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Research Paper

Alteration in levels of ADAMTS 13, D-Dimer, vWBF, Platelets, PT and APTT in newly infected tuberculosis patients

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ABSTRACT: *Tuberculosis remains a major public health concern globally. Tuberculosis as a disease is associated with chronic granulomatous inflammation which results in activation of the haemostatic system. Considering the importance of ADAMTS 13 and VWBF in the process of Haemostasis, this study is aimed to evaluate their status in tuberculosis patients. Blood was collected from newly diagnosed Tuberculosis subjects and control subjects. It was prepared accordingly for the various assays. Flourescence immune assay, automated cell counting and ELISA techniques were employed in analyzing the samples. The results reveal significantly higher levels of D-Dimer (418.10±111.75 ng/ml versus 209.82±78.73 ng/ml p=0.000.) and VWBF (14.78±5.52ng/ml versus 11.312±2.93ng/ml, p=0.000) in newly diagnosed Tuberculosis subjects compared to control subjects. There was no significant difference in ADAMTS 13 level (52.78±17.93ng/ml versus 56.84±16.49ng/ml, p=0.241) in newly diagnosed Tuberculosis subjects compared to control subjects. Platelet count, PT and APTT were significantly higher(p<0.05) in newly diagnosed Tuberculosis subjects compared to control subjects. This fact suggests that pulmonary TB may be associated with systemic activation of the vascular endothelium. The tuberculosis patient is in a state of hypercoagualability, therefore care givers should take note and possibly include treatment for coagulation disorders.*
KEYWORDS: *VWBF, ADAMTS 13, PLATELET, PT,APTT, D-Dimer*

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I. INTRODUCTION

Tuberculosis (TB) remains a major public health concern globally. Though an ancient disease, it is still among the top ten causes of mortality globally from a single infectious agent. In the year 2020, an estimated 10 million people fell ill with TB worldwide. 5.6 million weremen, 3.3 million women and 1.1 million children. TB is the 13th leading cause of death and the second leading infectious killer after COVID-19 (above HIV/AIDS)[1]. It is caused in man by the bacterium *Mycobacterium tuberculosis* [2]. The disease burden in Nigeria is rated as the highest in Africa [3], though on a global scale India accounts for one fifth of the global TB incidence according to the world Health organization in 2009 [4].The full blown infection is characterized as pulmonary tuberculosis or non-pulmonary tuberculosis. In pulmonary tuberculosis, the pulmonary aveoli and surrounding lymph glands are lodged by the bacilli resulting in lesion which cumulates to acute inflammatory reactions with accumulation of fluid and white blood cells around the aveoli [2].

The word Haemostasisrefers to an effective system in humans with the ability to stop loss of blood from sites of blood vessel injury through a series of enzymatic reactions. Haemostasis describes a fragile

balance between procoagulant as well as anticoagulant mechanisms involving an intricate series of events [5]. In the event of tissue injury due to infection, the body reacts in a process known as inflammation. This defense reaction attempts to remove or at least limit the spread of the offending agent, and in addition clear necrosed cells and tissues from the affected area [6]. Tuberculosis (TB) as a disease is a state of chronic granulomatous inflammation that arises from infection of Mycobacterium tuberculosis [7]. Report has it that inflammation results in activation of the haemostatic system, with the latter also affecting the activity of the former [8]. Coagulation process begins almost instantly after a damage to the endothelium lining a blood vessel. Exposure of blood to the subendothelial space 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 called primary hemostasis. Secondary hemostasis occurs simultaneously: additional coagulation (clotting) factors beyond factor VII respond in a cascade to form fibrin strands, which strengthen the platelet plug [9].

A Disintegrin and Metalloproteinase with Thrombospondin type 1 motif, member 13 (ADAMTS 13)—also called von Willebrand factor-cleaving protease (vWFCP)—is a zinc-containing metalloprotease enzynme that cleaves von Willebrand factor (vWf), a large protein involved in blood clotting. This enzyme is secreted into the blood and degrades large vWfmultimers, decreasing their activity. Shortage of ADAMTS 13 results in accumulation of UL-VWFM, which induces platelet clumping or thrombi under high shear stress [10, 11]. The marked imbalance between decreased ADAMTS 13 activity and increased production of UL-VWFM indicating a high-risk state of platelet microthrombi formation [12]. 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 [13].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 [14].

One major function of platelets is to contribute to hemostasis: the process of stopping bleeding at the site of interrupted endothelium. They gather at the site and, unless the interruption is physically too large, they plug the hole. First, platelets attach to substances outside the interrupted endothelium: *adhesion*. Second, they change shape, turn on receptors and secrete chemical messenger's activation. Third, they connect to each other through receptor bridges: aggregation [15]. Besides hemostasis, platelets are accepted to play a role also in inflammatory response [16, 17].Disorders of coagulation are disease states which can result in problems with hemorrhage, bruising, or thrombosis[18].

Considering the importance of ADAMTS 13 and VWBF in the process of coagulation and the reported elongation of the clotting process (PT and APTT) during TB infection [14, 19, 20, 21], we thought it wise to evaluate them values in tuberculosis patients.

II. MATRIALS AND METHODS

D-Dimer

Principles of test:

Ichroma D-Dimer is aflourescence immune assay (FIA) for the quantitative detection of human D-Dimer in plasma/whole blood The test use 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 florescence on detection antigen reflects amount of antigen captured and is processed by ichroma reader to show the D-dimer concentration in the specimen. The working range of ichroma D- dimer test is (50-10,000) ng/ml.

Platelets

Platelet count is by the Abacus 380 Haematology Analyzer.

Principle of test

The operating principle of the Abacus 380 autoanalyzer uses the impedance method also known as the coulter-method. This method counts/sizes cells by detecting and measuring changes in electrical impedance when a particle in a conducting liquid passes through a small aperture.

For each cell passing through the aperture, there is a constant direct current flowing between the external and internal electrodes and causes some changes in the impedance in the conductive blood cell suspension. These changes are recorded as increases in the voltage between the electrodes. The number of pulses is proportional to the number of particles. The intensity of each pulse is proportional to the volume of that particle. The volume distributions of the cells are displayed on diagrams.

ADAMTS 13 and Von Willebrand factor

The tests were done using the kits from Glory Science co. Ltd

Principle of test
The Gscience Human ADAMTS13 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro ELISA for the quantitative measurement of Human ADAMTS13 and Von Willebrand factor
in Serum/Plasma. This assay employs an antibody specific for Human ADAMTS13 coated on a 96-well plate. The stop solution changes the colourfrom blue to yellow and the intensity of the colour is measured is measured at 450ng using a spectrophotometer.

III. RESULTS

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 1 and table 1 gives some demographic features of the Tuberculosis subjects and controls.
In figure 1, 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.

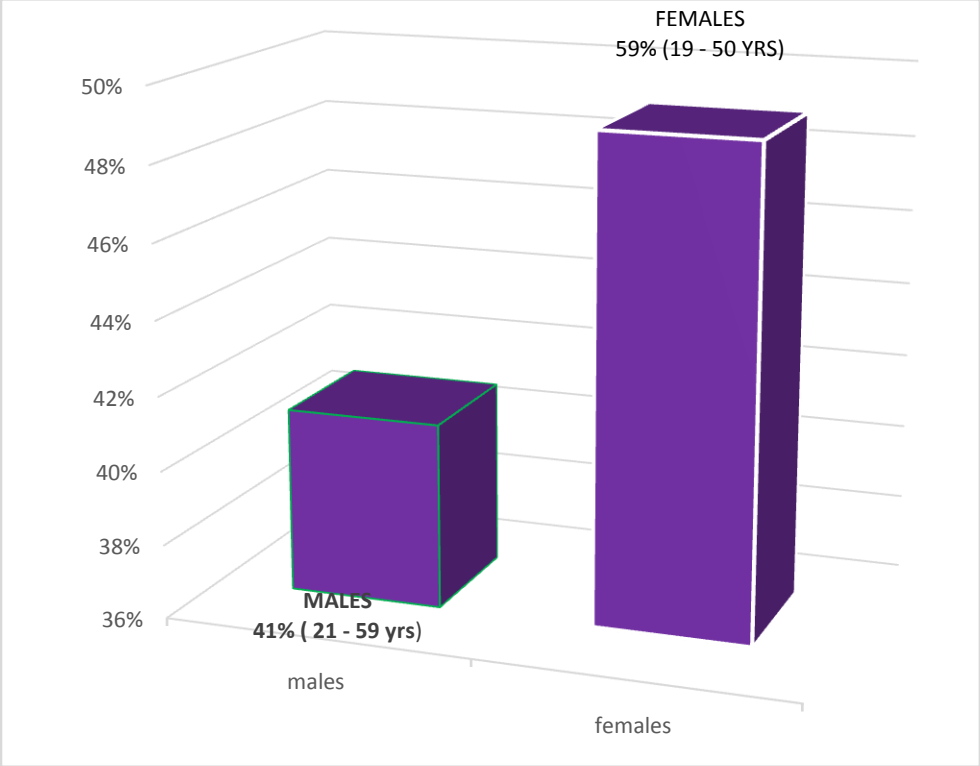

Figure 1aDemographic features of Male and female Tuberculosis subjects

Table 1. Is a continuation of the demographic features of Male and female Tuberculosis subjects and controls. It shows that the control subjects were made up of 21 males and 29 females having ages ranging from 19 to 48years and a median age of 34 years. That of the newly diagnosed TB subjects consists of 20 males and 30 females having ages ranging from 23 to 59 years of age and a median age of 41 years. Lastly the table reveals that the overall age range for all subjects was 19 59 years with a median age of 39.

Table 1Demographic features of Male and female Tuberculosis subjects and controls cont'd

	No of subjects			Age (yrs)	
Groups	**Total**	**Male**	**Female**	**Median**	**Range**
Newly infected	**50**	**20**	**30**	**41**	**23 - 59**
Controls	**50**	**21**	**29**	**34**	**19 - 48**
Total	**100**	**41**	**59**	**39**	**19 – 59**

Table 2.Shows the Mean ± SD of the measured haemostatic parameters of the control and the newly diagnosed TB subjects as well as the results of their comparism using t-test analysis.
The table reveals significantly higher levels of D-Dimer (418.10±111.75 ng/ml versus 209.82±78.73 ng/ml p=0.000.) and VWBF (14.78±5.52ng/ml versus 11.312±2.93ng/ml, p=0.000) in newly diagnosed Tuberculosis subjects compared to control subjects. Also the table reveals Platelet count PT and APTT tohavehigher levels(385.32±150.58 X 10^9 /l versus 210.64±61.36 X 10^9 /l, P= 0.000, 14.12±1.85 s versus 11.66±1.34 s, p=0.000 and32.84±4.9s 4versus 26.54±3.45s, p= 0.000 respectively) in newly diagnosed Tuberculosis subjects compared to control subjects. There was no significant difference in ADAMTS 13 level (52.78±17.93ng/ml versus 56.84±16.49ng/ml, p=0.241) in newly diagnosed Tuberculosis subjects compared to control subjects.

Table 2Mean±SD values of Haemostatic variables in Newly diagnosed Tuberculosis Subjects versus controls

Variables		Control Subjects	Newly diagnosed TB Subjects	t-value	P-value
	N=	50	50		
Plt (X 10^{12}/l)	Mean±SD	210.64±61.36	385.32±150.58	-7.596	0.000
D-Dimer (ng/ml)	Mean±SD	209.82±78.73	418.10±111.75	-10.77	0.000
ADAMT 13(ng/ml)	Mean±SD	56.84±16.49	52.78±17.93	-1.179	0.241
vWBF(ng/ml)	Mean±SD	11.312±2.93	14.78±5.52	-3.919	0.000
PT(sec)	Mean±SD	11.66±1.34	14.12±1.85	-7.544	0.000
APTT (sec)	Mean±SD	26.54±3.45	32.84±4.94	-7.389	0.000

KEY; ADAMTS 13; A DISINTEGRIN AND METALOPROTENASE WITH THROMBOSPODIN MOTIF MEMBER 13. VWF; VON WILLEBRAND FACTOR. PT; PROTHROMBIN TIME TEST. APTT; ACTIVATED PARTIAL THROMBOPLASTINE TIME TEST.

IV. DISCUSSION

This current studytakes a look at values of platelets, D-Dimer, ADAMTS 13, vWBF, PT and APTTin newly infected tuberculosis patients.

One parameters that increased in value was the platelets count. The mean ± SD for platelet of control values when compared with that of newly infected TB subjects, shows a statistically significant increase with a p value of P=0.000 applying the students t-test analysis. This rise in platelet number might be related to increased levels of interleukin 6 and may lead to a hypercoagulable state and deep vein thrombosis [22]. The same theory was postulated by Lieberth et al, 2015 [23]. Also, the increase in the platelet count has been reported to be correlated with the severity of tuberculosis and acute phase reactants [15].

Our findings are in agreement with the findings of Andrew and Edwin, 2013 who reported that Mycobacterium tuberculosis infection is associated with thrombocytosis [22]. Other researches that corroborated with ours on platelet value on TB patients include and Unsal*et al*, 2004 and Feng *et al*., 2011 [16, 24]

There was a significantly increased D-Dimer values when control was compared to newly diagnosed TB subjects.D-Dimer is a fibrin degradation product, whose higher levels in TB patients tells us about a high level of fibrinolysis. This might be triggered by higher levels of factors that promotes fibrinolysis. 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 an elevated level of D-dimer [25]. The same result was reported by Ekrem*et a*l 2006 and van *et al* [26, 27].ADAMTS-13 functions by specifically cleaving VWF multimers and regulates its platelet-tethering function.There was no significant difference in the level of ADAMTS 13 when controls levels was compared to newly infected subjects in our study. On the contrary there was an increase in the level of vWBF. It is known that during proinflammatoryconditions, the endothelium becomes activated resulting in attraction of leukocytes and thrombus formation. The large multimer von Willebrand factor, secreted by endothelial cells, is an acute phase protein and capable of binding platelets and clotting factors. Under normal circumstances ADAMTS13 regulates von Willebrand factor levels by proteolytical degradation of the multimers[28]. In this study, Pulmonary TB might have caused the decreased ADAMTS13 concentrations which most probably led to the enhanced levels of von Willebrand factor antigen. This fact suggests that pulmonary TB is associated with systemic activation of the vascular endothelium. Our findings agrees with Lieberth*et al* 2015 [22]. Furthermore the reduced nature of ADAMTS 13 might be as a result of the disease activating ADAMTS13 inhibitors.Furlan and Lämmle,2001 andTsai,2003 revealed that Since the discovery of ADAMTS13, specific epitopes on its surface have been shown to be the target of inhibitory antibodies [29,30].Deficiency of ADAMTS13 was initially discovered in Upshaw Schulman

Syndrome, the recurrent familial form of Thrombotic Thrombocytopenic Purpura [31]. Truncated levels of ADAMTS13 are also related with an increased risk of arterial thrombosis,including myocardial infarctionand cerebrovascular disease [32, 33, 34, 35].

V. CONCLUSION

The tuberculosis patient is in a state of hypercoagualability therefore care givers should take note and possibly include treatment for coagulation disorders.

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