Selvan et al | Article 9516
J Pure Appl Microbiol. 2025;19(1):669-681. doi: 10.22207/JPAM.19.1.56
Received: 29 April 2024 | Accepted: 19 February 2025
Published Online: 04 March 2025

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Influence of Anemia on the Prognosis of Patients with Tuberculosis: An Indicator of Severity

Pavithra Selvan[1]*, Nalini Jayanthi Nagesh[2] and Leela Kakithakara Vajravelu[1]

[1]Department of Microbiology, SRM Medical College Hospital and Research Centre, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu District, Tamil Nadu, India.
[2]Department of Respiratory Medicine, SRM Medical College Hospital and Research Centre, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu District, Tamil Nadu, India.

Abstract

Anemia stands out as a widespread health issue, playing a substantial role in the global disease burden. The significant occurrence of anemia in tuberculosis (TB) is concerning due to its correlation with delayed sputum conversion, severe manifestations of TB, TB-related mortality, and TB relapse. The current retrospective research aims to explore the prevalence of anemia amongst 117 recently diagnosed with pulmonary tuberculosis (PTB) and to determine potential risk factors that are related to the condition, within a tertiary care centre in South India. In the present study, the prevalence of anemia was found to be 75.2% in PTB patients and among them, 61.3% had mild anemia, 23.8% had moderate anemia and 14.9% had severe anemia. Moreover, normocytic normochromic anemia was the most common finding (58%). Multivariable logistic regression analysis showed that anemia was significantly associated with gender distribution, alcohol consumption, smoking habits, clinical suspicion with pallor, reduced hemoglobulin count, reduced red blood cell counts, increased leukocytes and neutrophil counts along with reduced lymphocytes, eosinophils and monocyte counts ($p < 0.05$*) suggesting their significant contribution of anemic risk in PTB patients. This association could be due to the impact of TB on the body's nutritional status and immune response. Therefore, anemia was shown to be much more common in individuals with the most severe clinical presentations of PTB, emphasizing that it may serve as an indicator of the severity of TB. Addressing anemia through targeted nutritional and medical interventions could potentially enhance immune competence and improve TB treatment outcomes.

Keywords: Pulmonary Tuberculosis, Anemia, Immune Response, Severity

***Correspondence:** jeevepavi123@gmail.com

Citation: Selvan P, Nagesh NJ, Vajravelu LK. Influence of Anemia on the Prognosis of Patients with Tuberculosis: An Indicator of Severity. *J Pure Appl Microbiol.* 2025;19(1):669-681. doi: 10.22207/JPAM.19.1.56

INTRODUCTION

Tuberculosis (TB), resulting from the *Mycobacterium tuberculosis* (MTB) bacterium, poses a significant and widely recognized public health crisis worldwide, standing as the primary cause of mortality attributed to a single infectious agent.[1] More than 10 million people remain infected with TB annually, making it the second most deadly infectious illness in the world, just ahead of COVID-19 and deadlier than HIV/AIDS. TB is currently the 13th biggest cause of death worldwide.[2,3] Despite being both preventable and treatable, TB claims the lives of 1.5 million individuals annually, solidifying its position as the foremost infectious cause of mortality globally.[4] India bears the brunt of the world's TB epidemic, accounting for 24% of cases worldwide and 29% of deaths from the disease as a result of many risk factors, such as diabetes mellitus (DM), smoking, malnourishment, and HIV/AIDS.[5] Despite these persistent efforts and notable advancements in managing TB infections, the disease continues to rank among the primary causes of mortality and morbidity worldwide, particularly in low- and middle-income nations like India, where a notable proportion of the global TB burden exists.[6]

Anemia ranks among the prevalent health conditions and serves as a significant risk factor increasing the burden of disease worldwide.[7] The World Health Organisation (WHO) estimates that anemia affects 40% of children between the ages of 6 and 59 months, 37% of pregnant women, and 30% of women between the ages of 15 and 49 worldwide.[8] Anemia represents a risk factor for TB and is frequently observed as a complication of the disease.[9] It can arise from various factors, including reduced production of erythropoietin, chronic inflammation, malnutrition, and blood loss. Notably, chronic inflammation and iron deficiency have been identified as primary contributors to the development of anemia in individuals with TB.[10]

Numerous individuals with active pulmonary tuberculosis (PTB) demonstrate reduced haemoglobin (Hb) levels, which can directly influence the morbidity associated with TB.[11] The occurrence of anemia among TB patients varied between 9.5% and 96%, with fluctuations dependent on the study's context.[12] Anemia has been linked to adverse TB outcomes and an increased risk of mortality among TB patients. Its prevalence is notably higher in HIV-associated TB compared to TB patients without HIV infection, indicating a connection between anemia and severe TB cases.[13] It has also been documented that patients with anemia exhibit a higher proportion of TB compared to non-anemic patients, with the highest burden of TB observed among those with severe anemia. This may result from the compromised immune status of anemic patients, leading to increased susceptibility due to significantly reduced cell-mediated immunity and diminished bactericidal function of leukocytes. This susceptibility makes anemic individuals more prone to infectious diseases, including TB, compared to non-anemic individuals.[14,15]

The elevated occurrence of anemia in TB is a concern due to its correlation with delayed sputum conversion, mortality associated with TB, severe TB forms like disseminated disease as well meningitis, and recurrence of TB.[16] Hence, diligent monitoring is appropriate as TB-related anemia typically presents as moderate and tends to improve with anti-TB medication.[17] Therefore, it is essential to understand the characteristics of anemia as a risk factor associated with severe complications of TB, in order to implement interventions aimed at addressing anemia among individuals with TB and advancing towards achieving the END TB strategy.[18] Hence, the current research aims to explore the prevalence of anemia amongst patients recently diagnosed with PTB and to determine potential risk factors that are related to the condition.

MATERIALS AND METHODS

Study design and location

The current retrospective research work has been conducted from January 2023 to December 2023 on the 117 newly diagnosed PTB patients admitted to the Respiratory medicine ward in SRM Medical College Hospital and Research Centre, Kattankulathur, Chengalpattu district, Tamil Nadu, India. As the study relied on pre-existing hospital and laboratory records routinely compiled, no additional consent was solicited from participants.

Study population

All adult patients (19 ≥ 70 years) who were hospitalized and newly diagnosed with active PTB infection were included in the investigation. The research excluded Extrapulmonary TB, pediatric TB and those who had received anti-tuberculosis treatment. Among 117 new PTB cases, patients who did not have anemia were considered as a control group for comparison with the PTB patients with anemia in this study.

Study protocol

The socio-economic and demographic characteristics were studied. PTB was diagnosed in a patient based on three criteria: microbiological confirmation (positive findings for Truenat or Gene Xpert MTB/RIF tests and acid-fast bacilli (AFB) smear), and radiological findings visible on X-ray and CT scan. The research also included patients with significant clinical suspicion and radiological findings but negative microbiological results for PTB. Patients' reports were evaluated for the presence of anemia by several data of the assessments conducted in a flow cytometry assay included hemoglobulin (Hb), erythrocyte sedimentation rate (ESR), Red Blood Cell (RBC) count, packed cell volume (PCV), platelet count, total iron binding capacity (TIBC), red cell distribution width (RDW), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), serum iron levels, folic acid levels, serum creatinine levels, serum ferritin levels, transferrin levels as well as white blood cells counts (Leukocytes, Neutrophils, Lymphocytes, Eosinophils and Monocytes) were conducted as necessary for all cases of anemia.

Definitions of various variables

Anemia was determined based on the criteria outlined in the WHO guidelines: Hb levels below 13 g/dL for men and below 12 g/dL for women. According to WHO guidelines, anemia severity was classified as follows: Haemoglobin values in the range of 11.0-12.9 g/dL for men and 11.0-11.9 g/dL for women are considered mild anemia, 8.0-10.9 g/dL for both sexes is considered moderate anemia, and 8.0 g/dL or less is considered severe anemia.[19]

Anemia was classified using the mean corpuscular volume (MCV) value as follows: microcytic (MCV <80 fL), normocytic (MCV between 80 and 100 fL), and macrocytic (MCV >100 fL). The mean corpuscular hemoglobin (MCH) value was utilized to categorize anemia as hypochromic (if MCH <26 pg) or normochromic (if MCH ≥26 pg).[20]

Data collection

In this study, information was gathered from the patient's case sheets, which included demographic details (such as age, sex, and BMI); behavioral aspects (such as alcohol and/or tobacco use, smoking, and other comorbidities); radiological results; microbiological, and hematological evaluations as mentioned above.

Statistical analysis

Statistical analysis was done using SPSS software (version 25.0, IBM Inc. Chicago). The odds ratio (OR) with 95% confidence intervals (Cis) was identified using a multivariable logistic regression model to identify factors which were associated with anemia status in PTB patients. The p-value < 0.05 was taken as significant.

RESULTS

General characteristics of pulmonary tuberculosis patients

In the current study, out of 3127 suspected PTB cases, 117 (3.7%) were newly diagnosed with PTB infection. PTB cases were more prevalent among males (67.5%) compared to females (32.5%). The PTB patients had an average mean age of 52 years and an interquartile range (IQR) of 46-55 years. Socioeconomic status reported that more cases were observed among individuals from the lower socioeconomic class (56.5%) and also, a significant proportion of PTB patients were illiterate (78.7%) (Table 1).

The present research work showed that a maximum of PTB patients were underweight (38.6%), highlighting malnutrition as a common risk factor for TB. Meanwhile, 31.7% had a normal BMI, 19.5% were overweight and only 10.2% were obese, suggesting that obesity is less common among PTB patients. Among PTB cases, 41.8%

Table 1. Baseline and clinical characteristics of the newly diagnosed PTB patients with and without anemia (n - 117)

Variables	Overall	Anemia status		p value	OR (95%)
		Present (n - 88)	Absent (n - 29)		
Sociodemographic profile					
Gender					
Male	79 (67.5%)	66 (76%)	13 (44.8%)	<0.0027*	3.692
Female	38 (32.5%)	22 (24%)	16 (55.2%)		(1.5367-8.8716)
Age					
19-30	25 (21.3%)	18 (20.5%)	7 (24.1%)	0.8467	NA
31-50	45 (38.6%)	33 (37.5%)	12 (41.5%)		
51-70	36 (30.7%)	29 (32.9%)	7 (24.1%)		
>70	11 (9.4%)	8 (9.1%)	3 (10.3%)		
Mean age 52 (46-55) years					
Type of residence					
Rural	39 (33.3%)	27 (30.6%)	12 (41.3%)	0.2892	0.627
Urban	78 (66.7%)	61 (69.1%)	17 (58.6%)		(0.263-1.492)
Socioeconomic status					
Middle class	51 (43.5%)	41 (46.6%)	10 (34.5%)	0.2562	1.6574
Lower class	66 (56.5%)	47 (53.4%)	19 (65.5%)		(0.6924-3.9673)
Literacy					
Literate	25 (21.3%)	19 (21.6%)	6 (20.6%)	0.9186	1.0556
Illiterate	92 (78.7%)	69 (78.4%)	23 (79.4%)		(0.3761-2.9628)
Nutritional status					
BMI (Kg/m²)					
Under weight (BMI <18.4 kg/m²)	45 (38.6%)	41 (46.6%)	4 (13.8%)		
Normal (BMI 18.5 kg/m² - 24.9 kg/m²)	23 (19.5%)	16 (18.2%)	7 (24.2%)	0.1266	NA
Overweight (BMI 25 kg/m² - 29.9 kg/m²)	37 (31.7%)	28 (31.8%)	9 (31.1%)		
Obese (BMI > 30 kg/m²)	12 (10.2%)	10 (11.4%)	2 (6.8%)		
Dietary intake					
Vegetarian	54 (46.1%)	41 (46.6%)	13 (44.8%)	0.8687	1.0736
Non-vegetarian	63 (53.9%)	47 (53.4%)	16 (55.2%)		(0.4620-2.4950)
Habits					
Alcohol usage					
Yes	49 (41.8%)	42 (47.7%)	7 (24.1%)	0.0262*	2.8696
No	68 (58.2%)	46 (52.3%)	22 (75.9%)		(1.1122-7.4038)
Smoking					
Yes	40 (34.2%)	23 (26.1%)	17 (58.6%)	0.0014*	0.2598
No	77 (65.8%)	65 (74.9%)	12 (41.4%)		(0.1037-0.6014)
Tobacco usage					
Yes	42 (35.8%)	34 (38.6%)	8 (27.5%)	0.2841	1.6528
No	75 (64.2%)	54 (61.4%)	21 (72.5%)		(0.6584-4.1492)

Table 1. Cont...

Variables	Overall	Anemia status		p value	OR (95%)
		Present (n - 88)	Absent (n - 29)		
Clinical Suspicion					
Cough					
Yes	110 (94%)	84 (95.4%)	26 (89.7%)	0.2555	2.4231
No	7 (6%)	4 (4.6%)	3 (10.3%)		(0.5090-11.5340)
Breathlessness					
Yes	55 (47%)	41 (46.5%)	14 (48.2%)	0.8753	0.9347
No	62 (53%)	47 (53.5%)	15 (51.8%)		(0.4034-2.1654)
Anorexia					
Yes	83 (71%)	63 (71.6%)	20 (68.9%)	0.7880	1.1340
No	34 (29%)	25 (28.4%)	9 (31.1%)		(0.4551-2.8257)
Weight Loss					
Yes	97 (82.9%)	73 (83%)	24 (82.5%)	0.9807	1.0139
No	20 (17.1%)	15 (17%)	5 (17.5%)		(0.3334-3.0835)
Fever					
Yes	48 (41%)	37 (42%)	11 (37.9%)	0.6973	1.1872
No	69 (59%)	51 (58%)	18 (62.1%)		(0.5017-2.8091)
Hemoptysis					
Yes	11 (9.4%)	9 (10.2%)	2 (6.8%)	0.5956	1.5380
No	106 (89.6%)	79 (89.8%)	27 (93.2%)		(0.3126-7.5666)
Pallor					
With Pallor	48 (41%)	47 (53.4%)	1 (3.4%)	<0.0001*	32.0976
Without Pallor	69 (59%)	41 (46.6%)	28 (96.6%)		(4.1811-246.404)
Risk Factors					
Hypertension	43 (36.7%)	34 (38%)	9 (31.1%)		
Diabetes Mellitus	65 (55.6%)	52 (59.1%)	13 (44.8%)		
Hyperlipidemia	29 (24.7%)	23 (26.1%)	6 (20.6%)		
Chronic Kidney Disease	5 (4.2%)	4 (4.5%)	1 (3.4%)		
Chronic Obstructive Pulmonary disease	19 (16.2%)	16 (18.1%)	3 (10.3%)		
HIV infection	2 (1.7%)	1 (1.2%)	1 (3.4%)	0.9377	NA
Hepatitis B infection	2 (1.7%)	1 (1.2%)	1 (3.4%)		
Hepatitis C infection	1 (0.8%)	1 (1.2%)	0 (0%)		
Liver cirrhosis	3 (2.5%)	2 (2.2%)	1 (3.4%)		

The result is significant at $p < 0.05$

consumed alcohol, 34.2% were smokers and 35.8% used tobacco. Moreover, cough (94%) was the most identified as the predominant clinical suspicion of PTB patients. Interpretation of risk factors among PTB patients showed diabetes mellitus (55.6%) was the most prevalent co-morbid condition, highlighting its strong association with TB (Table 1).

Sociodemographic and Clinical Characteristics of Pulmonary Tuberculosis Patients by Anemia Status

Table 1 presented the relationship between various sociodemographic and clinical factors of PTB patients based on anemia status. In the present study, the prevalence of anemia was found to 75.2% in PTB patients. A statistically significant association was found between gender

Table 2. Microbiological, Molecular and Radiological findings among PTB patients with and without anemia (n - 117)

Findings	Anemia		P value
	Present (n - 88)	Absent (n - 29)	
AFB Positive	47 (53.4%)	11 (37.9%)	0.5020
CBNAAT	78 (88.6%)	24 (82.7%)	
Radiological Findings			
Upper lung zone lesion	11 (12.5%)	2 (6.8%)	
Lowe lung zone lesion	17 (19.3%)	1 (3.4%)	
Consolidation	22 (25%)	5 (17.2%)	
Cavitary lesion	61 (69.3%)	1 (3.4%)	0.0183*
Pleural effusion	2 (2.2%)	2 (6.8%)	
Tree-in-bud sign	16 (18.1%)	4 (13.7%)	
Cavity lesion with consolidation	5 (5.6%)	1 (3.4%)	
Bronchiectasis	2 (2.2%)	0 (0%)	

The result is significant at $p < 0.05$

and anemia ($p < 0.0027$*) with males being more affected (76%) by anemia compared to females (24%), possibly due to biological factors, dietary habits or other risk factors contributing to a higher anemia prevalence in males. The prevalence of anemia was similar across different age groups, indicating that age does not play a major role in anemia risk among PTB patients ($p > 0.8467$). In this study, no significant difference was observed between rural and urban populations ($p > 0.2892$) suggesting that the residence type didn't influence anemia risk in PTB patients. Moreover, socioeconomic status ($p > 0.2562$), as well as literacy ($p > 0.9186$), also showed no significant association, suggesting that financial background and education do not determine anemia risk among PTB cases.

The present research found that underweight individuals had a higher percentage of anemia (46.6%) compared to the non-anaemic PTB patients (13.8%). Likewise, anemia was slightly more common among non-vegetarians (53.4%) than vegetarians (46.6%). This study showed a significant statistical association was observed between alcohol consumption and anemia ($p < 0.0262$*). Moreover, anaemia was more prevalent among alcoholics (47.7%) compared to non-alcoholics (24.1%). Also, a significant association was found between smoking and anemia ($p < 0.0014$*), with smokers having a lower proportion of anemia (26.1%) than non-smokers (74.9%). This suggests that alcohol consumption and smoking may contribute to anemia risk.

In this study, cough (95.4%), weight loss (83%), breathlessness (46.5%), anorexia (71.6%), fever (42%) and hemoptysis (10.2%) were identified as common in both anemic and non-anemic PTB patients. However, pallor was highly significant ($p < 0.0001$*), with 53.4% of anemic PTB patients when compared to only 3.4% of non-anemic individuals. This strong association confirms pallor is a key clinical indicator of anemia. However, BMI, dietary intake, tobacco usage, clinical suspicion, and risk factors among PTB patients didn't demonstrate any significant statistical correlation with anemia ($p > 0.05$).

Microbiological, Molecular and Radiological findings among PTB patients with and without anemia

Microbiological analysis showed that 53.4% of PTB patients with anemia were AFB positive compared to 37.9% without anemia ($p < 0.5020$), indicating no significant difference. CBNAAT positivity was slightly higher in anemia patients (88.6%) than in non-anemic (82.7%). Radiological findings revealed a significantly higher prevalence of cavitary lesions in anemic patients (69.3%) ($p < 0.0183$*). Moreover, other findings including upper and lower lung zone

Table 3. Hematological investigations of PTB cases with and without anemia (n - 117)

Investigations	Overall	Anemia		p value
		Present (n - 88)	Absent (n - 29)	
Hb (g/dl)	117 (100%)	8.94 ± 1.23	12.7 ± 1.3	<0.0001*
RBC Count (million/cumm)	117 (100%)	3.23 ± 0.37	4.57 ± 0.84	<0.0001*
PCV (%)	117 (100%)	25.20 ± 3.23	32 ± 4.98	<0.0001*
MCH (pg)	117 (100%)	26.89 ± 2.98	25.37 ± 2.71	0.0165
MCHC (%)	117 (100%)	32.48 ± 6.47	32.37 ± 0.79	0.9276
Platelet count (thousand/cumm)	117 (100%)	301.64 ± 124.7	294.57 ± 111.47	0.7742
Leukocytes, cells/mm^3	117 (100%)	8184 (6365-10487)	7172 (5899-8734)	<0.00001*
Neutrophil, cells/mm^3	117 (100%)	5601 (3874-7319)	4677 (3362-5969)	<0.00001*
Lymphocytes, cells/mm^3	117 (100%)	1463 (1048-1894)	1622 (1386-1998)	<0.00001*
Eosinophils, cells/mm^3	117 (100%)	111 (6-239)	161 (63-222)	0.00002*
Monocytes, cells/mm^3	117 (100%)	543 (327-765)	581 (397-741)	0.0072*
MCV				
Low	35 (29.9%)	28 (31.8%)	7 (3.4%)	
Normal	66 (56.4%)	54 (61.4%)	12 (41.4%)	0.0008*
High	16 (13.7%)	6 (6.8%)	10 (34.5%)	
RDW (%)				
Low	21 (17.9%)	2 (2.3%)	19 (65.5%)	
Normal	40 (34.2%)	31 (35.2%)	9 (31.1%)	0.0001*
High	56 (47.9%)	55 (62.5%)	1 (3.4%)	
TIBC				
Low	99 (84.6%)	74 (84.1%)	25 (86.2%)	
Normal	16 (13.7%)	13 (14.8%)	3 (10.3%)	0.6046
High	2 (1.7%)	1 (1.1%)	1 (3.5%)	
Serum Iron				
Low	94 (80.3%)	70 (79.5%)	24 (82.7%)	0.7052
Normal	23 (19.7%)	18 (20.5%)	5 (27.3%)	
Folic acid				
Low	56 (47.9%)	42 (47.7%)	14 (48.3%)	0.9091
Normal	51 (43.5%)	39 (44.3%)	12 (41.3%)	
High	10 (8.6%)	7 (8%)	3 (10.4%)	
Serum Creatinine (mg/dl)	117 (100%)	0.8 ± 0.57	0.97 ± 0.63	0.1775
Serum Ferritin levels				
Low	26 (22.2%)	14 (16%)	12 (41.4%)	
Normal	45 (38.5%)	29 (32.9%)	16 (55.2%)	0.0001*
High	46 (39.3%)	45 (51.1%)	1 (3.4%)	
Transferrin levels				
Low	70 (59.8%)	53 (60.2%)	17 (58.6%)	0.5097
Normal	40 (34.3%)	31(35.3%)	9 (31%)	
High	7 (5.9%)	4 (4.5%)	3 (10.3%)	
ESR				
Low	8 (6.8%)	3 (3.4%)	5 (17.2%)	
Normal	13 (11.1%)	6 (6.8%)	7 (24.1%)	0.0007*
High	96 (82.1%)	79 (84.1%)	17 (58.6%)	

The result is significant at $p < 0.05$.

Note: Hemoglobulin (Hb), Red Blood Cell (RBC), Packed Cell Volume (PCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Total Iron Binding Capacity (TIBC) and Erythrocyte Sedimentation Rate (ESR)

Table 4. Types of anemia in pulmonary tuberculosis patients (n - 88)

Type of anemia	No. of Cases
Mild (Hb 10.1-11.9 g/dl)	54 (61.3%)
Moderate (Hb 8.1-10 g/dl)	21 (23.8%)
Severe (Hb <8 g/dl)	13 (14.9%)
Microcytic hypochromic	25 (28.4%)
Microcytic normochromic	4 (4.5%)
Normocytic hypochromic	7 (8%)
Normocytic normochromic	51 (58%)
Macrocytic	1 (2.3%)

lesions, consolidation and tree-in-bud signs were more frequent in PTB patients with anemia, while pleural effusion was observed more in non-anemic patients (6.8%) (Table 2).

Hematological investigations of Pulmonary Tuberculosis Patients with and without anemia

Table 3 demonstrated a comparison of haematological parameters between PTB patients with and without anemia. The median Hb level recorded was 12.1 g/dL (interquartile range 10.3-13.4), ranging from a minimum of 5.8 g/dL to a maximum of 16.5 g/dL. The examination of haematological findings revealed that PTB patients with anemia exhibited lower mean values for Hb levels (8.94 ± 1.23 g/dl), RBC count (3.23 ± 0.37 million/cumm), PCV (25.20 ± 3.23%), platelet count (294.57 ± 111.47 thousand/cumm), and serum creatinine levels (0.8 ± 0.57). However, the mean MCH (26.89 ± 2.98 pg) and MCHC (32.48 ± 6.47%) were higher in PTB cases with anemia. Hence, the findings regarding RBC count, Hb levels, and PCV demonstrated a statistically significant correlation between anemia among PTB cases ($p = 0.0001$*). However, in terms of MCHC, MCH, platelet count, and serum creatinine levels, no statistically significant correlation was found with anemia among PTB cases ($p > 0.05$). The mean platelet count (301.64 ± 124.7) was greater in anemic patients with PTB instances in this research work. However, no statistically significant association with and without anemia was found among PTB cases ($p = 0.7742$).

This research highlighted that anemia PTB patients have a significantly higher total leukocyte count (8184 cells/mm^3) than non-anemic PTB patients (7172 cells/mm^3) ($p < 0.00001$*). Similarly, neutrophil levels were elevated significantly among anemia PTB patients (5601 cells/mm^3) compared to their non-anemic PTB patients (4677 cells/mm^3) ($p < 0.00001$*). In contrast, lymphocytes were significantly lower in anemic TB patients (1463 cells/mm^3) than in non-anemic PTB patients (1622 cells/mm^3) ($p < 0.00001$*). Eosinophil levels also showed a significant reduction in anemic TB patients (111 cells/mm^3) than in non-anemic PTB patients (161 cells/mm^3) ($p < 0.00002$*). Additionally, monocyte levels were also found to be significantly lower in anemic TB patients (543 cells/mm^3) than in non-anemic PTB patients (581 cells/mm^3) ($p < 0.0072$*). Hence, these findings suggest distinct alterations in the immune cell profile among anemic PTB patients, characterized by elevated leukocytes and neutrophils alongside reduced lymphocytes, eosinophils, and monocytes.

In the present study, 6.8% of PTB patients with anemia exhibited low MCV levels. The association between MCV levels and anemia in PTB cases was statistically significant $p < 0.0008$*. Regarding RDW levels in PTB cases with anemia, 2.3% had low RDW levels, while 35.2% had normal RDW levels, and 62.5% exhibited high RDW levels. This association was also statistically significant ($p < 0.0001$*). Likewise, there was no significant statistical difference observed in TIBC levels, serum iron levels and folic acid levels between anemic and non-anemic PTB patients ($p > 0.05$).

The analysis of serum ferritin levels revealed that 16% had low levels, 32.9% had normal levels, and 51.1% exhibited high levels. This distribution showed a statistically significant association with anemia in PTB cases ($p < 0.0001$*). The serum transferrin levels among PTB cases with anemia showed that 60.2% had low serum ferritin levels, 35.3% had normal levels and 4.5% cases had elevated levels. Also, the study found that 84.1% had high ESR levels, 6.8% cases had normal ESR levels and 3.4% cases had low ESR levels. A statistically significant association was observed between ESR levels and anemia in PTB cases ($p < 0.0007$*). However, TIBC levels and transferrin levels did not show any statistically significant association with and without anemia in PTB patients ($p > 0.05$).

Types of anemia in Pulmonary Tuberculosis patients

In this investigation, the analysis of anemia types among PTB patients reported that the majority had mild anemia (61.3%), followed by moderate anemia (23.8%), while 14.9% had severe anemia. Among morphological classifications, normocytic normochromic anemia was the most common (58%) followed by microcytic hypochromic anemia (28.4%). Normocytic hypochromic anemia accounted for 8%, while microcytic normochromic and macrocytic anemia was rare at 4.5% and 2.3%, respectively. These findings exhibit that most PTB patients had mild anemia with normocytic normochromic characteristics, indicating a multifactorial aetiology, possibly linked to nutritional deficiencies or chronic diseases (Table 4).

DISCUSSION

Anemia is commonly seen in chronic diseases such as TB. An elevated proportion of anemia among TB patients is attributed to inflammation and is referred to as anemia of inflammation (AI).[21] A significant number of individuals with active PTB display reduced Hb levels, directly affecting the morbidity associated with TB.[11] The impact of anemia varies among individuals with TB, serving as a risk factor for TB development and being linked to complications such as lung injury and unfavourable outcomes like delayed sputum conversion two months into TB treatment, as well as heightened mortality risk.[9,12,18]

The prevalence of anemia among newly diagnosed PTB cases was 75.2% in this study. This result was consistent with research conducted in Ghana by Asase *et al*. (68.8%) and Mukherjee *et al*. (71.8%) at Uttarakhand in a tertiary care centre, which had also indicated a comparable incidence of anemia among PTB cases.[10,22] However, a multi-centric prospective cohort study conducted by Araújo-Pereira *et al*. reported a lower prevalence of anemia among PTB patients.[23] Moreover, the study findings of Chhabra *et al*. in Punjab (88.5%) and Oliveira *et al*. from one of two TB referral hospitals in the city of Rio de Janeiro, Brazil (89.2%), demonstrated higher incidence of anemia than the present study findings among patients diagnosed to have PTB infection.[24,25] This association could be due to the impact of TB on the body's nutritional status and immune response. Chronic infections like TB can lead to inflammation and reduced nutrient absorption, which are contributing factors to anemia.[10]

MTB invasion activates T lymphocytes and monocytes, initiating an immune response that produces cytokines. Moreover, MTB strategically modulated inflammation, cytokine release, and iron homeostasis to support its survival. Also, hepcidin production further limits iron bioavailability by blocking dietary iron absorption and preventing iron release from macrophages, hepatocytes, and enterocytes, contributing to anemia of chronic disease and restricting erythropoiesis. Additionally, inflammatory cytokines shorten RBC lifespan and reduce plasma iron incorporation, further exacerbating anemia. While iron retention within macrophages serves as a host defence mechanism against MTB by depriving the bacteria of an essential nutrient, it also results in iron-restricted erythropoiesis, worsening anemia in TB-infected individuals. Thus, the interplay between MTB, immune and iron metabolism plays a crucial role in disease progression.[17,26,27]

In these current investigations, anemia was more predominantly reported among PTB cases in the age group between 31-50 years (38.6%) and these results were comparable to the study results of Mukherjee *et al*. showed that 33.7% had anemia of same age groups.[22] Also, the present study reported a higher prevalence of anemia among male PTB patients (76%) compared to female PTB patients (24%) which was identified to be in agreement with the reports of Mukherjee *et al*. 74.5%, Oliveira *et al*. 78.4%, and Mukherjee *et al*. 73.6%.[22,25,28] This is mostly attributed to the social behaviour of males as they interact more with friends and neighbours in addition to indulging in habits such as smoking and alcoholism hence increasing the chance of exposure to the airborne *Mycobacterium tuberculosis* bacilli.[29] In contrast, findings of Asase *et al*. demonstrated higher prevalence of anemia among female participants (56.9%) compared to males (43.1%).[10] The reason behind this gender disparity is likely due to physiological differences. Women are more susceptible to anemia due to menstrual blood loss

and higher iron requirements during pregnancy. Hormonal differences and dietary factors can also contribute to this disparity.[10]

Importantly, anemia is a major public health problem regardless of TB, and preferentially affects populations from low-middle income countries, demonstrating that such condition may be linked to a variety of multifactorial determinants, from nutritional deficiencies to co-infections.[12,24] The study highlighted malnutrition as a significant risk factor for anemia in PTB cases, with a high prevalence underweight PTB patients with anemia (46.6%) were malnourished (BMI < 18.5 kg/m^2). Similarly, Mukherjee *et al*. (59.1%) and Oliveira *et al*. (55.4%) demonstrated lower BMI in PTB anemic cases.[22,25] Also, the reports of a multi-centric study by Araujo-Pereira *et al*. highlighted that increased anemia severity was associated with a lower BMI which was concurrent with the present research findings.[23]

The results of this study revealed that all of these anaemic patients had at least one of the following characteristics: they were strictly vegetarians and belonged to the lower socioeconomic group. The impoverished people who live in and around the research region (Kattankulathur, Chengalpattu District, Tamil Nadu, India) often eat rice-based, high-carbohydrate foods and seldom eat foods high in iron. Due to their ignorance of the detrimental effects of anaemia, extra precautions had not been taken to raise their haemoglobin level.[30] The interconnection between TB and malnutrition is widely recognized, forming a complex bidirectional relationship that exacerbates both conditions.[31]

In this study, platelet count was found to be higher in anemia patients with PTB cases. This theory may be supported by the increased platelet counts seen in anemic individuals, as decreased iron availability in the bone marrow can lead to the generation of megakaryocytes with greater ploidy. However, in comparison to megakaryocytes with lower ploidy, those with greater ploidy may release more platelets.[13,32] It was found that 1 (1.1%) PTB cases with anemia had increased TIBC levels, 14 (16%) had lower serum ferritin levels, 53 (60.2%) had lower transferrin levels which suggested iron-deficiency anemia. These results were found to be in accordance with the study findings of Chhabra *et al*. here 2.29% of PTB patients with anemia were found with increased TIBC levels.[24] In this study, a majority of the anemic PTB cases exhibited elevated RDW levels among PTB cases (47.9%). The RDW test assesses the variability in RBC size or volume as part of a Complete Blood Count and it is employed in combination with other indicators of RBC, including mean corpuscular volume (MCV), to help determine the fundamental causes of anemia.[24,33]

The present study reveals significant haematological alterations in anemic PTB cases compared to their non-anemic PTB patients, suggesting a potential impact of anemia on immune response in PTB. The reports indicated a marked increase in total leukocyte count and neutrophil levels among anemic PTB patients suggesting an intensified inflammatory response in relation to MTB infection. Moreover, neutrophilia is commonly associated with TB as a response to MTB infection, which triggers an innate immune reaction. This aligns with the study findings of Hilda *et al*. suggesting that neutrophils play a crucial role in TB pathogenesis by producing reactive oxygen species and antimicrobial peptides, which may contribute to bacterial clearance but also tissue damage.[34]

Conversely, the significant reduction in lymphocyte levels among anemic PTB patients could reflect an impaired adaptive immune response. Since lymphocytes, particularly T cells, are critical for an effective immune response against TB, their reduced levels in anemic patients might contribute to a weakened immune defence mechanism, potentially leading to poorer disease outcomes or prolonged infection. Similarly, the observed decline in eosinophils among anemic PTB patients further supports the notion of immune dysregulation, as eosinophils play an important role in modulating immune responses and inflammation due to chronic infections like TB. Moreover, monocytes, which differentiate into macrophages essential for TB containment, were significantly lower in anemic PTB patients. This reduction in monocytes could impair macrophage function, leading to reduced phagocytosis and antigen presentation, thereby diminishing the host's ability to control the infection effectively. Therefore, these findings were

found to be in accordance with the study reports of de Mendonca *et al*.[3]

The study also examined iron levels and found that anemic PTB cases had low iron levels (79.5%) which indicate that though iron deficiency is common in PTB, it may not be the sole factor contributing to anemia in these patients. Similarly, folic acid levels showed no statistically significant association with anemic status in PTB cases, suggesting that folate deficiency is not a primary determinant of anemia in this study population and this was observed to be in concordance with the study findings of Mukherjee *et al*. and Chhabra *et al*.[2,24]

The results of this study addressing the inflammatory state demonstrated that ESR levels were higher in those with anemia (84.1%) compared to those without anemia (58.6%), since ESR tends to increase in response to anemia. These results were found to be in concordance with the study reports of Oliveira *et al*. in which ESR was increased in 87.2% of anemia PTB case when compared to non-anemic PTB cases (61.1%).[25] TB is both a chronic and destructive ailment. Consequently, levels of fibrinogen and gamma globulin rise, leading to increased rouleaux formation and subsequently elevated ESR levels. This phenomenon may aid in assessing the severity of the disease. Assessing ESR following treatment with antitubercular medications assists in evaluating prognosis and gauging the efficacy of the prescribed drugs.

The present study's findings on types of anemia prevalence were compared with the results reported by Araujo-Pereira *et al*. revealing a notable difference. Our study observed a lower proportion of mild anemia (42%) than the study reports of a multi-centric prospective cohort study conducted by Araujo-Pereira *et al*. which showed 61.3% of mild anemia.[23] These differences may reflect variations in population demographics, disease burden, nutritional status or healthcare access. The most prevalent kind of anemia reported in this study, accounting for 58% of cases, was found to be normocytic normochromic anemia and these results were in concordance with the study findings of Mukherjee *et al*. also reported similar incidence of normocytic normochromic anemia among PTB cases (56.9%).[22]

The limitations of the current research involve: the study was conducted at a single tertiary care centre with a limited patient population. Due to its retrospective study design, certain data, including serum hepcidin was unavailable to assess the aetiology of the anemia.

CONCLUSION

In the current investigation, anemia was prevalent in 75.2% of newly diagnosed PTB patients, and it has been concluded that iron metabolism was altered in PTB cases. Among them, the predominant type of anemia found was mild anemia and normochromic normocytic anemia. In addition, it was also identified that there was an increased frequency of malnutrition observed among anemia PTB cases. As a result, the high frequency of anemia in newly diagnosed PTB cases emphasizes the importance of the need to periodically screen all TB patients for anemia. This will not only improve the overall wellness of these patients but also improve mortality and morbidity in these patients. In light of this, anemia was shown to be much more prevalent in individuals who had the most severe clinical symptoms of TB, suggesting that it may be used as a biomarker to gauge the severity of the disease.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

The study was approved by the Institutional Ethical Committee, SRM Medical College Hospital and Research Centre, Tamil Nadu, India (8714/IEC/2023).

REFERENCES

1. Chauhan A, Parmar M, Dash GC, et al. The prevalence of tuberculosis infection in India: A systematic review and meta-analysis. *Indian J Med Res.* 2023;157(2&3):135-151.doi: 10.4103/ijmr.ijmr_382_23
2. World Health Organisation (WHO). Tuberculosis (TB). https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023. Accessed on April 28, 2024.
3. World Health Organization (WHO). Tuberculosis (TB). https://www.who.int/news-room/fact-sheets/detail/tuberculosis. Accessed April 28, 2024.
4. World Health Organisation (WHO). Tuberculosis (TB). https://www.who.int/health-topics/tuberculosis#tab=tab_1. Accessed on April 28, 2024.
5. Pande T, Huddart S, Xavier W, et al. Prevalence of diabetes mellitus amongst hospitalized tuberculosis patients at an Indian tertiary care center: A descriptive analysis. *PLoS One.* 2018;13(7):e0200838. doi: 10.1371/journal.pone.0200838
6. Padhi A, Agarwal A, Bhise M, Chaudhary A, Joshi K, Katoch CDS. Progress and challenges in achieving tuberculosis elimination in India by 2025: A systematic review and meta-analysis. *PLoS One.* 2024;19(3):e0301060. doi: 10.1371/journal.pone.0301060
7. Sappani M, Mani T, Asirvatham ES, Joy M, Babu M, Jeyaseelan L. Trends in prevalence and determinants of severe and moderate anaemia among women of reproductive age during the last 15 years in India. *PLoS One.* 2023;18(6):e0286464. doi: 10.1371/journal.pone.0286464
8. Anaemia. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/anaemia. Accessed April 29, 2024.
9. Luo M, Liu M, Wu X, et al. Impact of anemia on prognosis in tuberculosis patients. *Ann Transl Med.* 2022;10(6):329. doi: 10.21037/atm-22-679
10. Asase CS, Ebobabaara TB, Amoah BD. Prevalence of anaemia among symptomatic pulmonary tuberculosis patients at Weija Gbawe Municipal Hospital in Accra, Ghana. *World Journal of Advanced Research and Reviews*. 2023;20(1):796-807. doi: 10.30574/wjarr.2023.20.1.2071
11. Gil-Santana L, Cruz LAB, Arriaga MB, et al. Tuberculosis-associated anemia is linked to a distinct inflammatory profile that persists after initiation of antitubercular therapy. *Sci Rep.* 2019;9(1):1381. doi: 10.1038/s41598-018-37860-5
12. Barzegari S, Afshari M, Movahednia M, Moosazadeh M. Prevalence of anemia among patients with tuberculosis: A systematic review and meta-analysis. *Indian J Tuberc.* 2019;66(2):299-307. doi: 10.1016/j.ijtb.2019.04.002
13. de Mendonça EB, Schmaltz CA, Sant'Anna FM, et al. Anemia in tuberculosis cases: A biomarker of severity? [published correction appears in PLoS One. 2021 Mar 29;16(3):e0249545. doi: 10.1371/journal.pone.0249545.]. *PLoS One.* 2021;16(2):e0245458. doi: 10.1371/journal.pone.0245458
14. Ekiz C, Agaoglu L, Karakas Z, Gurel N, Yalcin I. The effect of iron deficiency anemia on the function of the immune system. *Hematol J.* 2005;5(7):579-583. doi: 10.1038/sj.thj.6200574
15. Gelaw Y, Getaneh Z, Melku M. Anemia as a risk factor for tuberculosis: a systematic review and meta-analysis. *Environ Health Prev Med.* 2021;26(1):13. doi: 10.1186/s12199-020-00931-z
16. Baluku JB, Mayinja E, Mugabe P, Ntabadde K, Olum R, Bongomin F. Prevalence of anaemia and associated factors among people with pulmonary tuberculosis in Uganda. *Epidemiol Infect.* 2022;150:e29. doi: 10.1017/S0950268822000103
17. Dasaradhan T, Koneti J, Kalluru R, Gadde S, Cherukuri SP, Chikatimalla R. Tuberculosis-Associated Anemia: A Narrative Review. *Cureus.* 2022;14(8):e27746. doi: 10.7759/cureus.27746
18. Abaynew Y, Ali A, Taye G, Shenkut M. Prevalence and types of anemia among people with tuberculosis in Africa: a systematic review and meta-analysis. *Sci Rep.* 2023;13:5385. doi: 10.1038/s41598-023-32609-1
19. World Health Organisation (WHO). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. https://iris.who.int/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf?sequence=22. Accessed on April 28, 2024.
20. Palmer L, Briggs C, McFadden S, et al. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. *Int J Lab Hematol.* 2015;37(3):287-303. doi: 10.1111/ijlh.12327
21. Kumar RNH, Rao CR, Maradi R, Umakanth S, Sanju SVC, Rao BPS. Impact of anemia on sputum smear conversion and treatment outcomes among newly diagnosed tuberculosis patients in Southern India. *Int J Mycobacteriol.* 2023;12(2):117-121. doi: 10.4103/ijmy.ijmy_24_23
22. Mukherjee A, Kaeley N, Dhar M, Kumar S, Bhushan B. Prevalence, characteristics, and predictors of tuberculosis associated anemia. *J Family Med Prim Care.* 2019;8(7):2445-2449. doi: 10.4103/jfmpc.jfmpc_311_19
23. Araujo-Pereira M, Nogueira BMF, Spener-Gomes R, et al. Anemia and anti-tuberculosis treatment outcome in persons with pulmonary tuberculosis: A multi-center prospective cohort study. *J Infect Public Health.* 2023;16(6):974-980. doi: 10.1016/j.jiph.2023.04.009
24. Chhabra S, Kashyap A, Bhagat M, Mahajan R, Sethi S. Anemia and Nutritional Status in Tuberculosis Patients. *Int J Appl Basic Med Res.* 2021;11(4):226-230. doi: 10.4103/ijabmr.ijabmr_76_21
25. Oliveira MG, Delogo KN, de Oliveira HMdMG, Ruffino-Netto A, Kritski AL, Oliveira MM. Anemia in hospitalized patients with pulmonary tuberculosis. *J Bras Pneumol.* 2014;40(4):403-410. doi: 10.1590/

S1806-37132014000400008
26. Ganz T. Molecular pathogenesis of anemia of chronic disease. *Pediatr Blood Cancer.* 2006;46(5):554-557. doi: 10.1002/pbc.20656
27. Dorhoi A, Kaufmann SHE. Pathology and immune reactivity: understanding multidimensionality in pulmonary tuberculosis. *Semin Immunopathol.* 2016;38(2):153-166. doi: 10.1007/s00281-015-0531-3
28. Mukherjee A, Kaushik R M, Sindhwani G, Kaushik R. Prevalence and characteristics of anemia in new cases of pulmonary tuberculosis in a tertiary care hospital in Uttarakhand, India. *SRHU Medical Journal.* 2017;1(1): 10-15
29. Akpan PA, Ogar RO, Nyong EA. Red cell parameters,iron, vitamin B12 and folate levels of pulmonary tuberculosis patients attending clinic at General Hospital, Akamkpa, Cross River state, Nigeria. *Afr J Lab Haem Transf Sci.* 2023;2(4):247-257. doi: 10.59708/ajlhts.v2i4.2331
30. Kumar PR, Sudha M, Udhaya V, Devi GD. Prevalence of Anemia among the Patients with Pulmonary Tuberculosis and Diabetes - In and Around Ongole -India. *Elixir Physio. & Anatomy.* 2021;161:55889-55892.
31. Musuenge BB, Poda GG, Chen PC. Nutritional Status of Patients with Tuberculosis and Associated Factors in the Health Centre Region of Burkina Faso. *Nutrients.* 2020;12(9):2540. doi: 10.3390/nu12092540
32. Kim S, Cho SY. Investigation of Iron Metabolism for Regulating Megakaryopoiesis and Platelet Count According to the Mechanisms of Anemia. *Clinical Laboratory.* 2018;64(3):329-332. doi: 10.7754/Clin. Lab.2017.170824
33. Baynes RD, Flax H, Bothwell TH, Bezwoda WR, Atkinson P, Mendelow B. Red blood cell distribution width in the anemia secondary to tuberculosis. *Am J Clin Pathol.* 1986;85(2):226-229. doi: 10.1093/ajcp/85.2.226
34. Hilda JN, Das S, Tripathy SP, Hanna LE. Role of neutrophils in tuberculosis: A bird's eye view. *Innate Immun.* 2020;26(4):240-247. doi: 10.1177/1753425919881176