4.634 and p of 0.001 respectively. Values for F XII was virtually unchanged as the Mean ± SD only slightly decreased from 6.20±1.49ng/ml to 6.15±1.35ng/ml (control to test subjects) giving an insignificant p value of p=0.883.

Table-1: Mean±SD values of Haemostatic variables in newly diagnosed Tuberculosis Subjects versus controls

Variables (Mean±SD)	Control Subjects (n=50)	Newly diagnosed TB Subjects (n=50)	t-value	P-value
PT(sec)	11.66±1.34	14.12±1.85	-7.544	0.000
APTT (sec)	26.54±3.45	32.84±4.94	-7.389	0.000
D-Dimer (ng/ml)	209.82±78.73	418.10±111.75	-10.77	0.000
F XII (ng/ml)	6.20±1.49	6.15±1.35	-3.874	0.883
F VII(ng/ml)	10.2±5.1	7.5±0.34	`-4.634	0.001

Key. Pt; prothrombin time test. Aptt; activated partial thromboplastine time. F vii; coagulation factor vii. F xii; coagulation factor xii

In this study of the values of some coagulation parameters in TB patients, the effects of tuberculosis were evidently demonstrated on the haemostatic parameters under study. One major finding in this study was that tuberculosis infection adversely affected almost all haemostatic parameters either as an increase or decrease in value.

On the parameters studied, an elongated time was seen in PT and APTT as well as significantly increased D-Dimer values when control was compared to newly diagnosed TB subjects. Prothrombin time test (PT) is a test that measures the functions of the coagulation factors of the extrinsic system While APTT measures the coagulation factors of the intrinsic system. These elongated values could be as a result of the derangement of the values of the coagulation factors. Our findings agrees with some researchers but are also not in line with other findings both within Nigeria and outside Nigeria. It agrees with Garip *et al.*(2004), where they reported a PT of a mean of 18.7s on TB patients. In Kano, Saidu *et al.* (2019) reports an elongated value of PT and APTT. This result is also consistent with the findings of Adtiyal *et al.* (2017) and Kartaloglu *et al.*, (2001) who demonstrated that TB patients have prolonged PT and APTT than in normal control subjects. In these studies that determined the effect of PTB on hemostasis among 50 participants in India, these authors proposed that various cytokines including tumor necrosis factor-alpha and interleukin 6 (IL-6) emerging from the TB granulomatous lesions were believed to influence the prolonged procoagulant biomarkers. The prolonged APTT was also thought to be due to phospholipid-dependent coagulation marker, known to be prolonged by antiphospholipid antibodies such as lupus anticoagulant (Osita *et al.* 2015). Researchers had noted increased presence of lupus anticoagulant in TB subjects (Klein *et al.*, 2005). However, this research work did not agree with the findings of Toppo *et al.* (2015) and Turken *et al.* (2002) that while studying the haematological changes in active pulmonary tuberculosis discovered that there was no significant change in the APTT values of the studied population.

D-Dimer is a fibrin degradation product. It's high levels in TB subjects tells us about a high level of fibrinolysis. This is anticipated because the endothelial damage orchestrated by the inflammation caused by TB disease is said to initiate coagulation and hence the formation of fibrins that ultimately leads to fibrinolysis ((Verhamme & Hoylaerts, 2009).). All the literatures reviewed on D-Dimer value were in line with our findings. Shen *et al.*, (2013), in their work on the potential role for D-dimer in the diagnosis of tuberculosis, reported a raised level of D-dimer. They informed that in tuberculosis pleural effusion (TPE), coagulation system was activated, and as a consequence, there is activation of the fibrinolytic system with increased levels of Pleural fibrin degradation products, including the D-dimer. The same result was reported by Ekrem *et al.* (2006).and Aditya (2017) who found D-Dimer positivity in 74 (57.8%) patients out of total study population of 128 subjects.

The study of the coagulation factors VII and XII is to further determine the relative contributions of the different clotting factors in the systemic procoagulant response during pulmonary TB (evident in the prolonged PT and APTT). We measured concentrations of the coagulation proteins affecting PT (F VII) and APTT (F XII). Of the factors affecting PT, factor VII decreased in value, while that of APTT (F XII) was virtually unchanged. The reason for the decreased value of coagulation factor F VII could be as a result of the disease affecting its site of production. The findings agree with that of Liegerth *et al.* (2015). Our findings about coagulation factor XII is that it remained unchanged and so could not have caused an elongation of APTT. Liegerth *et al.* (2015) also could not possibly gave a reason for the elongation of APTT as there were inconsistencies from the results of the values of the coagulation factors studied (some were elevated while others decreased in). However, Kerlin *et al.* (2004) opined that Possibly, APTT prolongation was the result of the inhibitory effects of high fibrinogen concentrations in TB patients considering that hyperfibrinogemia may suppress thrombin generation.

CONCLUSION

The results from this research work has revealed that Tuberculosis disease greatly alters almost all the haemostatic factors studied, favouring a state of hypercoagulability, therefore treatment/care givers should also note this state of tuberculosis patients and be handled accordingly.

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