Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/32qesa19

ASSOCIATION OF A-TNF AND IRON PARAMETERS IN ANEMIC AND NON-ANEMIC PTB

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ABSTRACT

Background: Tuberculosis has been major killer globally since the centuries and has now become a tenth leading cause of death worldwide. It is an air-borne chaos that induces systemic inflammation and have commonly linked with different comorbid clinical condition such as anemia. In chronic diseases such as pulmonary tuberculosis (PTB), inflammation due to bacterial burden play a vital role in Patho-physiology of anaemia.

Methodology: A comparative type of case control study. The study included 40 newly diagnosed anemic PTB (cases) and 40 newly diagnosed non anemic PTB (controls) of either gender having age group of 20-70 years. The PTB was confirmed by microscopic examination of sputum specimen for the detection of Acid-Fast Bacilli (AFB). Serum iron was analyzed by ferrozine method and α-TNF were analyzed by ELISA method. SPSS 20 version were used for the statistical analysis.

Results: Significantly higher levels of α-TNF (225.77±77.49) were observed in anemic PTB cases than that of non-anemic PTB cases ($p<0.001$). Significantly inverse correlation was observed between α-TNF and iron($p<0.05$) and positive association between α-TNF and Ferritin ($p<0.05$) in both the groups.

Conclusion: Increased α-TNF leads to higher bacterial burden and lower iron levels.

Keywords: Anemia; Pulmonary tuberculosis; α-tumor necrosis factor; Iron; ferritin.

Introduction:

Pulmonary tuberculosis is one of the major leading causes of death and morbidity in humans (1). Tuberculosis may be a pulmonary type infecting the lungs or an extrapulmonary type affecting other organs (2). World Health Organization (WHO) reported an incidence of 26.9 lakh patients of tuberculosis in India (3). Immunity is compromised in infectious diseases but when immunity is compromised like in the infection of human immuno-virus, there will be high chances of affliction with tuberculosis. In the infection of pulmonary tuberculosis, bacteria are attacking the body and activate systemic immunity leading to post-attack where there is a development of latent immunity and entering the final condition responsible for the manifestation of all clinical symptoms (4). Pulmonary tuberculosis is an infectious disease, where in inflammation is a common rule. But the

level of inflammation and extent of infection will reflect the severity of the disease. Tuberculosis has many co-morbidities and anemia is one of the common comorbidities of tuberculosis. One of the main reasons for the development of anemia is malnutrition. Anemia is a global issue, especially in developing countries like India. The large chunk of tuberculosis in anemia is mainly due to inflammation. Progression of anemia is a slow process in tuberculosis infection and might take several months or weeks to manifest and later hemoglobin stabilize (5,6). Numerous risk factors such as social factors, dietary and lifestyle habits may contribute to tuberculosis infection and also to the development of anemia. Anemia plays a Patho-physiological role in inflammatory disease like PTB (7). According to some prior studies prevalence of anemia is 32-94% in pulmonary tuberculosis. (8,9).

Ferritin is an iron storage protein wherein iron is stored when a surplus amount of iron is available and released iron when there is a requirement. Iron and ferritin levels fluctuate in pulmonary tuberculosis patients. Multifactorial pathophysiological conditions exist in anemia of chronic inflammation affecting both iron and ferritin levels (10). alpha-tumor necrosis factor-an essential component of host defense mechanism, despite of it also reflect the severity of tissue damage. (11) In the monitoring of the severity of pulmonary tuberculosis, the assessment of alpha-tumor necrosis factor and its correlation with Ferritin & iron to monitor disease severity and mechanism to changes in levels of iron parameters in pulmonary tuberculosis.

Materials and Methods

An analytical and observational type of study was conducted in the department of biochemistry, SBKSMI & RC, Dhiraj Hospital, Sumandeep Vidyapeeth, Vadodara, Gujarat with extended feasibility from GS Medical college, hapur, UP in the duration of May 2019-June 2020. Present study was approved from Sumandeep Vidyapeeth Institutional Ethical Committee (SVIEC/ON/MED/19029). A total of 80 confirmed diagnosed cases of pulmonary tuberculosis of either gender were enrolled for this study. Based on the hemoglobin levels (Females ≤ 12 g/dL and in Males and ≤13 g/ dL), a total of 80 were further classified into 40 anemic pulmonary tuberculosis (PTB) and 40 non-anemic pulmonary tuberculosis (PTB). The subjects with retreatment TB history, pregnant women, any surgical intervention, patients with chronic kidney disease, inflammation, patients with Cancer, heart disease and subjects with imperfect data report were excluded from the study.

Sample collection and processing: About 5 ml of venous blood was collected under all aseptic precautions from every participant and sample was dispensed into 2 different tubes. 3 ml in EDTA lavender tube for hemoglobin estimation and 2 ml in plain tube. Plain tube containing blood sample was centrifuged at 3000 RPM for 10 minutes to obtain serum for the analysis of iron, ferritin and α-TNF. Serum Iron were estimated by Ferrozine method by autoanalyzer, serum Ferritin (Catalogue no-DCM039-8) and Serum α-TNF (Catalogue no-EH0302) assessed by sandwich ELISA method in Dhiraj Hospital, Sumandeep Vidyapeeth University Vadodara, Gujrat, India clinical lab.

Statistical Analysis

Above collected data were presented in the form of Mean & SD (Mean ± SD). Test of significance (P-value) were analyzed by using unpaired student-t test. Relationship of α-TNF to ferritin & iron was determined by Pearson's correlation (two-tailed) analysis. The data was analyzed with descriptive statistics by using SPSS software version 16.0.The p-value <0.05 was considered as statistically significant.

Results:

In this study, a total of 80 newly diagnosed sputum AFB positive PTB subjects were categorized into anemic and non-anemic PTB based on their hemoglobin levels. The mean level of Hb were lower in anemic PTB subjects (9.76±1.39) compared to non-anemic PTB (13.16±0.61) ($p<0.001$) **[Table-1].** The mean level of serum Iron were lower in anemic PTB subjects (19.65±9.44) compared to non-

anemic PTB (30.58±13.14) ($p<0.05$). α-TNF levels were found to be significantly higher in anemic PTB group (225.77±77.49) as compared to non-anemic PTB (53.25±25.10) ($p<0.001$) **[Table-1].** Serum Ferritin was found significantly lower in anemic PTB (373.02±91.16) as compared to non-anemic PTB (265.57±102.36) ($p<0.001$) **[Table-1].**

Correlation analysis of α-TNF to serum Iron and serum ferritin were analyzed in anemic and non-anemic PTB and it was found statistically significantly negative correlation between α-TNF and serum iron in anemic PTB ($r=-0.515$, $p<0.001$) **(Fig-1)** and non-anemic PTB ($r=-0.515$,$p<0.001$) **(Fig-3).** A Positive significant association was observed between α-TNF and Ferritin in anemic PTB ($r=0.570$, $p<0.005$) **(Fig-2)** and non-anemic PTB ($r=0.411$, $p<0.005$) **(Fig-4).**

Table-1: Statistical evaluation of iron parameters and α-TNF in anemic and non-anemic PTB

Parameters	**Anemic PTB (n=40) (Mean ± SD)**	**Non-Anemic PTB (n=40) (Mean ± SD)**	***p-value***
Hb(g/dl)	9.76±1.39	13.16±0.61	<0.001*
Ferritin(ng/dl)	373.02±91.16	265.57±102.36	<0.000*
Iron(µg/dl)	19.65±9.44	30.58±13.14	<0.05*
α-TNF (pg/ml)	225.77±77.49	53.25±25.10	<0.000*

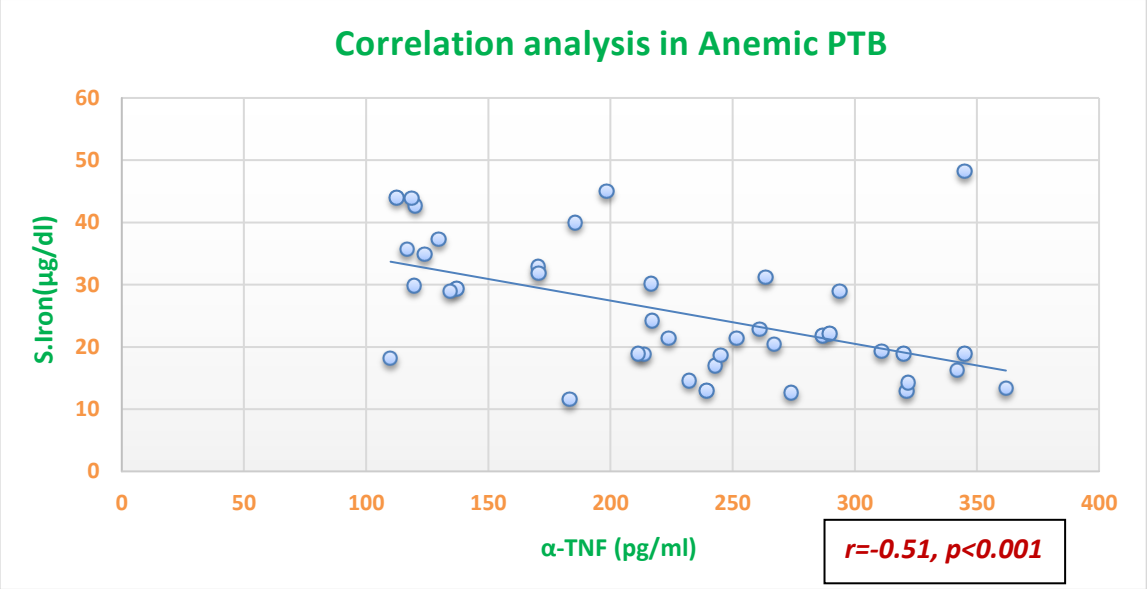

Fig-1-Correlation between α-TNF **and Iron in anemic PTB group.**

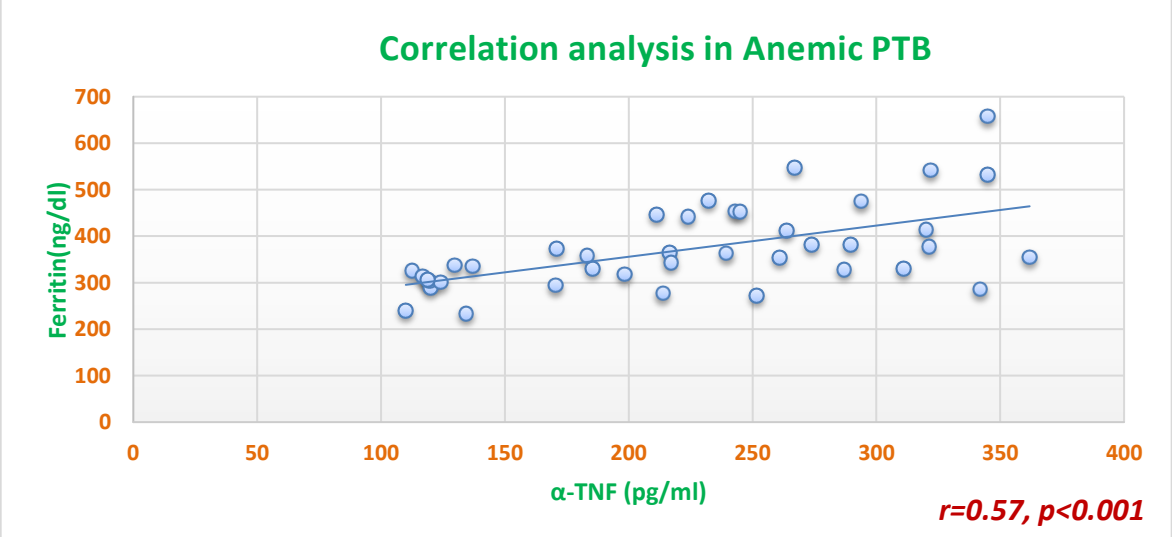

Fig-2-Correlation between α-TNF **and ferritin in anemic PTB group.**

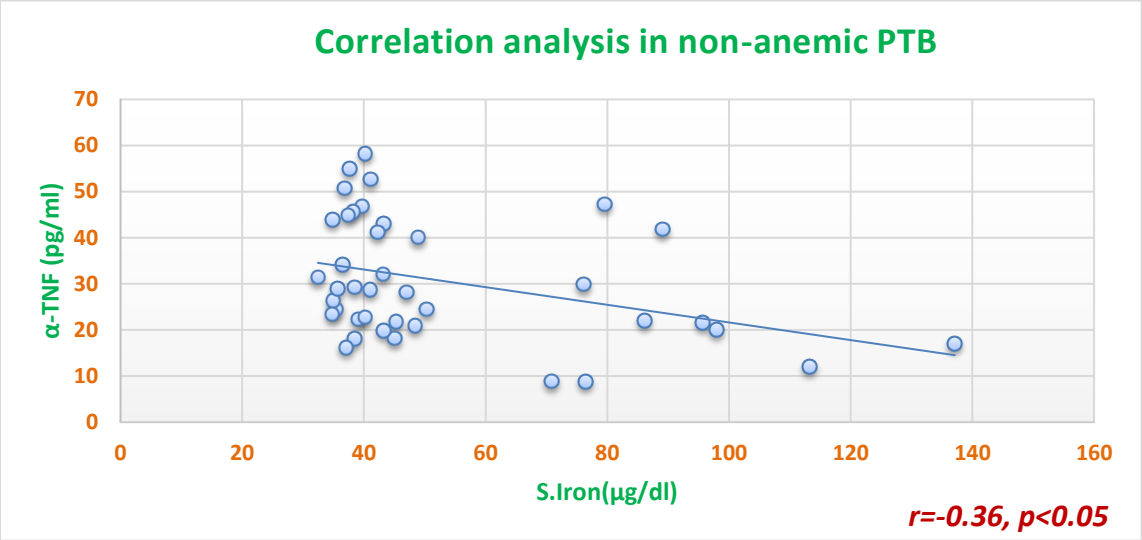

Fig-3-Correlation between α-TNF **and Iron in non-anemic PTB group.**

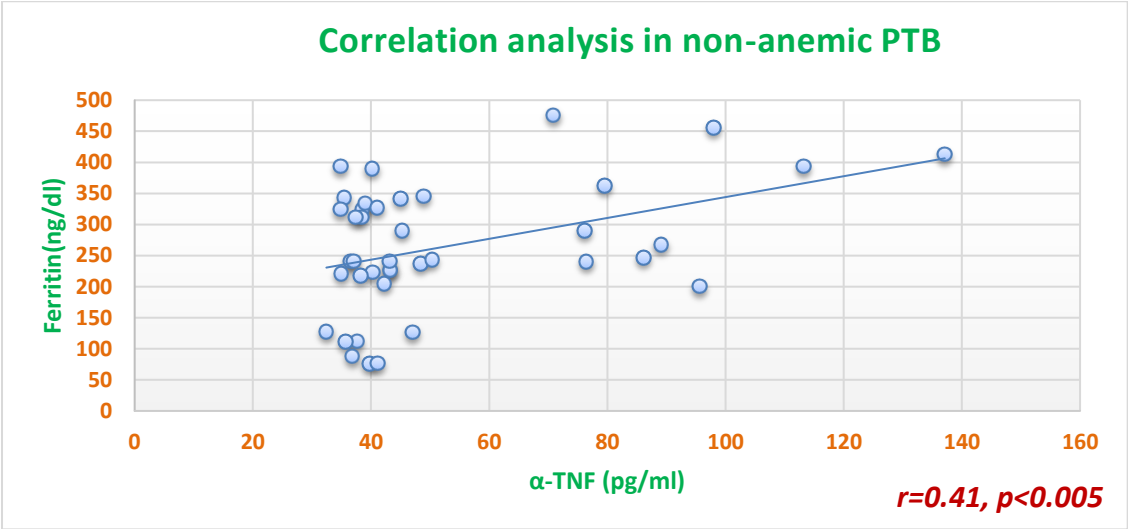

Fig-4-Correlation between α-TNF **and ferritin in non-anemic PTB group.**

Discussion:

Pulmonary tuberculosis accounts for approximately 80% of all forms of tuberculosis. It is a form of chronic granulomatous diseases characterized by caseating granulomas or pneumonia. (11) There are Multiple illnesses are entirely regulated by cell-mediated immunity. In this perspective, tuberculosis emerges as a disease of prime concern with its distribution at a global scale (12). Tuberculosis occurs when body white blood cells (WBCs) are unable to guard properly from this organism, which is a gram-positive acid-fast bacillus (Koch's bacilli), spreads through air from person to person. (13)

According to prior studies the specific mechanism of anemia in pulmonary tuberculosis is still unclear but some hypotheses anticipated that in disseminated tuberculosis, bone marrow participation with tubercular granuloma (14), fever due to nutritional deficiency (15), loss of appetite because of changes in metabolic system and haemoptysis. (16) The large chunk of tuberculosis in anemia is mainly due to anemia of inflammation (AI), reduced appetite and poor food intake which leads to diminished intake in the levels of different microminerals, mainly iron and selenium (17). Therefore, iron deficiency anemia and inflammatory anemia (IA) both might coincide in PTB subjects (18). In this study, we aimed to assess levels of α-TNF, serum Iron and Ferritin and their correlation in anemic and non-anemic pulmonary tuberculosis with same age matched criteria.

As per some prior studies they found lower degree of serum iron in anemic PTB subjects while degree of serum ferritin and α-TNF was higher in anemic of pulmonary tuberculosis group as compared to controls. [17,19,20].

In present study we found statistically significant differences in Ferritin and α-TNF in both the groups (Table-1). It was observed that α-TNF levels and serum ferritin levels were higher in anemic PTB (225.77±77.49, $p<0.0001$) as compared to non-anemic PTB (53.25±25.10, $p<0.001$). which clearly suggesting mycobacterium tuberculosis in PTB subjects stimulate the production of cytokines as- IL-1, IL-2 and α-TNF. which may be because of more bacterial burden or severity of disease. (21) Higher inflammation with higher α-TNF interfere decreased erythropoietin production which might leads anemia (22).

We observed slightly insignificant lower level of serum Iron in anemic PTB (19.65±9.44) compared to non-anemic PTB group (30.58±13.14) ($p<0.05$). In tuberculosis iron plays very important role by influencing both acquired and innate immune response and also required in bacterial replication in tuberculosis (22). Ferritin being an intracellular iron storage protein (23) which reflect iron storage in body (24). In the present study there is a significant inverse relationship between alpha tumor necrosis factor and serum Iron ($r= -0.51$, $p<0.001$) ($r= -0.36$,$p<0.05$)(Fig-1) in anemic PTB group and non-anemic PTB group respectively.

While analyzing an association, was established a significant positive association between ferritin and α-TNF in case and control ($r= 0.57$, $r=0.41$, $p<0.05$) group respectively. However, association of both were accordance with the study that α-TNF altered the iron metabolism which in-duces hypoferraemia (2,22) and increased ferritin production similarly observed by Kulkarni R.et al (18) for ferritin, inflammation, and Iron.α-TNF may be altering iron metabolism by various mechanism like-generation of reactive oxygen species (ROS) and free radicals which decreased RBC production (25) by decreasing EPO synthesis (2) and short-lived radicals like nitric oxide (NO) which modify iron homeostasis post transcriptionally through iron regulatory proteins (IRPs) (18). In pulmonary tuberculosis, α-TNF by retain of iron from microbes (2,18) and change the iron from transferring bound form to ferritin incorporated storage form might be a reason of disturbance in iron metabolism.

Conclusion:

- Increased α-TNF could give rise to higher bacterial burden and lower iron levels.
- Cytokine-mediated defense against microbial pathogens results in effective iron withholding within macrophages, influencing erythropoiesis and disrupting iron metabolism, which might be involved in the pathogenesis of anemia.

Future Scope of Study:

- Increased α-TNF in bacterial burden and iron regulation opens up several important areas for research and potential therapeutic interventions.
- To modulate α-TNF and iron homeostasis to better manage chronic infections, inflammatory diseases, and conditions like anemia of inflammation, with an emphasis on balancing immune response and iron availability to prevent unintended negative effects.

Conflict of Interest: All authors have declared that there is no any conflict of interest.

Acknowledgement: Special thanks goes to Dr Sadhna Ajay for critical review and help out to meet inclusion criteria for this study.

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