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Research Article
Inflammatory Mediators Released in Pulmonary Tuberculosis Enhance Hyper-Coagulable States: A Crucial Role of Tissue Factor

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Abstract

Background and Objective: Immune complexes and pro-inflammatory cytokines deduced from communicable diseases have been manifested to induce pro coagulopathy and tissue factor (TF) assertion in macrophages and the endothelial cells that remain at critical risk in tuberculosis (TB) patients. The current study was carried out among Sudanese patients with Pulmonary tuberculosis aimed to determine the long-term impacts of Tb infection on the coagulation cascade. **Materials and Methods:** A cross-sectional study was conducted among 30 patients who are already diagnosed with tuberculosis compared with the control group. Pulmonary Tuberculosis diagnosis of cases was emphasized in accordance with clinical examination, chest X-ray and positive Ziehl-Neelsen (ZN) smear. The questionnaire was used for the collection of demographic and baseline data. About 2.5 mL of venous blood was collected in trisodium citrate containers and 2.5 mL of blood was collected in EDTA container. SPSS version 21 statistical software was used for statistical analysis. **Results:** PLT count showed a significant difference ($p = 0.03$) with a mean (329.20×10^3 and 287.60×10^3 μL^{-1}) among patients and control, respectively. APPT shows a significant difference ($p = 0.00$), Mean of PLT decreased as the disease progressed (336.20 ± 36.02, 345.43 ± 16.02, 511.04 ± 42.02) showed a significant correlation between PLT count of test and duration of disease ($p = 0.00$). Additionally, a significant correlation between PLT count, MPV and APTT and the status of the patient's drug resistance was revealed ($p \leq 0.02$, 0.01 and 0.02). **Conclusion:** There is a significant alteration in coagulation parameters (PT, APTT and platelets count) among Sudanese pulmonary tuberculosis patients, which may indicate a feature of a hypercoagulable state.

Key words: Pulmonary tuberculosis, coagulation factors, inflammation, tissue factor, hypercoagulable

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tuberculosis (TB) is a contagious bacterial disease and Mycobacterium tuberculosis (MTB) is the most frequent biological agent, most infections will not cause symptoms, in which particular instance it is referred to as dormant tuberculosis. About 10% of latent infections develop into advanced TB, which ends up killing approximately half of those infected if remains untreated[1,2]. It is representing a global health problem in which its major etiology of morbidity and mortality globally, killing approximately 1.2 million people out of an estimated 10 million new cases in 2019[3], a high frequency of tuberculosis (TB) was an estimated in Sudan, with 50,000 approximate reported cases during 2009[4]. Pathophysiology in pulmonary tuberculosis is unsatisfactory to the burden of mycobacteria and patients can reveal with the extensive inflammatory process. Following inhalation, macrophages, the primary innate immune signal transduction host cells for bacterial multiplication are thought to phagocytose mycobacteria. When phagocytes get to be functional, they recruit other immune cell cells, like granulocytes[5,6], which are crucial early encounters in tuberculosis infection, putting on an act in pathogen eradication and also inflammatory processes[7].

Macrophages activate the inflammatory process to tuberculosis by phagocytic activity against the mycobacteria and the production of cytokines. The cytokines released assembled numerous enormous immune cells at the infection site[8,9]. Platelets are found at the site of TB disease as a consequence of platelet extravasation to the abscess or platelet biogenesis within the lung as a whole. Megakaryocytes have been encountered in the lungs and may be capable of increasing platelet development in response to certain stimuli. This would give credence to platelets acting as first responders in MT inflammation, placing them in close vicinity to any mycobacterial invaders. Furthermore, to MPV, the platelet indices PDW and PCT estimated by PC and MPV have been linked to atherosclerosis and thrombosis, along with tuberculosis[10,11].

Inflammatory response and coagulation are interconnected, with both the two methods linked by three major processes: Initiation of coagulation, downregulation of innate anticoagulants and decreased expression of fibrinolysis. Because pro-inflammatory cytokine TNF-α and interleukin-6 (IL-6) have been shown to involve in platelet development and high procoagulant occurrence, when injuries occur and haemorrhage starts, clotting factors are stimulated a sequentially (coagulation cascade) that results in blood clotting[12-14]. The abnormal expression of tissue factor (TF), the principal activator of the coagulation system has been linked to thrombotic abnormalities in a variety of diseases, along with bacterial infections, furthermore, to their deleterious effects, the TF-mediated extrinsic coagulation process may also serve as a preventive measure in protective immunity towards certain bacterial infections by minimizing pathogen burden and restricting pathogen spread[15,16]. The current study was carried out among Sudanese patients with Pulmonary tuberculosis and aimed to find out the subsequent effect of PTB on blood coagulation factors and to establish policies to be considered in the management of Sudanese patients with PTB.

MATERIALS AND METHODS

Study design: This was the descriptive cross-sectional study conducted in Port Sudan Teaching Hospital at Red Sea State-Sudan during the study period April-October, 2019 among 30 patients were already diagnosed with tuberculosis compared with the apparently control group. Pulmonary Tuberculosis (PTB) diagnosis was confirmed after clinical assessment, chest X-ray and positive Ziehl-Neelsen (ZN) stain. All new and old diagnosed cases as well as patients under tuberculosis therapy, with and out multidrug-resistant patients (MDR) were included in the study. The questionnaire was used for the collection of demographic and baseline data.

Blood samples: The subject's venous blood (2.5 mL in the proportion to 0.25 mL of anticoagulant) was drawn into a trisodium citrate container and a further 2.25 mL, 2.5 mL of blood was collected in EDTA container.

Instruments Mindray BC3000 plus: The WBC detector block employs the DC detecting approach to calculate the platelet count. The RBC detector block counts the RBCs and platelets by using the LDC detection approach[17].

Mean platelet volume (MPV) and platelet distribution width (PDW): MPV is machine-circulated management of the average size of platelet found in blood and is typically included in blood tests as part of the CBC:

Normal range = 7.5-11.5 Fl

For PDW it's a simple practical and specific marker of activation of coagulation. PDW is the variability in the size of platelets. Normally PDW increases with MPV[17].

Semi-automated coagulometer (Helena1)

Principle: Photo-optical, 1, 2 or 4 channel laser utilizing 450 nm wavelength. Suitable for icteric and lipemic samples. auto-start triggered by reagent addition. Which measurement clotting chromogenic and immune turbid meter[18].

Ethical consent: During the interview, each participant was asked to sign a written ethical consent form before the specimen was taken.

Data analysis: SPSS version 21 statistical software was used for statistical analysis. The results (PT, APTT and platelet count) were demonstrated as means standard deviations (SD) and the 95% confidence intervals (CIs) of the means were calculated. The t-test was used to determine categorical data differences between groups. Pearson's correlation analysis was used to determine the relationships between variables. The p-value of 0.05 level of significance was significant.

RESULTS

This cross-sectional study was conducted among pulmonary tuberculosis patients, all patients and the control group were tested for PLT count, MPV, PDW, PT and APTT. A total of 30 patients with Pulmonary Tuberculosis were enrolled, the majority of them 28 (93.3%) were male, their age the range between 10-40 years old (mean 30.5±3.8 SD) and the mean duration of the disease is 68.5 days, in addition to 30 healthy individuals as control group were recruited in this study, 10 (33%) of them were in age group more than 40 years. There is no statistically significant difference between gender and age between the case and control (p-value 0.061 and 0.235), all data were displayed in Table 1.

Clinical data of pulmonary tuberculosis was shown in Table 2, 46.7% of patients had Less than 60 days of infection and the majority of them (57 and 30%) had moderate to severe mycobacterium on Ziehl-Neelsen (ZN) smear, respectively. Only (27%) have multidrug resistance to mycobacterium.

Table 3 illustrated comparison of platelets indices and coagulation profile between pulmonary tuberculosis patients and control, PLT count shows a significant difference (p = 0.03) with a mean (329.20×10^3 and 287.60×10^3 μL^{-1}) among patients and control, respectively. There is no significant difference in MPV, with the mean 9.70±1.02 and 9.74±16.40 FL (p = 0.3). However, PDW show a significant difference (p = 0.00) the mean of the test = 15.91 FL while the mean of control = 9.86 FL. PT shows a significant difference (p = 0.00), the mean of the PT test = was 16.41 sec while the mean of control = was 14.21 sec. APPT show a significant difference (p = 0.00), the mean of the PT test = 38.31 sec while the mean of control = 35.11 sec. There was no

Table 1: Demographic data of study participants

Parameters	Case (n = 30) (%)	Control (n = 30) (%)	p-value
Gender			
Male	28 (93.3)	21 (70)	0.061
Female	2 (6.7)	9 (30)	
Age groups			
10-20 years	4 (13.3)	6 (20)	0.235
21-30 years	12 (40)	9 (30)	
31-40 years	8 (26.7)	5 (17)	
More than 40 years	6 (20)	10 (33)	
Total	30 (100)	30 (100)	

Table 2: Clinical data of pulmonary tuberculosis (PTB)

Parameters	Frequency (n = 30)	Percentage
Duration of tuberculosis		
Less than 60 days	14	46.7
60-120 days	11	36.7
More than 120 days	5	16.7
Severity of disease according to (ZN) smear		
+ (Mild)	4	13
++ (Moderate)	17	57
+++ (Sever)	9	30
History of treatments		
With multidrug-resistant	8	27
Without multidrug resistant	22	73

Table 3: Comparison of platelets indices and coagulation profile between pulmonary tuberculosis patients and control

Parameters	Patients	Control	p-value
PLT	329.20±86.08	287.60±63.63	0.03
MPV	8.29±1.02	11.34±16.40	0.30
PDW	15.91±0.70	9.86±1.26	0.001
PT	16.41±1.42	14.21±1.28	0.001
APTT	38.31±4.98	35.11±2.91	0.001
INR	1.26±0.10	1.29±0.20	0.51

T-test was used to calculate the p-value and *p>0.05 was considered significant

Table 4: Correlation with the duration of tuberculosis patient

Parameters	≤60 days (Mean±SD)	60-120 days (Mean±SD)	≥120 days (Mean±SD)	Correlation	p-value
PLT	336.20±36.02	345.43±16.02	511.04±42.02	-0.49	0.00*[a]
MPV	9.59±1.32	5.57±3.02	3.76±1.12	0.42	0.01*[b]
PDW	15.91±0.70	16.35±2.73	19.27±0.91	-0.06	0.73*[c]
PT	18.31±2.42	12.42±0.12	9.11±1.22	-0.03	0.86*[c]
APTT	39.54±2.12	42.31±8.22	49.24±0.42	-0.38	0.03*[d]
INR	1.26±0.10	0.88±0.30	0.52±1.24	0.02	0.89*[c]

*[a]Medium adverse correlation, *[b]Medium extrusive correlation, *[c]Nihilistic and *[d]Weak adverse correlation

Table 5: Correlation means of platelets and treatment status in tuberculosis patients

Parameters	Without multidrug-resistant (Mean±SD)	With multidrug-resistant (Mean±SD)	Correlation (Mean±SD)	p-value
PLT	329.20±86.08	459.20±23.10	-0.4	0.02*[a]
MPV	10.16±1.02	4.43±0.07	0.47	0.01*[b]
PDW	16.51±0.40	18.38±0.73	-0.08	0.69*[c]
PT	16.41±1.42	8.37±2.12	-0.01	0.92*[c]
APTT	38.31±4.98	43.21±2.18	-0.42	0.02*[d]
INR	1.26±0.10	0.88±0.23	0.02	0.89*[c]

*[a]Medium adverse correlation, *[b]Medium extrusive correlation, *[c]Nihilistic and *[d]Weak adverse correlation

significant difference in the International normalized ratio, the mean of the INR test = was 1.26 while the mean of control = was 1.29 (p = 0.51).

Mean of PLT decreased as disease progress (336.20±36.02, 345.43±16.02, 511.04±42.02) show significant correlation between PLT count of test and duration of disease (p = 0.00) medium adverse correlation r^{-} = -0.49. Show significant correlation between MPV of test and duration of disease (p = 0.01) medium extrusive correlation r^{-} = 0.42. There is no significant correlation between PDW of test and duration of disease (p = 0.73) nihilistic correlation r^{-} = 0.06. A significant correlation between APTT of test and duration of disease (p = 0.03) weak adverse correlation r^{-} = -0.38, no significant correlation between INR of test and duration of disease (p= 0.89) Nihilistic correlation r^{-} = 0.02. All data were summarized in Table 4.

Table 5 displays the correlation means of platelets and treatment status in Tuberculosis patients, where a significant correlation between PLT count, MPV and APTT and status of patient's drug resistance was revealed (p≤0.02, 0.01 and 0.02) with medium adverse correlation r^{-} = -0.40, Medium extrusive correlation 0.47 and weak adverse correlation -0.42).

DISCUSSION

The stimulation of the coagulation and fibrinolytic pathways as consequences of a variety of bacterial infections is a crucial component of both pathways. Immune complexes and many other factors elicited by various infectious diseases have been shown to stimulate pro-coagulant tissue factor (TF) expression in monocytes/macrophages and the endothelium, which do not express TF in a normal healthy state. The present study was performed in Port Sudan Teaching Hospital and aimed to find out the subsequent effect of PTB on blood coagulation and establish policies to be considered in the management of Sudanese patients with PTB.

A statistically significant increase in platelet count among patients compared to the control group was revealed (p-value 0.003), this finding is inconsistent with previous investigations which have shown that variations in platelet count, especially during TB infection, may be related to mortality and severity of the condition[19,20]. So platelets contribute to TB immunopathology, in which tissue damage in tuberculosis is induced by enzymes, principally matrix metalloproteinases (MMPs) that are released into the blood by leukocytes and stromal cells and are responsible for

extracellular matrix degeneration[21]. As well as patients with tuberculosis tend to have greater platelet counts, which directly relate to pain intensity as well as a hypercoagulable manner. Human TB granulomas contain platelets and platelet-associated gene transcripts are significantly greater in TB patients than in controls. Platelets most likely stimulate other immune cells, particularly monocytes, leading to higher activation markers, MMP secretion and phagocytic cells[22,23].

Elevated Prothrombin time reported in this study has previously been identified in patients with pulmonary TB [24,25], it is considered to be prolonged by cytokines and mediators released from a tuberculosis abscess. When compared to the control group, the patient has considerably higher platelet manufacturing capacity. According to other statistics, cytokines have risen in inflammatory responses and these cytokines (primarily IL-6) enhance platelet development[1,23]. In addition, inflammatory cytokines (such as bacteria lipopolysaccharide, thromboxane A2 and others) stimulate platelets. Platelet production and initiation trigger the activation of the secondary hemostatic paths (formation of thrombin). The interplay between activated platelets and subendothelial cells provokes adherence and the discharge of the cytokine Interleukin-1 (IL-1) by inflammatory white blood cells, which improves endothelial cell adhesion[24]. Tissue thromboplastin, which is connected to FVIIa, initiates the fibrin pathway in activated endothelial cells. The findings demonstrate that PT and APTT times were significantly longer in cases and platelet counts were markedly higher in cases compared with controls. Our results were consistent with a study conducted by Eldour *et al.*[26] in Sudan (North Kordofan State) in January 2014, which revealed significant PT ($p = 0.00$), APTT ($p = 0.02$) and Platelet count ($p = 0.00$) among TB patients.

With regard to platelet count and coagulation profile with the duration of disease (state of acute and chronic course), platelet count as well as investigated coagulation profile and alters have been revealed in tuberculosis patients. Patients with active tuberculosis have an overwhelming amount of alpha granules[27] that encompass pro-inflammatory mediators such as tumour necrosis factor-alpha (TNF) and interleukin-1 beta (IL-1), both of which are recognized to be highly expressed in TB patients. Patients with severe tuberculosis, on the other hand, had lengthened platelets and an increased number of alpha and dense granules. These contain a variety of mediators such as adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin and ionized calcium, which is implicated in the coagulation cascade. These discrepancies may represent specific functional responsibilities at various stages of the disease, with acute TB exhibiting a predominantly pro-inflammatory phenotype and chronic disease exhibiting a combined inflammatory-thrombotic state[25].

Interestingly current y findings noted that there was a statistically significant hypercoagulable state among patients in multi Drug-resistant group in term of platelets count, MPV and prolonged APTT, this is the satisfying result as disease status become worsen and the immune system is exhausted as well as overall devastating immune-compromised status due to reduction in anti-inflammatory cytokines production[28]. Platelets are a crucial element of the innate immune response to tuberculosis, according to adequate documentation. For better management of tuberculosis patients, anti-platelet and anti-inflammatory NSAIDs drugs are prescribed to be used in TB, which are appealing since they are by now confirmed and have well-established safety profiles. Results suggested that they can also help promote tuberculosis control.

Investigation of PLT count, PLT indices, PT and PTT must be done routinely for patients with Pulmonary Tuberculosis to help for good diagnosis and prognosis of patients, study with a large sample size must be applied. Advances tests for the determination of hypercoagulability among patients with TB should be done in further studies including (D. dimer, fibrinogen level, factor VIII, Antithrombin III. Protein C and Plasminogen activator inhibitor-1).

CONCLUSION

There is a significant alteration in coagulation parameters (PT, APTT, PDW and platelets count) among Sudanese pulmonary tuberculosis patients in comparison with healthy subjects, which may indicate a feature of a hypercoagulable state. A significant correlation between APTT of test and duration of disease ($p = 0.03$) and a significant correlation between PLT count, MPV and APTT and status of patient's drug resistance, thus reflecting the role of the compromised immune system in the study participants.

SIGNIFICANCE STATEMENT

Mycobacterium tuberculosis is accompanied by coagulation activation in the bloodstream as evidenced by enhanced fibrinolytic levels in the blood. A few reports are available and a limited sequence has noted a connection between tuberculosis and venous thromboembolism, implying that these patients are in a procoagulant state.

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