Original Article

Anaemia of chronic disease among pulmonary tuberculosis patients is associated with inflammatory marker at the start of intensive phase

Jovita Leon[1], Sonali Sarkar[1], Debdatta Basu[2], Nivedita Nanda[3], Noyal Mariya Joseph[4]

[1]Department of Preventive and Social Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, [2]Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, [3]Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, [4]Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India

Abstract

Background: Tuberculosis (TB) disrupts iron balance through systemic inflammation. Pulmonary tuberculosis (PTB) is linked to diverse anaemia types, necessitating intricate haematological and biochemical assessments for diagnosis. This study aims to describe the prevalence of anaemia of chronic disease (ACD), iron deficiency anaemia (IDA) among PTB patients and factors associated with these types of anaemia. **Methods:** A cross-sectional analysis was conducted from community-based cohort study involving sputum-positive PTB patients from 2018 to 2020 in urban Puducherry. Participants were enrolled from 10 primary health centres within 2 weeks of initiating anti-tubercular treatment (ATT). Blood samples were collected for assessing haematological and biochemical parameters. The sTfR/log ferritin ratio was used to distinguish between ACD and IDA. Data were captured using Epicollect5 and analysed using STATA V14. **Result:** Of the 176 PTB patients included, 63.07% (111/176) had anaemia, with ACD being the predominant type (84.6%, 94/111). The C-reactive protein (CRP) levels were higher among the anaemic group [40.77 (16.66-58.51) mg/dl vs 24.65 (14.23-47.26) mg/dl] and higher among the ACD as compared to IDA [46.9 (22.3-61.2) vs 20.8 (13.0-39.1) mg/dl]. Undernourished [adjusted prevalence ratio (APR) =3.43; confidence interval (CI): 1.21-9.69] and patients having low risk of dependence on tobacco [APR = 1.52; CI: 1.10-2.11] had higher risk of ACD. Female patients had higher risk of IDA [APR = 4.95, $P < 0.01$]. **Conclusion:** The largest proportion of the PTB participants with anaemia had ACD. Acute-phase reactant and inflammatory marker are increased among newly diagnosed new sputum smear-positive (NSP) PTB participants at the start of ATT. Addressing inflammation is needed for combating anaemia in PTB patients.

Keywords: Acute-phase reactant, anaemia, anaemia of chronic disease, iron deficiency anaemia, tuberculosis, new sputum smear-positive, pulmonary tuberculosis

Address for correspondence: Dr. Sonali Sarkar, Professor, Department of Preventive and Social Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India.
E-mail: sarkarsonaligh@gmail.com

Received: 21-08-2023 **Revised:** 19-10-2023
Accepted: 17-11-2023 **Published:** 22-04-2024

Access this article online

Quick Response Code:

Website: http://journals.lww.com/JFMPC

DOI: 10.4103/jfmpc.jfmpc_1374_23

Introduction

Tuberculosis (TB) remains a significant global health threat, causing substantial morbidity and mortality.[1,2] Despite the World Health Organization (WHO)'s efforts, TB affects millions annually, with 10.6 million cases and 1.6 million deaths in 2021.[3,4]

How to cite this article: Leon J, Sarkar S, Basu D, Nanda N, Joseph NM. Anaemia of chronic disease among pulmonary tuberculosis patients is associated with inflammatory marker at the start of intensive phase. J Family Med Prim Care 2024;13:1316-27.

India reports a fifth of cases.[5] TB and anaemia are intertwined, increasing risks and treatment challenges.[6] Anaemia worsens TB outcomes, including sputum conversion, mortality and post-TB issues such as lung damage.[7-9]

Anaemia affects 32.8% of the world's population, with the prevalence being higher in women (33.9%) than in males (31.8%), and it is mostly brought on by inadequate dietary iron consumption and its poor bioavailability.[10-12] Anaemia can also result from maternal haemorrhage, sickle cell disorder, thalassaemia and chronic kidney disease, but chronic parasitic infection and inflammation are the main causes of iron deficiency anaemia (IDA) and anaemia of chronic disease (ACD), respectively.[10,13]

Anaemia is known to be more common (60–80%) in patients with pulmonary TB (PTB) than in the general population.[14] Anaemia in patients with TB has been linked to various factors including malnutrition, bone marrow suppression, haemoptysis, failure of iron utilization, iron deficiency (ID) and chronic inflammation.[15-19] Among these, chronic inflammation and ID are the primary drivers contributing to the presence of anaemia among TB patients.[9,20] Patients with chronic immunological activation, such as PTB and HIV, are more likely to have ACD compared to IDA.[20] Infection frequently induces a significant acute-phase response, resulting in iron sequestration, limited erythropoiesis and cytokine-mediated inflammation-related anaemia. Assessing the extent of iron deficiency contributing to anaemia in PTB patients proves challenging.[21]

The primary objective of this study was to find out the prevalence of types of anaemia and its association with iron parameters such as C-reactive protein (CRP) at the start of treatment which helps us to understand the interplay between inflammation and iron metabolism in TB-associated anaemia.

Materials and Methods

Study design, population and setting

The study participants were residents of Puducherry, enrolled between 2017 and 2019 as part of a community-based prospective cohort study. Adult PTB patients with new sputum smear positivity (NSP) from primary healthcare centres (PHC) within 10–15 km of the tertiary care medical college hospital were included, excluding individuals on anti-tubercular treatment (ATT) for over 3 weeks, pregnant/nursing women, HIV-positive, physically challenged, blood-transfused or with chronic diseases.

Puducherry is a union territory with a population of 1,248,000 in 2011.[22] There are 27 designated microscopy centres (DMCs) and seven TB units (TUs) in Puducherry. After being screened for comorbidities, individuals receiving a TB diagnosis at the DMCs are directed to their local PHC for the start of treatment.

The sample size was determined based on the prevalence of anaemia of inflammation (75.9%) at the start of ATT using OpenEpi version 3.01, with an α-error of 0.05, absolute precision of 7% and non-response rate of 20%, and the final sample size was calculated as 176.[23] We enrolled participants consecutively as they were registered in the National Tuberculosis Elimination Programme (NTEP) register in Puducherry.

Study procedure

During enrolment, 190 NSP participants were registered from selected PHCs under NTEP; 14 participants were excluded [Figure 1]. We enrolled 176 participants after obtaining written informed consent. Interviews gathered sociodemographic data (age, sex, residence, marital status, education, socioeconomic status and occupation), and risk behaviours (alcohol and smoking) were assessed using structured and validated questionnaires. The Alcohol Use Disorders Identification Test-Consumption (AUDIT-C) scale is a brief screening tool that evaluates alcohol consumption patterns. It consists of 10 questions, each assigned a score, and the total score indicates the level of alcohol consumption. The Fagerström Test for Nicotine Dependence scale assesses the degree of nicotine dependence in smokers. This scale includes six questions that contribute to a total score, which helps categorize the level of nicotine dependence, guiding interventions for smoking cessation. Nutritional status, body mass index (BMI) and mid-upper arm circumference (MUAC) were assessed using weight in kg/m2 and measuring tape, respectively.

For biochemical (iron) and haematological parameters (CBC), 5 ml venous blood was drawn using a BD Vacutainer: 3 ml for biochemical analysis and 2 ml in ethylenediaminetetraacetic acid (EDTA) tube for complete blood count (CBC). Serum was obtained by centrifuging the blood in the serum tube at 3000 rpm for 10 minutes and then stored at -20°C for analysis.

Haematological and biochemical tests, including CBC parameters (haemoglobin (Hb), red blood cell (RBC), haematocrit (Hct), mean corpuscular volume (MCV), mean haemoglobin (MCH), mean haemoglobin concentration (MCHC) and red cell

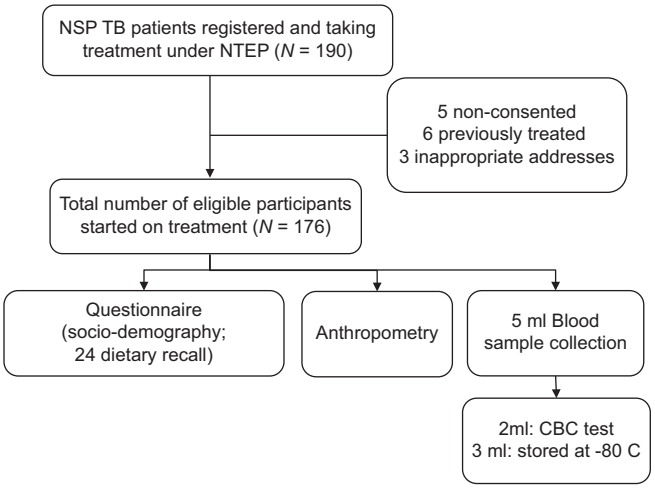

Figure 1: Study algorithm

distribution width (RDW)) on an XTi 4000i autoanalyzer (Sysmex, Kobe, Japan) and iron-related parameters (serum ferritin, transferrin and iron) on a Beckman Coulter AU680 autoanalyzer (California, USA), were conducted at a South Indian tertiary care hospital's Pathology and Biochemistry departments. Soluble transferrin receptor (sTfR) was measured using chemiluminescence (Beckman Coulter version AU5800), and CRP was determined with a Calbiotech ELISA kit. The sTfR index was calculated by applying the log (base-10) function to both ferritin and sTfR values. Total iron-binding capacity (TIBC) and transferrin saturation (TSAT) were calculated using the specified formulas:

TIBC = transferrin × conversion factor (1.4),[24]

TSAT = (serum iron/TIBC) ×100.[25]

Operational definitions

Alcohol use: Consumption of any form of alcohol (one standard drink) in the past year was assessed using AUDIT-C.[26]

Tobacco use: Use of smoke or smokeless form of tobacco in the past year was assessed using the Fagerstrom Test for Nicotine Dependence (FTND) tool.[27]

Anaemia: Hb levels less than 13 g/dL and 12 g/dL for males and females, respectively.[28]

MUAC: male <23 cm and female <22 cm considered low.[29]

Classification of types of anaemia based on iron profile [Figure 2]

IDA: *Hb low and serum ferritin <15; serum ferritin low/normal and sTfR index is >1.5*.[30]

ACD: *Hb low, serum ferritin increased and sTfR index is <1.5*.[31]

Iron deficiency without anaemia (IDWA): *Hb normal, ferritin low/normal and sTfR index is >1.5*.[30,31]

Ethical statement

Every participant in the study provided written informed permission, and the authors followed the Declaration of Helsinki, with approval from the university's ethics committee (JIP/IEC/2017/0151)

Statistical analysis

Data entry was conducted in Epicollect5, and analysis was performed using STATA version 14 (STATA Corp LP, College Station, TX, USA). Categorical variables were summarized using frequency (n) and percentage (%). Continuous variables were summarized using mean with SD or median with interquartile range (IQR) based on the normality of data. The differences in the level of haematological and biochemical parameters between ACD, IDA and no anaemia groups were analysed using the analysis of variance (ANOVA) with Tukey's multiple comparison test and Kruskal–Wallis test with Dunn's multiple comparisons. The prevalence of types of anaemia was expressed as proportion with 95% CI. Variables with a *P* value < 0.2 in bivariate analysis were entered into the multivariable log-binomial regression model to determine factors that are independently associated with the ACD and IDA and expressed as adjusted prevalence ratio (APR). The variables included for ACD were age, gender, ration card, AUDIT-C and FTND, diabetes mellitus (DM) and BMI; for ID were age, gender, religion, education, occupation, ration card, marital status, AUDIT-C score, FTND, DM, BMI and MUAC. The Spearman rank test was performed to assess the correlation between iron parameters and Hb status. The *P* value of 0.05 was considered statistically significant.

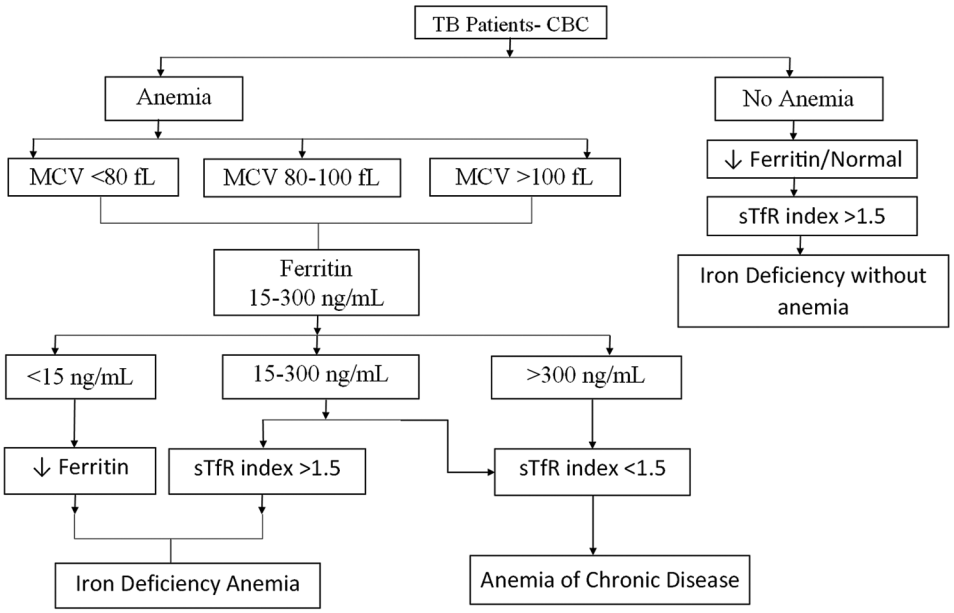

Figure 2: Algorithm used for differentiating the types of anaemia

Results

Baseline characteristics of the NSP PTB patients

Among the study participants, males accounted for 73.2% (129) and females 27.8%. The median age of the study participants was 43 years (IQR 34–52 years). About three-fourths (71.0%) of the participants were aged between 30 and 60 years. The majority of them were Hindus (88.1%), and 86% had at least basic formal education. More than half were employed (55.1%), and about two-thirds (64.2%) were from the below poverty line. Alcohol use, smoking, DM and undernourishment (BMI <18.5 kg/m^2) were reported in 44.9%, 38.6%, 46% and 44.9% of the participants, respectively [Table 1].

Prevalence and types of anaemia observed among the NSP PTB patients

The prevalence of anaemia was 63.1% (95% CI: 55.4%–75.2%). About 83% of those with anaemia were females. ACD was the most common type (94/111, 84.6%) followed by IDA (15/111, 13.5%) among the anaemics. Mixed anaemia (ACD + IDA) and megaloblastic anaemia were observed in one participant each. Among the non-anaemics, iron deficiency was found in three participants (2.63%) [Figure 3].

Association of the haematological parameters with ACD, IDA and no anaemia in study participants

The differences in mean (SD) of haematological parameters such as Hb, RBC, Hct, MCV, MCH, MCHC and RDW of the NSP PTB patients were statistically significant between the three groups, namely ACD, IDA and no anaemia based on one-way ANOVA test. On *post hoc* test with Bonferroni, differences in all the haematological parameters were found to be statistically significant between ACD vs IDA, except RBC and Hct [Figure 4]. When the haematological parameters were compared between non-anaemic vs ACD and non-anaemic vs IDA groups, differences in all the parameters such as Hb, RBC, HCT, MCH, MCHC and RDW were statistically significant except MCV [Table 2].

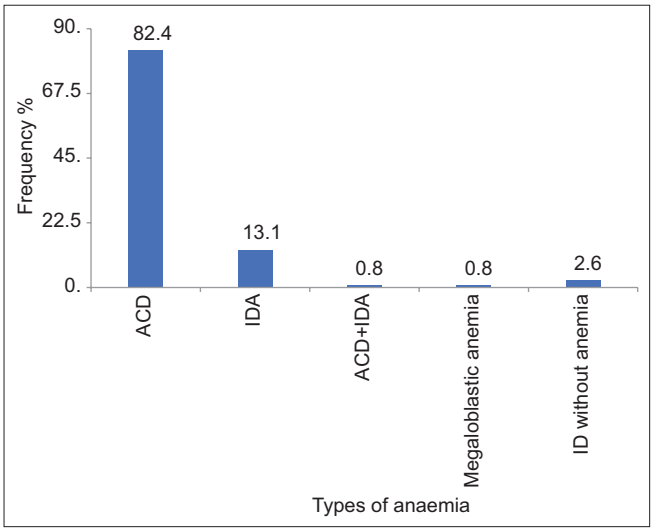

Figure 3: ACD: anaemia of chronic disease; IDA: iron deficiency anaemia; ACD + IDA: anaemia of chronic disease and iron deficiency anaemia; ID: iron deficiency

Association of the biochemical parameters with ACD, IDA and no anaemia in study participants

The differences in the median (IQR) of parameters such as TSAT, log of ferritin, sTfR, sTfR index and CRP between the three groups: ACD, IDA and no anaemia, were statistically significant based on the Kruskal–Wallis test. In *post hoc* group comparisons by the Dunn test, we found a significant difference in the levels of ferritin, TSAT, log ferritin, sTfR and ratio of sTfR between the ACD and IDA [Figure 5]. Ferritin levels were significantly lower in the IDA group compared to non-anaemic, whereas there was negligible difference between the ACD and non-anaemic, the level being slightly lower in the ACD [Table 3].

Correlation between Hb and biochemical parameters

Hb was positively correlated with ferritin ($r = 0.2$; $P < 0.001$) and TSAT ($r = 0.39$; $P < 0.001$) in the anaemic TB patients, the correlations being low and moderate, respectively. There was a weak negative correlation between CRP levels and Hb ($r = -0.15$; $P = 0.03$) in the anaemic TB patients [Figure 5].

Factors associated with ACD and IDA

Binary logistic regression was performed to identify the factors independently associated with ACD and IDA. Very low dependence on tobacco (FTND score 3–4) and undernutrition (BMI < 18.5 kg/m^2) was significantly associated with ACD. In the univariate analysis age, gender and type of ration card signifying the socioeconomic status of the TB patients were found to be associated with ACD, which was not associated after adjusting for other covariates [Tables 4-7].

Female patients were significantly associated with IDA (APR = 4.95; 95% CI 1.54–15.87). In the univariate analysis age, occupation, marital status and DM were found to be associated with IDA, which was overcome after adjusting for other covariates.

Discussion

Iron is required by pathogens and the host as well for their survival.[32-34] Pathogens use iron to survive inside their hosts, either by competing with them for intracellular iron or by creating molecules with high iron affinity.[35,36] Exploring the relationship between anaemia and iron parameters is difficult.

As per the WHO classification, 63.1% of PTB patients in our study had anaemia at the time of diagnosis of TB. The prevalence from other studies in India by Mukerjee *et al.* (71.8%) and Dileepan *et al.* (75.5%) and from Indonesia by Sahiratmadja *et al.* (63%) was comparable to our findings.[2,19,35]

In our study, ACD was the predominant type of anaemia as compared to IDA [95 (84.6%) vs 15 (13.5%)]. Others [Mario

Table 1: Distribution of anaemia among new sputum smear-positive pulmonary tuberculosis patients at the start of anti-tubercular treatment in Puducherry, South India

Variables	Total no. of participants *n*=176		
	Anaemia *n*=111 *n* (%)	No anaemia *n*=65 *n* (%)	Total
Gender			
Male	72 (55.8)	57 (44.1)	129
Female	39 (82.9)	08 (17.0)	47
Age in years			
18-30	26 (72.2)	10 (27.7)	36
30-60	72 (57.6)	53 (42.4)	125
>60	13 (86.6)	02 (13.3)	15
Religion			
Hindu	97 (62.5)	58 (37.4)	155
Christian	08 (57.1)	06 (42.8)	14
Muslim	06 (85.7)	01 (14.2)	7
Education			
No formal education	18 (72.0)	07 (28.0)	25
Primary (class 1–12)	56 (64.3)	31 (35.6)	87
Senior secondary (undergraduation and above)	37 (57.8)	27 (42.1)	64
Occupation			
Employed	50 (51.5)	47 (48.4)	97
Unemployed	61 (77.2)	18 (22.7)	79
Ration card			
Yellow card	32 (50.7)	31 (49.2)	63
Red card	79 (69.9)	34 (30.0)	113
Marital status*			
Married	78 (61.9)	48 (38.1)	126
Unmarried	26 (68.4)	12 (31.5)	38
Others	7 (58.3)	05 (41.6)	12
AUDIT-C scale†			
Lower risk	70 (65.4)	37 (34.5)	107
Higher risk	6 (7)	2 (2)	8
Increasing risk	27 (62.7)	16 (37.2)	43
Possible dependence	8 (44.4)	10 (55.5)	18
FTND‡ (applicable only for male participants)			
Very low	45 (49.4)	46 (50.5)	91
Low	13 (81.2)	03 (18.7)	16
High	07 (70.0)	03 (30.0)	10
Very high	01 (25.0)	03 (25.0)	04
Medium	06 (75.0)	02 (25.0)	08
DM§			
Yes	42 (51.8)	39 (48.1)	81
No	69 (72.6)	26 (27.3)	95
BMI‖			
Underweight (<18.5 kg/m^2)	67 (84.8)	12 (15.1)	79
Normal (18.5–22.9 kg/m^2)	34 (50.0)	34 (50.0)	68
Overweight (23–24.9 kg/m^2)	04 (30.7)	09 (69.2)	13
Obesity (≥25 kg/m^2)	06 (37.5)	10 (62.5)	16
MUAC¶			
Normal	49 (51.5)	46 (48.4)	95
Deficient	62 (76.5)	19 (23.4)	81

*Divorced; separated; widow; widower; †AUDIT-C scale : high risk (16-19), increasing risk (8-15), low risk (0-7), possible dependence (20+); ‡Fagerstrom Tolerance Test: high (6-7), low (3-4), medium (5), very high (8-10), very low (0-2); §DM: diabetes mellitus; BMI: body mass index; MUAC: mid-upper arm circumference; ¶MUAC male: <23 cm; MUAC female : <22 cm considered low

Oliveria (75.9%),[23] Minchella PA *et al.*, (36%),[37] Bashir A (34%),[38] Jerry Hella *et al.*, (59.8%)[11]] have also reported ACD as the most common type of anaemia among the PTB patients from various parts of the world, while the proportions range widely, probably because definitions applied to differentiate ACD and IDA varied.[37] However, Sheila *et al.* from their study in Tanzania reported IDA to be the most common type of anaemia (37%).[4]

ACD arises from chronic illnesses characterized by a persistent underlying inflammatory condition, leading to

Table 2: Distribution of haematological parameters observed among the ACD, IDA and normal study participants at the start of treatment

Haematological parameters	ACD Mean (SD)	IDA Mean (SD)	No anaemia Mean (SD)	*P*	ACD vs IDA	No anaemia vs ACD	No anaemia vs IDA
Hb g/dL	10.94 (1.31)	9.58 (1.42)	14.06 (1.28)	<0.001	0.001	<0.001	<0.001
RBC µL	4.18 (0.55)	4.20 (0.64)	5.04 (0.62)	<0.001	1.000	<0.001	<0.001
HCT %	34.10 (3.97)	31.22 (3.90)	41.99 (3.89)	<0.001	0.029	<0.001	<0.001
MCV fl	81.93 (7.11)	75.39 (10.84)	83.35 (7.42)	0.001	0.007	0.781	0.001
MCH pg	26.38 (2.78)	22.48 (3.21)	27.92 (2.60)	<0.001	<0.001	0.003	<0.001
MCHC gm%	32.13 (1.44)	30.54 (1.58)	33.54 (1.42)	<0.001	<0.001	<0.001	<0.001
RDW %	15.48 (2.59)	17.18 (2.48)	13.98 (1.34)	<0.001	0.027	0.001	<0.001

Hb: Haemoglobin; RBC: Red blood cell, HCT: Haematocrit; MCV: Mean corpuscular volume; MCH: Mean haemoglobin; MCHC: Mean haemoglobin concentration; RDW-CV: Red cell volume distribution width; fl: Femtolitre; pg: picogram; %: Percentage

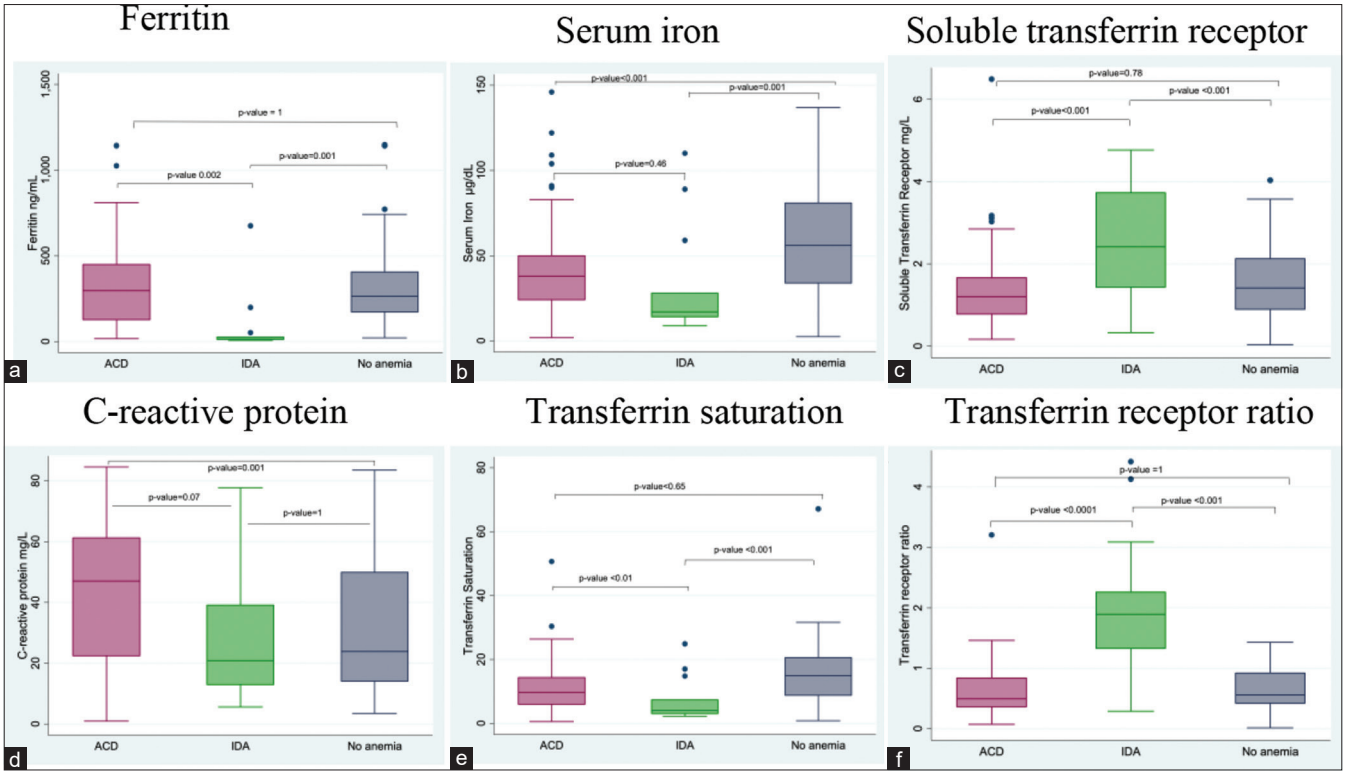

Figure 4: Box plot shows the distribution of biochemical parameters among the anaemic tuberculosis patients at the start of the treatment. A *P*-value <0.05 is considered statistically significant, (a)Ferritin, (b) Serum Iron, (c) Soluble Transferrin Receptor, (d) C-reactive protein, (e) Transferrin Saturation, (f) Transferrin receptor ratio

inflammation-related issues such as shortened erythrocyte lifespan, impaired erythrocyte iron absorption, and reduced sensitivity to or availability of erythropoietin.[11,39] IDA primarily stems from inadequate dietary intake and malabsorption due to food insecurity and reduced appetite among PTB patients. IDA and ACD-related IDA both result from disruptions in iron balance, with the latter often being linked to inflammation.[7,40]

The Hb, HCT, MCH and MCHC levels were low among the anaemic PTB participants. The mean (SD) Hb was 10.74 (1.39) g/dL in anaemic and 14.11 (1.30) g/dL in non-anaemic participants, which is similar to findings from other Indian studies. RDW at the start of ATT was higher among the anaemic patients. Haematological changes that occur are often associated with body's immune response of TB infection. A reduction in Hb levels in anaemic TB patients may be related to the severity of TB infection and inflammation, which may have an impact on erythropoiesis, but also due to iron deficiency.[15]

Distinguishing IDA from ACD relies on criteria involving lowered Hb, HCT, MCV and MCHC, and raised RDW for IDA. ACD shares similar criteria except for normal MCHC, with normal or increased RDW. Our study confirmed these criteria, revealing significant differentiation between groups through *post hoc* analysis. Notably, though RDW and HCT did not show statistical significance, lower levels were evident in ACD and IDA.

Irrespective of the anaemic status, serum iron, TSAT and sTfR index were low in all PTB patients, whereas TIBC and CRP levels

Table 3: Distribution of biochemical parameters observed among the ACD, IDA and normal study participants at the start of treatment

Biochemical parameters	ACD Median (IQR)	IDA Median (IQR)	No anaemia Median (IQR)	*P*
Ferritin ng/mL	297 (127-450)	14 (11-27)	265 (172-406)	<0.001
Transferrin mg/dL	267 (230-312)	315 (250-391)	284.5 (255-331)	0.020
Serum iron µg/dL	38 (24-50)	17 (14-28)	56 (34-81)	<0.001
TIBC µg/dL	373.8 (322-436.8)	441 (350-547.4)	398.3 (357-463.4)	0.020
TSAT %	9.7 (6.0-14.3)	4.1 (3.1-7.4)	14.9 (8.7-29.5)	<0.001
Log ferritin	2.4 (2.1-2.6)	1.1 (1.0-1.4)	2.4 (2.2-2.6)	<0.001
CRP mg/L	46.9 (22.3-61.2)	20.8 (13.0-39.1)	23.8 (14.1-49.8)	<0.001
sTfR mg/L	1.2 (0.7-1.6)	2.4 (1.4-3.7)	1.4 (0.8-2.1)	0.001
Ratio of sTfR	0.49 (0.35-0.83)	1.8 (1.3-2.2)	0.5 (0.4-0.9)	0.001

TIBC: Total iron-binding capacity; TSAT: Transferrin saturation; sTfR: Soluble transferrin receptor; ng: Nanogram; mL: Millilitre; µg: microgram; dL: Decilitre; mg: Milligram; L: Litre

Table 4: Sociodemographic factors associated with ACD among the newly diagnosed pulmonary tuberculosis patients at the start of the treatment *n*=176

Variables	Total	Risk factors for ACD		PR 95%CI	APR 95%CI	Adjusted *P*
		ACD (*n*=94)	No ACD (*n*=82)			
Age in years						
18-30	36	18 (50)	18 (50)	1		
31-60	125	66 (52.8)	59 (47.2)	1.05 (0.73-1.52)	1.19 (0.82-1.72)	0.82
>60	15	10 (66.67)	05 (33.33)	1.33 (0.82-2.16)	1.42 (0.86-2.33)	0.86
Gender						
Male	129	67 (51.94)	62 (48.06)	1		
Female	47	27 (57.45)	20 (42.55)	1.10 (0.82-1.48)	1.22 (0.87-1.71)	0.23
Ration card						
Red	113	67 (59.29)	46 (40.71)	1.38 (1.00-1.91)		
Yellow	63	27 (42.86)	36 (57.14)	1		

ACD: Anaemia of chronic disease; 1 reference

Table 5: Lifestyle risk factors and nutritional status associated with ACD among the newly diagnosed pulmonary tuberculosis patients at the start of the treatment *n*=176

Variables	Total	Risk factors for ACD		PR 95%CI	APR 95%CI	Adjusted *P*
		ACD (*n*=94)	No ACD (*n*=82)			
AUDIT-C scale[†]						
Lower risk	107	56 (52.34)	51 (47.66)	1		
Higher risk	08	05 (62.5)	03 (37.5)	1.19 (0.67-2.10)		
Increasing risk	43	26 (60.47)	17 (39.53)	1.15 (0.85-1.56)		
Possible dependence	18	07 (38.89)	11 (61.11)	0.74 (0.40-1.38)		
FTND[‡] (applicable only for male participants)						
Very low	91	40 (43.9)	51 (56.04)	1		
Low	16	13 (81.25)	03 (18.75)	1.84 (1.32-2.57)	1.52 (1.10-2.11)	0.01
High	10	07 (70.00)	03 (30.00)	1.59 (0.99-2.54)	1.13 (0.75-1.71)	0.54
Very high	04	01 (25.00)	03 (75.00)	0.56 (0.10-3.15)	0.51 (0.11-2.36)	0.39
Medium	08	06 (75.00)	02 (25.00)	1.70 (1.07-2.70)	1.43 (0.92-2.21)	0.10
DM[§]						
Yes	81	40 (49.38)	41 (50.62)	0.86 (0.65-1.15)		
No	95	54 (56.84)	41 (43.16)	1		
BMI[‖]						
Underweight (<18.5 kg/m^2)	79	58 (73.42)	21 (26.58)	3.91 (1.39-10.95)	3.43 (1.21-9.69)	0.02
Normal (18.5–22.9 kg/m^2)	68	29 (42.65)	39 (57.35)	2.27 (0.79-6.54)	2.05 (0.70-5.94)	0.18
Overweight (23–24.9 kg/m^2)	13	04 (30.77)	09 (69.23)	1.64 (0.44-6.05)	1.49 (0.41-5.34)	0.53
Obesity (≥25 kg/m^2)	16	03 (18.75)	13 (81.25)	1		

1 reference; ACD: anaemia of chronic disease; [‖]BMI: body mass index; [†]AUDIT-C scale: high risk (16-19), increasing risk (8-15), low risk (0-7), possible dependence (20+); FTND: [‡]Fagerstrom Test for Nicotine Dependence: high (6-7), low (3-4), medium (5), very high (8-10), very low (0-2)

were high. Similar observation was reported by Bashir A *et al.*, Mishra *et al.* and Chandan *et al.*[38,41,42] However, it was noted that Bashir A *et al.* reported low TIBC, which might indicate functional deficit of availability of iron.[38,43]

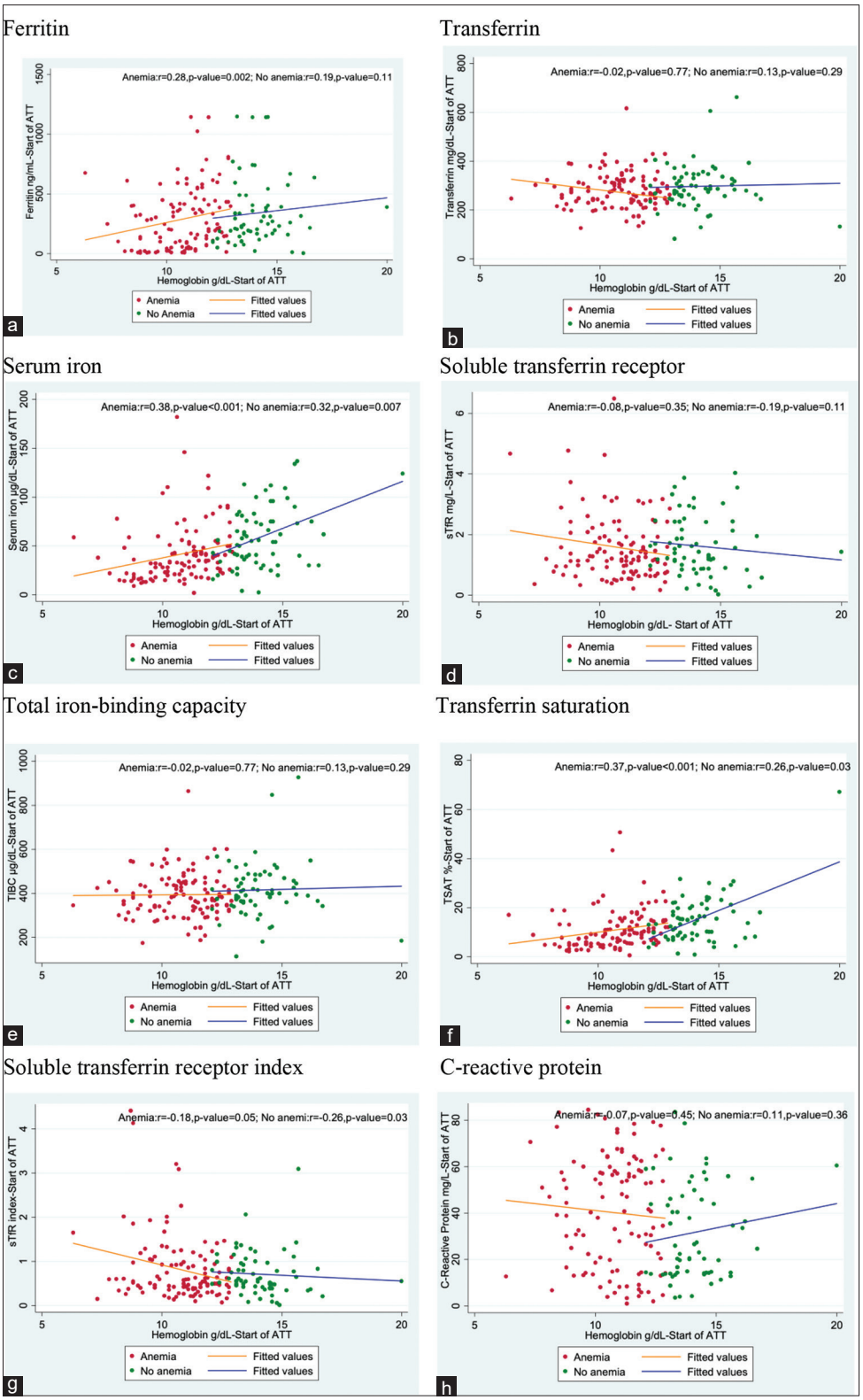

Figure 5: Spearman's correlation plot between biochemical parameters and haemoglobin levels is shown Anemic and No anemia tuberculosis patients at the start of treatment. A P value <0.05 is considered significant, (a) Ferritin vs Haemoglobin, (b) Transferrin vs Haemoglobin, (c) Serum iron vs Haemoglobin , (d) Soluble transferrin receptor vs Haemoglobin, (e) Total iron-binding capacity, vs Haemoglobin, (f) Transferrin saturation vs Haemoglobin, (g) Soluble Transferrin receptor ratio vs Haemoglobin, (h) C-reactive protein vs Haemoglobin

In our study, weak positive correlations with statistical significance were observed between ferritin, serum iron, TSAT and Hb, suggesting a slight Hb increase as iron parameters rise. Limited association might stem from PTB-related inflammation. CRP displayed weak negative correlation with Hb, implying high inflammation linked to Hb reduction,

Table 6: Sociodemographic associated with IDA among the newly diagnosed pulmonary tuberculosis patients at the start of the treatment *n*=176

Variables	Total	Risk factors for IDA		PR 95%CI	APR 95%CI	Adjusted *P*
		IDA (*n*=15)	No IDA (*n*=161)			
Age in years						
18-30	36	08 (22.22)	28 (77.78)	5.55 (1.93-15.93)	1.96 (0.37-10.24)	0.42
31-60	125	05 (04)	120 (96)	1	1	
>60	15	02 (13.33)	13 (86.67)	3.33 (0.71-15.70)	2.92 (0.30-28.44)	0.35
Gender						
Male	129	04 (3.1)	125 (96.9)	1		
Female	47	11 (23.4)	36 (76.6)	7.54 (2.52-22.55)	4.95 (1.54-15.87)	0.00
Religion						
Hindu	155	12 (7.74)	143 (92.26)	1.08 (0.15-7.73)		
Christian	14	1 (7.14)	13 (92.86)	1		
Muslim	7	2 (28.57)	05 (71.43)	4 (0.43-36.91)		
Education (ISC)						
No formal education	87	5 (5.75)	82 (94.25)	1		
Primary, middle stage and secondary	64	8 (12.5)	56 (87.5)	2.17 (0.74-6.33)		
Senior secondary education and bachelors or masters or equivalent	25	2 (8)	23 (92)	1.39 (0.28-6.74)		
Occupation						
Employed	97	05 (5.15)	92 (94.85)	1		
Unemployed	79	10 (12.66)	69 (87.34)	2.45 (0.87-6.88)	0.90 (0.37-2.17)	0.82
Ration card						
Red	113	11 (9.73)	102 (90.27)	1.53 (0.51-4.61)		
Yellow	63	04 (6.35)	59 (93.65)	1		
Marital status						
Married	126	06 (4.76)	120 (95.24)	1		
Unmarried	38	07 (18.42)	31 (81.58)	3.86 (1.38-10.81)	1.12 (0.25-5.01)	0.87
Others	12	02 (16.67)	10 (83.33)	3.5 (0.79-15.47)	1.63 (1.31-20.35)	0.70

IDA: Iron deficiency anaemia; PR: Prevalence risk; APR: Adjusted prevalence risk

Table 7: Lifestyle risk factors and nutritional status associated with IDA among the newly diagnosed pulmonary tuberculosis patients at the start of the treatment *n*=176

Variables	Total	Risk factors for IDA		PR 95%CI	APR 95%CI	Adjusted *P*
		IDA (*n*=15)	No IDA (*n*=161)			
AUDIT-C scale[†]						
Lower risk	107	13 (12.15)	94 (87.85)	1		
Higher risk	8	1 (12.5)	07 (87.5)	1.02 (0.15-6.89)		
Increasing risk	43	0	43 (100)	1		
Possible dependence	18	1 (5.56)	17 (94.44)	0.45 (0.06-3.28)		
DM[§]						
Yes	81	02 (2.47)	79 (97.53)	0.18 (0.04-0.77)	0.27 (0.49-1.50)	0.13
No	95	13 (13.68)	82 (86.32)			
BMI[‖]						
Underweight (<18.5 kg/m^2)	79	08 (10.13)	71 (89.87)	1.37 (0.47-4.01)		
Normal (18.5–22.9 kg/m^2)	68	05 (7.35)	63 (92.65)	1		
Overweight (23–24.9 kg/m^2)	13	0	13 (100)	1		
Obesity (≥25 kg/m^2)	16	02 (12.5)	14 (87.5)	1.7 (0.36-7.98)		
MUAC[¶]						
Normal	95	06 (6.32)	89 (93.68)	1		
Deficient	81	09 (11.11)	72 (88.89)	1.75 (0.65-4.73)		

DM[§]: diabetes mellitus; BMI[‖]: body mass index; low risk (0-7); possible dependence (20+); MUAC[¶]: mid-upper arm circumferences. 1 references; PR: prevalence risk; APR: adjusted prevalence risk. There are less participants diagnosed with IDA in the FTND group; therefore, we have not included in the PR table. [†]AUDIT-C scale: high risk (16-19); increasing risk (8-15)

affirming inflammation's Hb impact. Elevated ferritin, TIBC, transferrin and sTfR may counter Mycobacterium tuberculosis (MTB) multiplication by limiting iron availability, responding to infection.[46,48]

Our study also found a small number of cases with IDWA. Diagnostic iron parameters such as ferritin, serum iron, TIBC, transferrin and TSAT[44,45] are affected by chronic illness, complicating their interpretation.

While ferritin levels decrease in IDA, inflammation can elevate them, similar to serum iron, TIBC and transferrin, making it complex to interpret in Fe-deficient patients with infections or inflammation.[46] Patients with PTB often exhibit elevated serum ferritin levels, which can indicate oxidative stress.[47] Transferrin, a marker for nutritional status and a negative acute-phase protein, tends to decrease during infections and is influenced by protein intake and malnutrition.[31] Stress and immune mediator cytokine synthesis by the liver can further impact the synthesis of proteins such as albumin and transferrin levels.[42] Additionally, Fe deficiency prompts increased sTfR release from erythroblasts.[48]

sTfR is a transmembrane protein expressed in cells that require iron. These receptors are not affected by any inflammatory condition and are raised during Fe-deficient state.(39) Studies have shown sTfR is a better indicator of Fe deficiency when associated with inflammation. This is consistent with our finding that highest mean sTfR is found in the IDA [(2.65 (1.4)] when compared to the ACD group [(1.39 (0.8)]. This comparison explains that low Hb and high sTfR in IDA compared with ACD indicate inflammation related to anaemia and may be due to iron deficiency in PTB patients with anaemia.

This finding is in accordance with the research by Abedin *et al.*, who also found an increase in sTfR in the ACD patient's group.[49,50] In our study, sTfR alone did not distinctly separate ACD and IDA due to overlapping values. We then used the sTfR/log ferritin index, estimating body iron across various stores. The index decreased in ACD and increased in IDA, facilitating accurate differentiation. ACD correlated with alcohol, smoking and undernutrition, while IDA was predominantly linked to gender among sociodemographic characteristics.

As previously reported,[46] ferritin is an acute-phase reactant and its level is not reliable at the time of infection; hence, the type of anaemia can be differentiated by sTfR index. Our results demonstrate that the addition of sTfR and sTfR index to the serum ferritin measurement substantially improves the detection of ACD and IDA, particularly in PTB diagnosis where routine markers for iron status such as Hb, MCV, ferritin, Fe, Tf, TIBC and TSAT provide equivocal results that may delay diagnosis of combined IDA and ACD. This study, investigating ACD during the initiation of ATT, offers a focused and valuable examination. As anaemia has implications on the TB treatment outcomes, it is necessary to address and control anaemia in TB patients. However, treatment with Fe supplementations can be a double-edged sword as it can fuel MTB growth. We propose that type of anaemia be evaluated in all TB patients at the start of treatment and managed adequately based on whether it is due to Fe deficiency or inflammation. Due to the heightened inflammatory response in TB patients, iron supplements might not effectively correct infection-related anaemia. Instead, treatment strategies targeting the infection or inflammation itself, such as anti-tumour necrosis factor (TNF) therapy, have been recommended.[7,41]

The strength of the study is the use of sTfR index in the evaluation of the types of anaemia, which clearly delineates the anaemia of chronic disease from anaemia due to iron deficiency. This study has certain limitations as well. The patients were from the urban areas, and the levels of inflammation may vary in those from the rural areas. There could have been causes of inflammation other than TB in these patients, which we did not explore. Despite this limitation, the study offers vital insights into ACD's characteristics, emphasizing the importance of routine screening and management during ATT. It also calls for further research into the connections between TB, ACD and treatment timing.

Conclusion

The study concludes that iron metabolism is altered during TB infection. Hence, anaemia among PTB patients is mainly due to ACD compared to iron deficiency. The parameters such as ferritin, TIBC, transferrin and sTfR were not able to differentiate between the types of anaemia in PTB patients as their levels change in response to infection. The sTfR/log ferritin index was a better measure for differentiation between the ACD and IDA.

Ethical consideration

The study was approved by the Institute Ethics Committee (IEC: JIP/IEC/2017/0151), the State TB Control Officer (STO), the Government of Puducherry, and the State Task Force Operational Research Committee (STFORC), NTEP, Puducherry. Written informed consent was obtained from all the study participants. The authors adhered to the principles in the Declaration of Helsinki.

Acknowledgement

The authors extend their appreciation to the participants and laboratory technicians involved in the study for their valuable contributions and technical assistance.

Data availability

The dataset used for the study is available with the corresponding author on a reasonable request.

Financial support and sponsorship

We thank all the participants for providing blood samples and Tuberculosis Health Visitor for their support. The authors would like to thank the tertiary care institute in Puducherry for providing an intramural grant (JIP/Res/Intra-PhD/phs1/33/2017-2018) for the completion of this study.

Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. Global tuberculosis report 2020 [Internet]. [cited 2023 Aug 13]. Available from: https://www.who.int/publications-detail-redirect/9789240013131.
2. Sahiratmadja E, Wieringa FT, van Crevel R, de Visser AW, Adnan I, Alisjahbana B, *et al.* Iron deficiency and NRAMP1 polymorphisms (INT4, D543N and 3'UTR) do not contribute to severity of anaemia in tuberculosis in the Indonesian population. Br J Nutr 2007;98:684-90.
3. World Health Organization. Global tuberculosis report 2021 [Internet]. [cited 2023 Aug 13]. Available from: https://www.who.int/publications-detail-redirect/9789240037021.
4. Isanaka S, Mugusi F, Urassa W, Willett WC, Bosch RJ, Villamor E, *et al.* Iron deficiency and anaemia predict mortality in patients with tuberculosis. J Nutr 2012;142:350-7.
5. Sharma N, Basu S, Chopra KK. Achieving TB elimination in India: The role of latent TB management. Indian J Tuberc 2019;66:30-3.
6. Baluku JB, Namiiro S, Nabwana M, Muttamba W, Kirenga B. Undernutrition and Treatment Success in Drug-Resistant Tuberculosis in Uganda. Infect Drug Resist 2021;14:3673-81.
7. Dasaradhan T, Koneti J, Kalluru R, Gadde S, Cherukuri S Priya, *et al.* Tuberculosis-Associated Anaemia: A Narrative Review. Cureus 14(8):e27746.
8. Nagu TJ, Spiegelman D, Hertzmark E, Aboud S, Makani J, Matee MI, *et al.* Anaemia at the Initiation of Tuberculosis Therapy Is Associated with Delayed Sputum Conversion among Pulmonary Tuberculosis Patients in Dar-es-Salaam, Tanzania. PLOS ONE 2014;9:e91229.
9. Luo M, Liu M, Wu X, Wu Y, Yang H, Qin L, *et al.* Impact of anaemia on prognosis in tuberculosis patients. Ann Transl Med 2022;10:329.
10. Zimmermann MB, Chaouki N, Hurrell RF. Iron deficiency due to consumption of a habitual diet low in bioavailable iron: a longitudinal cohort study in Moroccan children. Am J Clin Nutr 2005;81:115-21.
11. Hella J, Cercamondi CI, Mhimbira F, Sasamalo M, Stoffel N, Zwahlen M, *et al.* Anaemia in tuberculosis cases and household controls from Tanzania: Contribution of disease, coinfections, and the role of hepcidin. PLoS ONE 2018;13:e0195985.
12. Balarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian S. Anaemia in low-income and middle-income countries. The Lancet 2011;378:2123-35.
13. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, *et al.* A systematic analysis of global anaemia burden from 1990 to 2010. Blood 2014;123:615-24.
14. Ratnaningsih T, Sukirto NW, Wahyuningsih AT. Soluble Transferrin Receptor (sTfR) Identifies Iron Deficiency Anaemia (IDA) in Pulmonary Tuberculosis Patients. Acta Medica Indones 2020;52:334-43.
15. Adzani M, Dalimoenthe NZ, Wijaya I. Profile of Anaemia on Lung Tuberculosis at Dr. Hasan Sadikin General Hospital and Community Lung Health Center Bandung. Althea Med J 2016;3:137-40.
16. Lee SW, Kang YA, Yoon YS, Um SW, Lee SM, Yoo CG, *et al.* The prevalence and evolution of anaemia associated with tuberculosis. J Korean Med Sci 2006;21:1028-32.
17. van Lettow M, West CE, van der Meer JWM, Wieringa FT, Semba RD. Low plasma selenium concentrations, high plasma human immunodeficiency virus load and high interleukin-6 concentrations are risk factors associated with anaemia in adults presenting with pulmonary tuberculosis in Zomba district, Malawi. Eur J Clin Nutr 2005;59:526-32.
18. Das BS, Devi U, Mohan Rao C, Srivastava VK, Rath PK, Das BS. Effect of iron supplementation on mild to moderate anaemia in pulmonary tuberculosis. Br J Nutr 2003;90:541-50.
19. Mukherjee A, Kaushik R, Sindhwani G, Kaushik R. Prevalence and characteristics of anaemia in new cases of pulmonary tuberculosis in a tertiary care hospital in Uttarakhand, India 2019 Feb 6;
20. Kerkhoff AD, Meintjes G, Opie J, Vogt M, Jhilmeet N, Wood R, *et al.* Anaemia in patients with HIV-associated TB: relative contributions of anaemia of chronic disease and iron deficiency. Int J Tuberc Lung Dis 2016;20:193-201.
21. Friis H, Range N, Kristensen CB, Kæstel P, Changalucha J, Malenganisho W, *et al.* Acute-phase response and iron status markers among pulmonary tuberculosis patients: a cross-sectional study in Mwanza, Tanzania. British journal of nutrition 2009;102:310-7.
22. TB Elimiation Mission PUDUCHERRY UT_2020.pdf [Internet]. [cited 2023 Aug 13]. Available from: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://tbcindia.gov.in/WriteReadData/10TBElimiationMissionPUDUCHERRYUT_2020.pdf.
23. Oliveira MG, Delogo KN, Oliveira HM, Ruffino-Netto A, *et al.* Anemia in hospitalized patients with pulmonary tuberculosis. J Bras Pneumol 2014;40:403-10.
24. Elsayed ME, Sharif MU, Stack AG. Transferrin Saturation: A Body Iron Biomarker. Adv Clin Chem 2016;75:71-97.
25. Al-Rubaie HA, Al-Bayaa IM, Al-Amiri YA. The Value of Soluble Transferrin Receptor and Soluble Transferrin Receptor-ferritin Index in Discriminating Iron Deficiency Anaemia from Anaemia of Chronic Disease in Patients With Rheumatoid Arthritis. The Open Rheumatology Journal 2019;13(1).
26. World Health Organization, Babor TF, Higgins-Biddle JC, Saunders JB, Monteiro MG. AUDIT: the alcohol use disorders identification test : guidelines for use in primary health care [Internet]. World Health Organization; 2001 [cited 2023 Aug 13]. Report No.: WHO/MSD/MSB/01.6a. Available from: https://apps.who.int/iris/handle/10665/67205.
27. Fagerstrom KO, Heatherton TF, Kozlowski LT. Nicotine addiction and its assessment. Ear Nose Throat J 1990;69:763-5.
28. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity [Internet]. World Health Organization; 2011 [cited 2023 Aug 13]. Report No.: WHO/NMH/NHD/MNM/11.1. Available from: https://apps.who.int/iris/handle/10665/85839.
29. Guidance Document - Nutritional Care & Support for TB patients in India: Central TB Division [Internet]. [cited 2023 Aug 13]. Available from: https://tbcindia.gov.in/index1.php?lang=1&level=3&sublinkid=4731&lid=3277.
30. Das Gupta A, Abbi A. High serum transferrin receptor level in anaemia of chronic disorders indicates coexistent iron deficiency. Am J Hematol 2003;72:158-61.
31. Infusino I, Braga F, Dolci A, Panteghini M. Soluble transferrin receptor (sTfR) and sTfR/log ferritin index for the diagnosis of iron-deficiency anaemia. A meta-analysis. Am J Clin Pathol 2012;138:642-9.
32. Núñez G, Sakamoto K, Soares MP. Innate Nutritional Immunity. J Immunol Baltim Md 1950 2018;201:11-8.

33. Doherty CP. Host-Pathogen Interactions: The Role of Iron1,23. J Nutr 2007;137:1341-4.
34. Schaible UE, Kaufmann SHE. Iron and microbial infection. Nat Rev Microbiol 2004;2:946-53.
35. Prevalence And The Progress of Anaemia Associated With Newly Diagnosed Pulmonary Tuberculosis Patients: A Cohort Study | International Journal of Current Research [Internet]. [cited 2023 Aug 14].
36. Weiss G, Goodnough LT. Anaemia of Chronic Disease. N Engl J Med 2005;352:1011-23.
37. Minchella PA, Donkor S, Owolabi O, Sutherland JS, *et al.* Complex anemia in tuberculosis: the need to consider causes and timing when designing interventions. Clinical Infectious Diseases 2015;60:764-72.
38. Bashir BA, Abdallah SA, Mohamedani AA. Anemia among patients with pulmonary tuberculosis in Port Sudan, Eastern Sudan. Int J Recent Sci Res 2015;6:4128-31.
39. Davis SL, Littlewood TJ. The investigation and treatment of secondary anaemia. Blood Rev 2012;26:65-71.
40. Begum S, Latunde-Dada GO. Anaemia of Inflammation with An Emphasis on Chronic Kidney Disease. Nutrients 2019;11:2424.
41. Mishra S, Taparia P, Yadav D. Study of Iron Metabolism in Pulmonary Tuberculosis Patients. Int J Health Sci Res 2018;8:70-7.
42. Sharma C, Badyal A. Role of iron metabolism in pulmonary tuberculosis: a case control study in Katra. Int J Sci Rep. 2021;7:298-301.
43. Bahi GA, Bamba A, M'Boh GM, Aké-Edjeme A, *et al.* Evaluation of the Hematological and Biochemical Markers of Iron Metabolism in Pulmonary Multidrug-Resistant Tuberculosis (MDR-TB). J Trop Med Health [Internet]. 2018 Feb 9 [cited 2023 Aug 15].
44. Kernan KF, Carcillo JA. Hyperferritinemia and inflammation. Int Immunol 2017;29:401-9.
45. Baer AN, Dessypris EN, Krantz SB. The pathogenesis of anaemia in rheumatoid arthritis: a clinical and laboratory analysis. Semin Arthritis Rheum 1990;19:209-23.
46. Punnonen K, Irjala K, Rajamäki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood 1997;89:1052-7.
47. Huang HH, Yan HC, Han CL, Yu FC, *et al.* Association of in vitro oxidative stress, serum ferritin concentration and C-reactive protein in febrile emergency room patients. Clin Investig Med Med Clin Exp 2005;28:48-54.
48. Punnonen K, Irjala K, Rajamäki A. Iron-deficiency anaemia is associated with high concentrations of transferrin receptor in serum. Clin Chem 1994;40:774-6.
49. Yoon SH, Kim DS, Yu ST, Shin SR, Choi DY. The usefulness of soluble transferrin receptor in the diagnosis and treatment of iron deficiency anaemia in children. Korean J Pediatr 2015;58:15-9.
50. Zein El Abedin MY, Saleh MT, El Wakkad AS, Fathy GA, Awadallah R. Serum transferrin receptor and transferrin receptor/ferritin ratio distinguish iron deficiency in anemia of chronic disorders in children Egypt. Med. J. Natl. Res. Centr 2003;2:75-92.