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Some hemostatic parameters of patients with pulmonary tuberculosis infection attending Aminu Kano Teaching Hospital Kano, Nigeria

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ABSTRACT

Objective: Tuberculosis (TB) is a major public health problem in Nigeria. This study was aimed at providing information on pattern of some hemostatic parameters of pulmonary TB (PTB)-infected patients.

Materials and Methods: This is a comparative cross-sectional study of 102 participants comprising 51 TB-infected individuals and 51 healthy individuals as control. Five milliliters of blood were drawn from each subject and 3 ml was transferred into 0.3 ml of trisodium citrate (3.8%) anticoagulant plastic tube for the analysis of prothrombin and activated partial thromboplastin time using standard techniques while the remaining 2 ml was then transferred into an ethylenediaminetetraacetic acid container (of what concentration) for platelet count and morphology.

Result: The median interquartile range of the PLC, platelet morphology, prothrombin time (PT), and APPT was determined and comparative analysis using Chi-square was made and found to be statistically significant (P = 0.001, 0.001, and 0.001, respectively). There was no statistically significant association between hemostatic parameters (PT, activated partial thromboplastin time with kaolin [APTTK], and platelet count) and body mass index. A Kruskal–Wallis H test showed that there was a statistically significant difference in PT, APTTK, and platelet count between the different categories of patient's *Mycobacterium tuberculosis* load ($P < 0.005$). Chi-square statistics revealed the association between APTTK and platelet count, with anti-TB drugs regimen to be statistically significant. However, there was no statistically significant association between PT and anti-TB drug regimen.

Conclusion: This study revealed that PTB affects hemostasis by prolongation of PT and APTTK, it is also associated with giant platelet formation and intravascular platelet aggregation.

Keywords: Prothrombin time, Thromboplastin time, Hemostasis, Hemoptysis

INTRODUCTION

Tuberculosis (TB) is a disease of the ancient world and still of much relevance today; particularly in developing countries, where it constitutes a major public health threat.[1] Besides the pulmonary system, it can affect the bones, the central nervous system, and many other organs and systems in the body.[2] It is caused by a closely related group of organisms, all of which form the *Mycobacterium tuberculosis* complex. These organisms include *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti,* and *Mycobacterium canettii*.[3] Pulmonary TB (PTB) is an infection of the lower respiratory tract, initiated by the inhalation

of *M. tuberculosis* contained aerosolized sputa.[4] The hallmarks of the active disease are protracted cough, fatigue, loss of weight and appetite, and night sweats. Hemoptysis, secondary infection by Fungi, and permanent lung damage are few of the complications of PTB.[5] Nigeria ranks first in Africa and fourth among the 22 high TB burden countries in the world. No fewer than 460,000 cases of TB are reported annually in Nigeria.[6] The coinfection of PTB with human immune deficiency virus has in the recent past compounded the epidemiology, clinical outcome, diagnosis, and treatment of the disease worldwide,[7] this has led to the different reports made for this condition in Nigeria.[8] Hemoptysis is one of the major presentations related to vascular walls disorders.[9] A number of earlier studies reported the occurrence of thrombotic complications, particularly disseminated intravascular coagulation (DIC) and deep vein thrombosis, in TB patients. The aberrant expression of tissue factor, the primary activator of coagulation cascade, is known to be responsible for thrombotic disorders in many diseases including bacterial infections.[10] However, severe infection and inflammation almost invariably lead to hemostatic abnormalities, ranging from insignificant laboratory changes to severe DIC. Therefore, the objective of this study is to assess the effect of TB on blood coagulation parameters among patients attending Aminu Kano Teaching Hospital, Nigeria.

MATERIALS AND METHODS

This is a comparative cross-sectional study among patients with PTB attending Aminu Kano Teaching Hospital Kano and apparently healthy subjects drawn from who served as control. A total of 102 adult participants were enrolled in the study, 51 were patients with PTB on treatment (definitively diagnosed with PTB using sputum smear microscopy, Genexpert test, chest radiograph, clinical history, and physical examination) the test group and the remaining 51 were apparently healthy blood donors serving as control. Activated partial thromboplastin time and prothrombin time (PT) were conducted on plasma obtained from venous whole blood collected into trisodium citrate (3.8%) at a ratio of 1:9; and samples were stored at −20°C till the time of analysis. The kits used were obtained from K Labkit, Chemelex Company, Barcelona, Spain, and manufacturer's instructions were strictly adhered to. Platelet count was performed manually using improved Neubauer counting chamber after a dilution of 1 in 20 with 1% (10 g/L) ammonium oxalate, the preparation was examined microscopically using a ×40 objective and ×10 eyepiece. Platelet morphology was ascertained from a well-made thin peripheral blood film stained with Leishman's stain. *M. tuberculosis* load information was extracted from the hospital record of the patients. The body mass index (BMI) was calculated from height and weight measured for each participant by the researchers.

Ethical clearance was obtained from the ethical committee of Aminu Kano Teaching Hospital (NHREC/21/08/2008/AKTH/EC/2305). In addition, all the subjects signed a written informed consent to participate in the study with an assurance of confidentiality.

Statistical analysis

The data obtained were presented in tables as percentages and median. Chi-square, Fisher's exact test, Mann–Whitney U-test, Kruskal–Wallis H test, and Spearman correlation were used to test for the level of significance using SPSS version 23. Significance level was set at $P \leq 0.05$ and 95% confidence interval.

Table 1: Socioanthropometric attributes of participants.

Parameter	Test (*n*=51)	Control (*n*=51)	*P* value
Gender *n* (%)			
Male	32 (63)	49 (96)	0.010[1]
Female	19 (37)	2 (4)	
Median age (IQR)	34 (27–44)	29 (25–33)	0.036[2]
Median body mass index (IQR)	16 (15–18)	21 (19–22)	0.001[2]

[1]Determined using Chi-square, [2]Determined using unpaired sample *t*-test. IQR: Interquartile range

Table 2: Distribution of hemostatic parameters among participants.

Parameter	Test *n*=51 (%)	Control *n*=51 (%)	*P* value
PT			
Normal	30 (59)	45 (88.2)	0.001
Prolonged	21 (41)	5 (11.8)	
APTTK			
Normal	0	40 (78.4)	0.001
Prolonged	51 (100)	11 (21.6)	
PLT count			
High	37 (73)	8 (14)	0.001
Normal	14 (27)	43 (86)	
Low	0	0	
PLT morphology			
Normal	1 (2)	51 (100)	0.001
Large	39 (76)	0	
Dimorphic	8 (16)	0	
Large+agg	3 (6)	0	

P-value determined using Chi-square for prospective data.
KEY; PT: Prothrombin time, APTTK: Activated partial thromboplastin time with kaolin, PLT: Platelet, normal: Values within 11–16 s and 30–39 s for PT and APTTK, prolonged: Values >normal, large: Predominant of the platelets are larger than one-third of the diameter of normal red cell, dimorphic: Blood film shows a picture of mixed platelet population at near similar proportion, large+agg: Large platelets and aggregates of platelets were found

Table 3: Comparison of hemostatic parameters between the test and control.

Variables	Median (IQR)		
	Test	Controls	*P*-values*
PT (seconds)	18 (15–20)	15 (15–16)	0.023
APTTK (seconds)	46 (40–86)	34 (29–35)	0.012
Platelet count ($\times 10^9$/L)	400 (333–488)	230 (185–288)	0.001

IQR: Interquartile range, PT: Prothrombin time, APTTK: Activated partial thromboplastin time. **P*-value determined using two independent samples Mann–Whitney U-test

RESULTS

The test group have reduced body mass index compared with the control group, 16 and 21 kilogram per meter square $P = 0.001$. The median age of the participants was 34 and 29 yr respectively for the test and control group Table 1.

As depicted in Table 2, the test group has higher proportion of participants with prolonged PT and activated partial thromboplastin time with kaolin (APTTK) values; 41% against 10% and 100% against 20%, respectively. This prolongation of the PT and APTTK values in a portion of the test group has affected the overall averages in the group compared to the control; 18 (15–20) s against 15 (15–16) s and 46 (40–86) s against 34 (29–35) s; $P = 0.00$ and 0.00, respectively [Table 3]. However, PT and APTTK correlated weakly with BMI of the participants; Spearman's $\rho = 0.088$ and -0.082; and $P = 0.539$ and 0.565, respectively [Table 4]. Paradoxically, however, PT and APTTK appeared to be influenced by the mycobacterial load of the participants as semi-quantitatively determined using Genexpert machine (a nucleic acid amplification technique for *M. tuberculosis*-specific gene sequence) Kruskal–Wallis H test; $P = 0.003$ and 0.001, respectively [Table 5].

The platelet count varied significantly between the two groups; 400 (333–488) $\times 10^9$/L and 230 (185–288) $\times 10^9$/L; $P = 0.000$ [Table 2]. However, not all the test participants have elevated counts: 73% compared to 14% in the control group [Table 1]. The morphology of platelets was dramatically altered among the test participants with 98% having either giant platelets or platelet aggregates, this picture is not seen in the control group [Table 1]. Platelet count appears to be affected by the *M. tuberculosis* load with the group with very low and high *M. tuberculosis* load groups having the least and most elevated count 334 (282–376), 399 (307–419), 404 (393–450), and 501 (478–511), respectively, $P = 0.00$ [Table 3]. Platelet count correlates poorly with BMI in patients with TB [Table 3].

Table 4: Relationship between body mass index and hemostatic parameters.

Hemostatic parameters	Spearman's ρ	*P*-value
PT (s)	0.088	0.539
APTTK (s)	−0.082	0.565
Platelet count ($\times 10^9$/L)	0.128	0.370

ρ: Correlation coefficient

DISCUSSION

The result of this study has shown that the TB patients had both deranged extrinsic and intrinsic pathways of coagulation as indicated by prolonged PT and activated partial thromboplastin time (APTTK) than normal healthy individuals, normal values = 11–16 s and 30–39 s, respectively. The result is consistent with the findings of Kutiyal *et al*.[11] who demonstrated that TB patients have prolonged PT and APTTK than in normal control subjects. A similar report was also documented by Kartaloglu *et al*.[12] However, this contradicted the findings of Toppo *et al*.[1] in a study 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 thought to influence the prolonged procoagulant biomarkers. The prolonged APPT was also believed to be due to phospholipid-dependent coagulation marker, known to be prolonged by antiphospholipid antibodies such as lupus anticoagulant.[13] Researchers had documented increased presence of lupus anticoagulant in TB patients.[14]

This study also revealed an increase in platelet count in our patients compared to our control group. This agrees with the report of Kutiyal *et al*.,[11] Kartaloglu *et al*.,[12] and Bashir *et al*.,[15] in which they suggested that the thrombocytosis was due to elevation in thrombopoietin seen in the acute phase of the TB infection, they also stated that IL-6 secreted by activated T-cell in TB patients has thrombopoietic activity which stimulates megakaryocytes to secret platelets continuously.[12]

The difference in platelet morphology between TB patients and normal control individuals was also revealed to be statistically significant by this study. TB patients had macrocytic thrombocytes while control individual had a normal thrombocyte picture. Large platelets are juvenile platelet present in blood circulation due to hyperproduction

Table 5: Comparison of hemostatic profile with *Mycobacterium tuberculosis* load.

Hemostatic parameters {median (IQR)}	*Mycobacterium tuberculosis* load				H statistic (df)	*P*-value
	Very low mtb load (>28 ct range)	Low mtb load (22–28 ct range)	Medium mtb load (16–22 ct range)	High mtb load (<16 ct range)		
PT (s)	15.5 (15–18.5)	15.5 (14–18)	19 (17–20)	20 (18–26.5)	13.65 (3)	0.003
APTTK (s)	41.5 (38.8–46.8)	45.5 (41–79)	46 (41–66)	94 (77.3–102.3)	16.39 (3)	0.001
PLT count×10^9/L	334 (282–376.3)	399 (307.5–419.3)	404 (393–45)	501 (478.8–511)	20.49 (3)	0.000

P-value determined by Kruskal–Wallis H test, Statistically significant values, mtb: *Mycobacterium tuberculosis*, ct range: Range cycle threshold value for the real-time polymerase chain reaction

of platelet by bone marrow of megakaryocytes which liberate younger platelet.[16] Large platelets are bigger than normal platelet and cannot clot properly and as a result, patients may bleed from even small injuries, develop substantial bruising, and may experience internal bleeding that is difficult to stop because the blood does not clot.[16]

This study found out no statistically significant association between hemostatic parameters (PT, APTTK, and platelet count) and BMI. Even though all the participants with TB were underweight (<18.5 kg/m^2), the hemostatic parameters correlated poorly with the BMI. The proposed mechanisms of weight loss in TB may not have an effect on the synthesis of the clotting factors. The inflammatory cytokines involved in cachexia are elevated in TB, namely, IL-6, tumor necrosis factor, and interferon-gamma. These cytokines directly exert catabolic effects on skeletal muscles and adipocytes by utilizing the popular ubiquitin proteasome pathway[17] and inhibition of MyoD a transcription factors essential for fast twitch muscle development and inhibition of mRNA for myosin heavy chain production.[18] The end result of muscle proteolysis is the generation of small peptides which are used by the liver in acute-phase reactants production such as the coagulation proteins,[18] but we found out that there was a statistically significant difference in PT, APTTK, and platelet count between the different categories of patient's *M. tuberculosis* load. A greater proportion of patient had a decreased TB load. Cross-tabulation between hemostatic parameters and mycobacterium load showed that patients with decreased TB load have normal PT, normal platelet count, and slight prolongation in activated partial PT. High TB load is associated with severe prolongation in APTTK: As high as 120 s. Chi-square statistics showed these to be statistically significant ($P < 0.05$). This is because the higher the number of mycobacterium bacilli is more associated with deranged hemostatic parameters than when the load is reduced.[13]

CONCLUSION

This study revealed that PTB affects hemostasis by prolongation of PT and APTTK; it is also associated with giant platelet formation and intravascular platelet aggregation.

Authors' contributions

Saidu H and Muhammad H conceived the idea designed the work; Muhammad H performed experiment; Saidu H, Garba N, Danladi S.B, and I.A Aliyu supervised the conduct of the experiment; and Muhammad H and Saidu H analyzed the data and wrote the manuscript. Garba N, Danladi S.B, and I.A Aliyu proofread the manuscript before submission.

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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