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Pattern of Some Haemostatic Parameters Among Patients with Tuberculosis Attending Aminu Kano Teaching HospitalSaidu H.¹, Muhammad A.H.*¹, Garba N.¹, Danladi S.B.¹ and Aliyu I.A.¹Department of Medical Laboratory Science, Bayero University Kano¹
Author for Correspondence *: mohdadamu2@yahoo.com**Abstract**

Tuberculosis (TB) is a major public health problem in Nigeria. This aim of this study was to investigate the pattern of some haemostatic parameters among patients with pulmonary tuberculosis (PTB). This comparative cross-sectional study included 102 participants comprising 51 TB- infected subjects and 51 healthy controls. Sterile 5ml syringe was used to withdraw 5ml of blood specimen from each subject the 3ml of blood was transferred in to 0.3ml of trisodium citrate (3.8%) anticoagulant plastic tube and the plasma was obtained by centrifugation at 2500rpm for 15 minutes and then stored at -20°C. The remaining 2ml of blood was transferred in to an EDTA container for platelet count and platelet morphology. The median interquartile range of the PLC, platelet morphology, PT and APTT were determined and comparative analysis was carried out using chi square analysis. A p-value < 0.05 was considered to be statistically significant. There is no statistically significant association between haemostatic parameters (PT, APTT and platelet count) and body mass index. A Kruskal-Wallis H test showed that there was a statistically significant difference in PT, APTT and platelet count between the different categories of patients with mycobacterium tuberculosis (<0.005). Chi-square statistics revealed a statistically significant association between APTT and platelet count with anti-tuberculosis drugs regimen. However, there is no statistically significant association between PT and anti-tuberculosis drug regimen. This study revealed that PTB infection is associated with prolongation of PT and APTT, giant platelet

formation and intravascular platelet aggregation.

Keywords: *Prothrombin time, Thromboplastin time, haemostasis, haemoptysis***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 (Toppo *et al.*, 2015). Beside the pulmonary system, it can affect the bones, the central nervous system and many other organ systems in the body (Akpan *et al.*, 2016). It is caused by a closely-related group of organisms, all of which form the Mycobacterium tuberculosis complex. These organisms include *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microfti* and *M. Canetti* (Iseman, 2000). Pulmonary tuberculosis (PTB) is an infection of the lower respiratory tract, initiated by the inhalation and subsequent of aerosolized droplets of respiratory secretions containing *Mycobacterium tuberculosis* (MTB) onto lung alveolar surfaces (Ferri, 2010). The hallmarks of the active disease are protracted cough, fatigue, loss of weight and appetite and night sweats. Haemoptysis, secondary infection by fungi and permanent lung damage are few of the complications of PTB (Fraser, 2000). Nigeria ranks first in Africa and fourth among the 22 high TB burden countries in the world. No fewer than 460,000 cases of tuberculosis are reported annually in Nigeria (WHO, 2008). The co-infection of pulmonary tuberculosis with human immune deficiency virus (HIV) has in the recent past compounded the epidemiology, clinical outcome, diagnosis and treatment of the disease

worldwide (CDC, 2011). This has led to the different reports made for this condition in Nigeria (Saidu *et al.*, 2016). Hemoptysis is one of the major presentations related to vascular walls disorders (Saed and Hameed, 2008). A number of earlier studies reported the occurrence of thrombotic complications, particularly disseminated intravascular coagulation and deep vein thrombosis, in tuberculosis (TB) patients. The aberrant expression of tissue factor (TF), the primary activator of coagulation cascade, is known to be responsible for thrombotic disorders in many diseases including bacterial infections (Kothari *et al.*, 2012). However, severe infection and inflammation almost invariably lead to haemostatic abnormalities, ranging from insignificant laboratory changes to severe disseminated intravascular coagulation (DIC). Therefore, the objective of this study was to assess the effect of TB on some blood coagulation parameters among patients attending Aminu Kano teaching hospital, Nigeria.

Materials and Method

This is a comparative cross-sectional study among patients with pulmonary tuberculosis and normal healthy individuals attending Aminu Kano Teaching Hospital Kano. A total of 102 adult participants were enrolled in the study, 51 were patients with pulmonary tuberculosis on treatment (definitively diagnosed with PTB using sputum smear microscopy, GeneXpert test, chest radiograph, clinical history and physical examination) and the remaining 51 were apparently healthy blood donors. Activated partial thromboplastin time and prothrombin time were conducted on plasma obtained from venous whole blood collected into trisodium citrate (3.8%) at a ratio of 1:9. Samples were stored at -20°C till the time of analysis. The kits were obtained from K Lab Company, Barcelona, Spain and manufacturer's instructions were strictly adhered to. Platelet count was performed manually using Improved Neubauer counting chamber covered with a thick cover glass. The blood sample was collected into EDTA and diluted 1 in 20 with 1%(10g/l) ammonium oxalate, the preparation was examined microscopically using a $\times 40$ objective and $\times 10$ eyepiece. Platelet morphology was ascertained

from a well-made thin peripheral blood film stained with Leishman's stain. Mycobacterium tuberculosis load and drug regimen information was extracted from the hospital record of the patients. The BMI was calculated from height and weight measured for each participant by the researchers.

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

Statistical Analysis

The data was edited and cleaned using Microsoft excel spread sheets, measures of central tendency and dispersion, percentages and frequencies were all obtained using the Microsoft excel. Normality test was conducted using SPSS version 23. Chi square, Fishers exact test, Man Whitney U test, Kruskal Wallis H test and Spearman correlation were all performed using SPSS version 23. Significance level was set at $p \leq 0.05$ and a 95% confidence interval.

Results

As depicted in table 1, the test group had higher proportion of participants with prolonged PT and APTT values; 41% against 10% and 100% against 20% respectively. This prolongation of the PT and APTT values in a portion of the test group have affected the overall averages in the group compared to the control; 18 (15 - 20) seconds against 15 (15 - 16) seconds and 46 (40 to 86) seconds against 34 (29 to 35) seconds ($p = 0.00$ and 0.00 respectively) as shown in table 2. However, PT and APTT correlated weakly with body mass index of the participants (Spearman's $\rho = 0.088$ and -0.082 ; $p = 0.539$ and 0.565 respectively as shown in table 3. Paradoxically however, PT and APTT appeared to be influenced by the mycobacterial load of the participants as semi-quantitatively determined using GeneXpert analyzer (a nucleic acid amplification techniques for mycobacterium tuberculosis specific gene sequence). Kruskal Wallis H test p value = 0.003 & 0.001 respectively as shown in table 4.

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 had 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 compared to the control group which showed normal platelet morphology (table 1). Platelet count appears to

be affected by the *Mycobacterium tuberculosis* load with the group with very low and high *Mycobacterium tuberculosis* load groups having the least and most elevated count 334(282-376), 399(307-419), 404(393-450) and 501(478-511) respectively ($p=0.00$). Platelet count correlate poorly with BMI in patients with tuberculosis as shown in table 4. Table 5 shows the association of anti-tuberculosis drug regimen and haemostatic parameters.

Table 1. Distribution of haemostatic parameters among participants.

Parameter		Test-n (%)	Control-n (%)	p- value
PT	Normal	30 (59)	45 (90)	0.000
	Prolonged	21 (41)	5 (10)	
APTT	Normal	0	40 (80)	0.000
	Prolonged	51 (100)	7 (20)	
PLT Count	High	37 (73)	7 (14)	0.000
	Normal	14 (27)	43 (86)	
	Low	0	0	
PLT	Normal	1 (2)	51 (100)	0.000
Morphology	Large	39 (76)	0	
	Dimorphic	8 (16)	0	
	Large+Agg	3 (6)	0	

Key: *p-value: determined using chi square for prospective data.*

PT=Prothrombin time

APTT= activated partial thromboplastin time with kaolin

PLT=platelet

Agg=Aggregate

Table 2 Comparison of haemostatic parameters between the test and control

Variables	Median (IQR)		p- value *
	Test	Controls	
PT (seconds)	18 (15 to 20)	15 (15 to 16)	0.000
APTT (seconds)	46 (40 to 86)	34 (29 to 35)	0.000
Platelet count ($\times 10^9/L$)	400 (333 to 488)	230 (185 to 288)	0.000

Key

IQR = Interquartile range

PT = Prothrombin time

APTT = Activated Partial Thromboplastin Time

p-value= value determined using two independent sample Mann-Whitney U test*

Table 3: Relationship between body mass index and haemostatic parameters

Haemostatic parameters	r_s	p- value
PT	0.088	0.539
APTT	- 0.082	0.565
Platelet count	0.128	0.370

Table 4: Comparison of haemostatic profile with mycobacterium tuberculosis load

Parameter	Very low(n=)	Low(n=)	Medium(n=)	High(n=)	H score	p- value#
PT	21.88	18.42	32.61	36.54	13.645	0.003 ¹
APTT	15.79	24.89	23.44	39.79	16.393	0.001 ¹
PLT count	334	399	404	501	20.492	0.000 ¹

Key

=determined by Kruskal-Wallis H test,

1 = Statistically significant values

Table 5: Association of anti-tuberculosis drug regimen and haemostatic parameters

Parameter	RZHE (%) n=27	RH (%) n= 24	p- value#
PT			
Normal	14 (52)	16 (67)	0.283
Prolonged	13 (48)	8 (33)	
APTT			
Normal	3 (11)	10 (47)	0.012
Prolonged	24 (89)	14 (53)	
PTL count			
Normal	3 (11)	0 (0.0)	0.006
Low	0 (0.0)	11 (46)	
High	24 (89)	13 (54)	

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 Prothrombin time (PT) and activated partial Thromboplastin time (APTT) compared to normal healthy individuals, normal values=11-16secs and 30-39secs respectively. The result is consistent with the findings of Aditya *et al.* (2017) who demonstrated that TB patients have prolonged PT and APTT compared to normal controls. Similar report was also documented by Kartaluglu *et al.* (2001). However, this contradicted the findings of Toppo *et al.* (2015) in a study that determine the effect of pulmonary tuberculosis on haemostasis among 50 participants in India. These authors proposed that various cytokines including tumour necrosis factor alpha (TN α), interleukin 6 (IL-6) emerging from the TB granulomatous lesions were thought to influence the prolongation of procoagulant biomarkers. The prolongation of APPT was also believed to be due to the fact that APTT is a phospholipid dependent coagulation marker, known to be prolonged by anti-phospholipid antibodies such as lupus anti-coagulant (Osita *et al.*, 2012). Researchers had documented increased presence of lupus anti-coagulant in TB patients (Klein *et al.*,2005).

This study also indicated an increase in platelet count in our subjects compared to our control group. This finding is in agreement with a previous report (Kutiya *et al.*, 2017; Bashir *et al.*, 2014; Kartaloglu *et al.*,2001) which they suggest that the thrombocytosis was due to elevation in thrombopoietin seen in the acute phase of the TB infection. Interleukin 6 secreted by activated T-cell in TB patients has thrombopoietic activity which stimulates megakaryocytes to secrete platelets continuously (Kartaloglu *et al.*,2001).

The difference in platelet morphology between TB patients and normal control individuals was found out to be statistically significant ($p=0.000$). TB patients had a large thrombocyte while control individual had a normal thrombocyte picture. Large platelets are juvenile platelet present in blood circulation due to hyperproduction of platelet by bone marrow of

megakaryocytes which liberates younger platelet (Famodu *et al.*, 2004). Large platelets are bigger than normal platelet and cannot clot properly and as a result, patient 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 (Famodu *et al.*, 2004).

We observed that there was no statistically significant association between haemostatic parameters (PT, APTT and platelet count) and body mass index. This may be due to the fact that all our clients were already on anti-TB drug.

But we found out that there was a statistically significant difference in PT, APTT and platelet count between the different categories of patient's mycobacterium tuberculosis load. A greater proportion of patient had a decreased TB load. Cross-tabulation between haemostatic parameters and mycobacterium load showed that patients with decreased TB load have normal Prothrombin time, normal platelet count and slight prolongation in activated partial Prothrombin time. High TB load is associated with severe prolongation in APTT: as high as 120 seconds. Chi-square statistics showed these to be statistically significant ($p < 0.05$). This is because the higher the number of mycobacterium bacilli are more associated with deranged haemostatic parameters than when the load is reduced (Osita *et al.*, 2012).

Conclusion

This study revealed that PTB infection is associated with prolongation of PT and APTT as well giant platelet formation and intravascular platelet aggregation

Conflict of Interest: The authors unanimously declare no conflict of interest

Authors Contribution

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

Reference

- Akpan, P.A. and Josephine, O.E. (2016). Some Haematological Parameters of Tuberculosis (TB) Infected Africans: The Nigerian Perspective. *Journal of Natural Sciences Research*;2: 2224-3186.
- Andrews, R.K. and Berndt, M.C. (2004). Platelet physiology and thrombosis. *Thrombosis Research*, 114:447-453
- Andus, T., Bauer, J. and Gerok, W. (1991). Effects of cytokines on the liver. *Hepatology*;13: 364-375.
- Bashir, A.B., Ageep, A.K., Abufatima, A.S. and Mohamedani, A.A. (2014). Reactive thrombocytosis and erythrocyte sedimentation rate in patients with pulmonary tuberculosis. ;5(3):29-34.
- Boekel, E. and Bartels, P. (2002). Abnormally short activated partial thromboplastin times are related to elevated plasma levels of TAT, F1+2, D-dimer and FVIII: C. *Pathophysiology of Haemostasis and Thrombosis*;32(1):137-142.
- Centers for Disease Control and Prevention (2011). *Controlling TB in Correctional Facilities Atlanta*: Department of Human services & Centers for Disease Control and Prevention.
- Cheesbrough, M. (2010). *District laboratory practice in tropical countries*, part 2 (2nd edition) haematological tests. Cambridge University Press, Cape Town South Africa: 317-347.
- Erem, C., Deger, O., Bostan, M., Orem, A., Sonmez, M., Ulusoy, S. and Telatar, M. (2003). Plasma lipoprotein (a) levels in Turkish NIDDM patients with and without vascular diabetic complications. *Acta Cardiology Sinica*; 54(1):203-207.
- Famodu, A.A., Ajayi, O.I., Awodu, O.A. and Nguepi, P. (2005). Effect of pulmonary tuberculosis on plasma fibrinogen and fibrinolytic activity. *Haematology*;8(3): 423-426.
- Ferri, F.F. (2010). Ferri's differential diagnosis: a practical guide to the differential diagnosis of symptoms, signs, and clinical disorders (2nd ed.). Philadelphia, PA: x (2nd ed.). Philadelphia, PA: Elsevier/Mosby.
- Fibrinogen. *Journal of Laboratory and Clinical Medicine*; 104:223-237.
- Fraser, R.S. (1993). Pulmonary aspergillosis: pathologic and pathogenetic features. *Pathology Annual*;28: 231-277.
- Girma, J.P., Kalafatis, M., Pietu, G., Lavergne, J.M., Chopek, M.W., Edgington, T.S., Gokce, N., Holbrook, M., Duffy, S. J., Demissie, S., Cupples, L.A. and Biegelsen, E. (2001). Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. *Hypertension*;38(6):1349-1354.
- Heemskerk, J.W., Bevers, E.M. and Lindhout, T. (2002). Platelet activation and blood coagulation. *Thrombosis Haemostasis*; 88: 186-193.
- Hoffbrand, A.V., Poul, A. and Moss, H. (2011). *Essential haematology* (6thed), Blackwell, London UK: 314-360.
- Howard, L., Karpatkin, M. and Karpatkin, S. (1984). Studies of the origin of platelet associated. *Journal of Laboratory and Clinical Medicine*; 104(2):223-237.
- Iseman, M.D. (2000). A clinician's guide to tuberculosis. Philadelphia: Lippincot, Williams and Wilkins, New Delhi: Allied Chambers India Ltd.: 352.
- Kartaloglu, Z., Cerrahoglu, K., Okutan, O., Ozturk, A. and Aydilek, R. (2001). Parameters of Blood coagulation In Patients with Pulmonary Tuberculosis. *Journal International Medicine*;2:2.
- Klein, S.K., Slim, E.J., Kruif, De M.D., Keller, T.T., Ten Cate, H., van Gorp, E.C. and Brandjes D.P. (2005). Is chronic TB infection associated with venous thrombotic disease? A systematic review. *Netherland Journal of Medicine*; 63(4):129-136.
- Konstantinos, A. (2010). *Testing for tuberculosis*. Australian Prescriber;33(1): 12-18.
- Kothari, H., Rao, L.V.M., Vankayalapati, R. and Pendurthi, U. (2012). Mycobacterium tuberculosis Infection and Tissue Factor Expression in Macrophages. PLoS ONE; 7(9): e45700.
- Kutiyal, A.S., Gupta, N., Garg, S. and Hira, H.S. (2017). A Study of Haematological and Haemostasis Parameters and Hypercoagulable State in Tuberculosis Patients in Northern India and the Outcome with Anti-Tubercular Therapy. *Journal of*

- Clinical Diagnosis Research*;11(2): OC09–OC13.
- Lippi, G., Franchini, M., Targher, G., Montagnana, M. and Salvagno, G. L. (2008). Epidemiological association between plasma fibrinogen and shortened APTT. *Clinical Biochemistry*; 42(1-2):118–120.
- McFarlane, I.A. (1997). *Textbook of Diabetes*, (2nded.) Oxford: Blackwell Publishing: 640-660.
- Nwankwo, E.K., Kwaru, A., Ofulu, A. and Babashani, M. (2007). Haematological changes in tuberculosis in Kano, Nigeria. *Journal of Medical Laboratory Science*; 14(2):35-39.
- Ogawa, T., Uchida, H., Kusumoto, Y., Mori, Y., Yamamura, Y., Hamada, S. (1991). Increase in tumour necrosis factor alpha and interleukin 6 secreting cells in peripheral blood mononuclear cells from subjects infected with *Mycobacterium tuberculosis*. *Infection and Immunology*; 59:3021-3025.
- Opal, S.M., Kessler, C.M., Roemisch, J. and Knaub, S. (2002). Antithrombin, heparin, and heparan sulfate. *Critical Care Medicine*; 30(5): S325-331.
- Osita, O.S., Amilo, G.I., Ositadinma, I.M., Obi, E., Akawu, B. and Martina, N.A. (2016). Overt increase in lupus anticoagulant level in TB patients co-infected with pulmonary infection. A prospective cohort study. *Kanem Journal of Medical Sciences*; 1(10): 21-27.
- Owens, A.P. and Mackman, N. (2010). Tissue factor and thrombosis. *Thrombosis and Haemostasis*; 104:432-439.
- Pepys, M.B., Hirschfield, G.M. (2003). Correction protein; a critical update: *Journal Clinical Investigation*; 111(12): 1805-1812.
- Saeed, A. and Hameed, G.H.H.M. (2008). Seasonality in pulmonary tuberculosis among migrant workers entering Kuwait. *BMC Infectious Diseases*; 8:3.
- Saidu, H., Ahmad, A.E., Garba, N., Dalhatu, A., Mukhtar, A. and Abdullahi, I.N. (2016) *occurrence of multi-drug resistant tuberculosis among patients with suspected tuberculosis in Katsina state north western Nigeria. Bayero Journal of Medical Laboratory Science*;1(1):113 -118.
- Simeone, R., Bobard, A.L., Bitter, W., Majlessi, L., Brosch, R. and Enninga, J. (2012) Phagosomal rupture by Mycobacterium tuberculosis results in toxicity and host cell death. *PLoS Pathology*; (2): e1002507.
- Stiene-Martin, E.A., Lotspeich-Steininger, C.A. and Koepke, J.A. (1998). *Clinical Hematology: Principles, Procedures, Correlations*, (2nd ed.), Lippincott Publishers, Philadelphia:599-611.
- Toppo A., Varma S., Khare R.L. and Malhotra, Y. (2015) Study of Bleeding and Coagulation Profile in Patients of Pulmonary Tuberculosis in a Tertiary Care Hospital in Chhattisgarh. *International Journal of Contemporary Medical Research*; 2(4):932-937.
- Triplett, D.A. (2000). Coagulation and bleeding disorders: Review and update. *Clinical Chemistry*; 46:1260-1269.
- Tripodi, A., Chantarangkul, V., Martinelli, I., Bucciarelli, P., Mannucci, P.M. (2004). A Shortened activated partial thromboplastin time is associated with the risk of Venous thromboembolism. *Blood*; 104(12): 3631–3634.
- Valerie, L.N. (2009) Prothrombin time and partial thromboplastin time assay considerations. *Clinical Laboratory Medicine*; 29(2): 253–263.
- WHO Report (2008). Global tuberculosis control: Surveillance, planning and financing.

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