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Diagnostic evaluation of alpha tumor necrosis factor and iron profile in newly diagnosed pulmonary tuberculosis with and without anemia

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***Abstract*---**Tuberculosis is the 9th leading cause of deaths worldwide. In chronic diseases such as pulmonary tuberculosis (PTB), inflammation due to bacterial burden play a vital role in patho-physiology of anaemia. Functions of erythropoietin (EPO) is affected by inflammation. The present study was an analytical type of case control study. The study included 100 newly diagnosed anaemic PTB cases and 50 newly diagnosed non anaemic PTB controls. PTB was confirmed by microscopic examination of sputum specimen for the detection of Acid-Fast Bacilli (AFB). Both cases and controls were subjected to iron profile and serum α-TNF were analysed by ELISA method. Significantly higher levels of α-TNF(214.56±82.30) was observed in anemic PTB cases than that of non-anemic PTB cases ($p<0.001$). Iron profile was also found significantly higher in anaemic PTB group in non anaemic PTB group. Significantly inverse correlation was observed between α-TNF and iron ($r=-0.257$, $p<0.05$) and α-TNF and Ferritin ($r=-0.202$, $p<0.05$) and Iron and Ferritin in both the groups. Increased α-TNF associated with increased bacterial burden and relatively decreased Iron. Cytokine-mediated defence against

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microbial pathogen causes effective withholding of iron within macrophages which affects erythropoiesis.

Keywords---cytokine, anemia, inflammation, pulmonary tuberculosis, α-tumor necrosis factor.

Introduction

Tuberculosis is an infectious disease widely spread worldwide. (1,2). Several previous studies reported that 90% population during their lifetime remain asymptomatic even after infection (1). Out of 90% infected people only 5-10% people developed active TB symptomatically and left over infected people resist the infection [3,2]. As according to previous studies pulmonary tuberculosis(PTB) is a chronic infectious disease.During the course of this disease there is high chance of development of inflammatory anemia(IA) which ultimately playing a role in pathophysiology of this disease (4). 32-94 % of individuals of tuberculosis are anemic according to previous studies (5,6). In pulmonary tuberculosis, anemia may occur due to chronic inflammatory consequences despite bone marrow suppression or blood loss (7), Stimulation of cytokines and alpha tumor necrosis factor cause blunted response of erythropoietin(8).It is recognized that innate and adaptive immune responses evokes due to communication of mycobacterium with host [9].In mycobacterium infection, macrophages is activated by reaction of α-TNF signaling with cytokines synthesis and non-activated macrophages remain the habitat for Mtb (2).

Worldwide iron deficiency anemia(IDA) is a common nutritional deficiency especially in developing countries(6).In IDA increased blood volume and chronic blood loses can cause poor iron absorption(10) and anemia of inflammation in infectious disease (bacterial, fungal,viral) patients like tuberculosis.(11)In Tuberculosis iron has a fundamental role because it is required for Mtb replication(12). The concentration of iron binding protein, transferrin and serum iron varies in different physiological and pathological conditions (13). Both inflammatory anemia and IDA may coexist in PTB patients. (14). Altered iron metabolism due to cytokines considered as factor of pathogenesis of chronic inflammation anemia. Cytokines withhold the iron from microbes which indirectly affect the erythropoiesis which required iron supply for RBCs (7,14). Also a-TNF induces a decrease in mature erythroblasts and raise of apoptosis of immature erythroblasts However, the exact mechanism of anemia remains unclear. (14). Erythropoiesis involves the communication of erythropoietin and iron. In essence, erythropoietin act as the accelerator which drives erythropoiesis and Iron act as the fuel for the new RBCs production. When both are coupled, RBCs production moves rapidly and efficiently. If any one of the factor is absent results anemia (15).

Materials and Methods

The Present study was a type of analytical case control study. Samples were collected from GSMCH (GSMCH2019/IEC/Approval/019). Analysis of the samples were carried out in the biochemistry department and central clinical

laboratory of SBKSMI&RCand Dhiraj General Hospital (DGH) Sumandeep Vidhyapeeth, Piperia, Vadodara, Gujrat, India fromMay 2019 to May 2020 after approval from Institutional Ethics Committee(SVIEC/ON/MED/PhD/19029).A total of 150 newly diagnosed age and sex matched pulmonary tuberculosis subjects were enrolled for the study, which was further categorized into 100 anemic PTB subjects comprised as cases and 50 non anemic PTB subjects as controls. Diagnosis of PTB of cases and controls wasbased on clinical presentation and microscopic examination of sputum for Acid-Fast Bacilli (AFB) detection and by CBNAAT by the attending pulmonologist. After describing the study in detail, the written consent was taken from all the patients before enrolment.

Inclusion criteria

100 newly diagnosed anaemic PTB cases:18-70 years of both male and females having Hb levels < 13 g/dL in males and < 12 g/ dL in females.(16)
50 non anaemic PTB controls:18-70 years of both genderhaving Hb levels≤13 g/dL for males and ≤12 g/ dLfor females. (16)

Exclusion criteria

Subjects with prior anti TB treatment history, any surgical intervention, pregnant women, patients with chronic inflammation, kidney disease, patients with HIV, heart disease, Cancer and subjects with in-complete data report were excluded from the study.

Sample collection and processing

5ml fasting venous blood sample under aseptic precautions were drawn from subjects, sample was separated into two parts: In the 1st part 4ml blood was collected in plain vacutainer and left to clot for 30 minutes, after 30 minutes serum is separated by centrifugation and stored at -20° C for α-TNF, serum ferritin, serum Iron and TIBC analysis. In the 2nd part 2ml of blood was collected in EDTA vial for CBC count simultaneously using automated cell counter.CBC were estimated by flow cytometry method by automated cell counter Nihon Kohden (3 part cell counter). Total Iron binding Capacity (TIBC) and serum Iron were estimated by Ferrozine method by autoanalyser with coral SR diagnostic available commercial test kits. Serumα-TNF(Catalogue no-EH0302) and serum Ferritin(Catalogue no-DCM039-8) estimated by sandwich ELISA methodin the clinical laboratory of Dhiraj Hospital, Sumandeep Vidyapeeth University Vadodara, Gujrat, India.

Statistical analysis

The data was analysed with descriptive statistics by using SPSS software version 16.0. , results were presented as mean±SD. Statistical significance of results evaluate by student independent sample t-test. Association between α-TNF, ferritin, iron and Hb were determined by Pearson's correlation (two-tailed) analysis. The p-value <0.05 was considered as statistically significant.

Results

In the study 100newly diagnosed sputum positive anemic PTB subjects with mean age of 42.0±14.5 years (age range 18-70) and 50 newly diagnosed sputum positive non anemic PTB controls 42.98±14.21 years (age range 18-70 years) were included. The data of present study was presented in the form of mean and standard deviation (SD) as (Mean±SD). The mean level of Hb were lower in anemic PTB cases (9.78±1.53) compared to non-anemic PTB controls(13.13**± 0**.63) ($p<0.001$)[Table-1]. Serum Iron was found to be significantly lower(24.85±9.32) in case group and higher (33.46±12.69) in control group($p<0.05$)[Table-1].α-TNF levels was found to be significantly higher in anaemic PTB group(214.56±82.30) as compared to controls (55.32±24.18)($p<0.001$)[Table-1]. TIBC (256.76±61.40) levels were found to be significantly lower in anaemic PTB group as compared to control group(342.86±102.32) ($p<0.001$).

Correlation between α-TNF, serum Iron, serum ferritin and Hb were measured in anemic and non anemic PTB cases and controls[Table-2].It was seen that statistically significantly negative correlation between α-TNF and serum iron in cases ($r=-0.248$,$p<0.05$) shown in (Fig-1)while no correlation was observed in control group(Table-2). positive significant correlation was found between α-TNF and Ferritin($r=0.262$,$r=0.339$, $p<0.001$) in cases (Fig-3) and control groups (Table-2). Statistically significant inverse correlation between serum α-TNF and Hb level ($r=-0.202$,$p<0.05$) in anemic PTB cases and ($r=-0.288$, $p<0.05$) in non anemic PTB controls was observed(Fig-2) [Table-2]. Furthermore, we observed statistically insignificant correlation between serum Iron and Hb level ($r=0.100$, $p>0.05$) in PTB cases and ($r=-0.113$, $p>0.05$) in non anemic PTB controls and significantly positive correlation between serum Iron and TIBC ($r=0.243$, $p<0.05$) in anemic PTB group While no correlation was seen in nonanemic PTB group shown in [Table-2].

Parameters	AnemicPTB Control(n=100) (Mean ± SD)	Non Anemic PTB patient (n=50) (Mean ± SD)	p-value
Hb (g/dl)	9.78±1.53	13.13± 0.63	0.000 *
Ferritin	421.81± 145.02	259.57 ±103.01	0.000*
Iron	24.85±9.32	33.46±12.69	0.027*
α-TNF	214.56±82.30	55.32±24.18	0.000*
TIBC	256.76±61.40	342.86±102.32	0.000*

PARAMETERS		α-TNF r value	Iron r-value	Ferritin r-value	Hb r-Value
TIBC	Case	0.142	0.243*	0.031	0.082
	Control	-0.135	-0.090	0.034	0.136
α-TNF	Case	—	-0.248*	0.262**	-0.202*
	Control	—	-0.006	0.339*	-0.288*
Iron	Case	—	—	-0.224*	0.100
	Control	—	—	-0.403**	-0.113
Ferritin	Case	—	—	—	-0.113
	Control	—	—	—	0.185

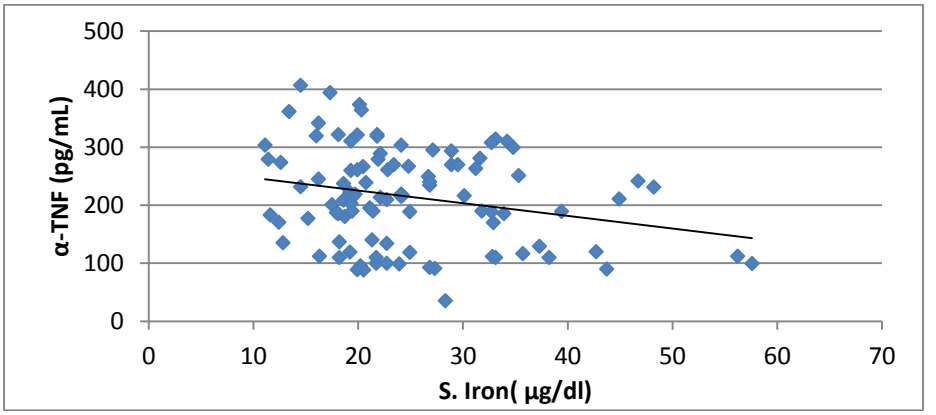

Fig 1. Correlation between α-TNF and Iron in anemic PTB group

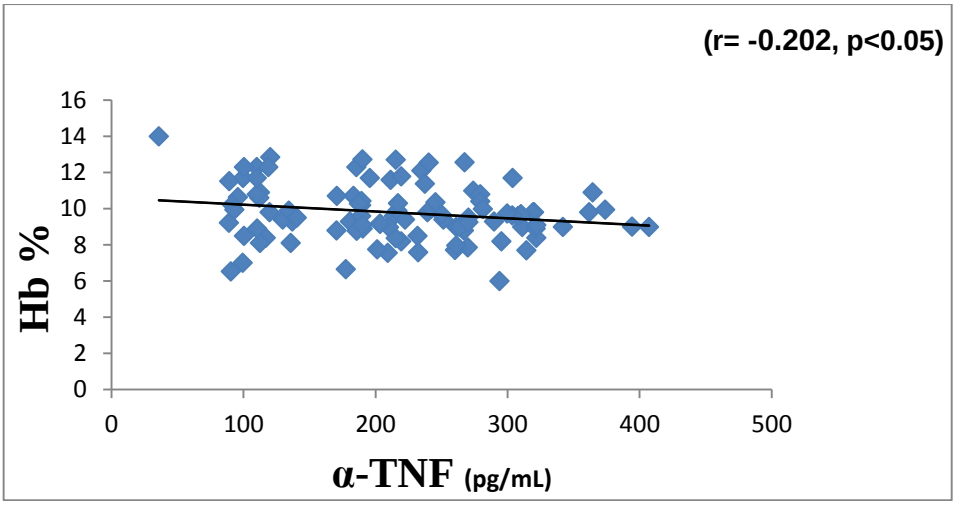

Fig 2. Correlation between Hb and α-TNF in anemic PTB group

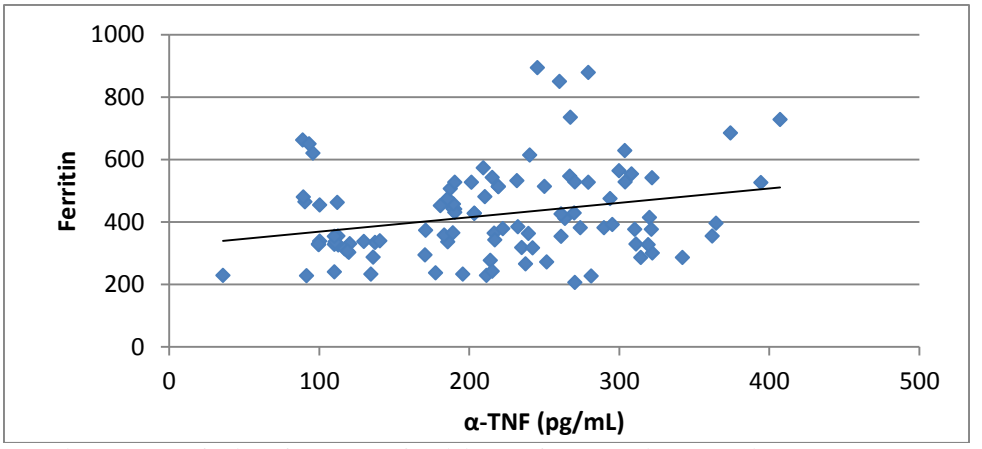

Fig 3. Correlation between ferritin and α-TNF in anemic PTB group.

Discussion

Anemia is very common complication in PTB (6). In pulmonary tuberculosis the specific mechanism of anemia is not known, but several hypotheses have been proposed like- in disseminated tuberculosis bone marrow participation with tubercular granuloma(17), loss of appetite and fever due to nutritional deficiency(7)blood loss due to haemoptysis(17) etc.. Therefore, both anemia due to chronic inflammation and iron deficiency anemia may coexist in patients of PTB (14).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.

The findings of our study are in correlation with that of previous observations that there were decreased levels of Iron, TIBC and Hb while α-TNF and ferritin levels were increased in TB patients [7,18,19]. In this study we have found statistically significant differences in α-TNF, Iron, Ferritin, Hb and TIBC between both the groups (Table-1). It was found that α-TNF levels were higher in this study as compared to control group ($p<0.001$). when compared with some previous studies who observed that α-TNF levels was higher compared to healthy individuals(14,20,21) and serum ferritin level was higher in both the groups($p<0.001$) of this study. PTB is a chronic infectious disease correlated to increased stimulation of cytokines as α-TNF, IL-6, IL-1 and IL-10.(20) which might be due to disease severity (22) and bacterial burden.(20) Increased cytokine intervene with impaired erythro-poiesis and decreased RBC survival results associated with anemia[21].

Serum Iron(Fe) levels were seen significantly lower in anemic PTB group as compared to control group($p<0.05$). Hypoferremia in disease or inflammation allied to anemia appears to be the consequence of complex network of immune cells, cytokines, and acute-phase proteins [23]. Ferritin concentration in blood is considered as a precise indicator of Fe stores in body [23],however, the stimulation of ferritin synthesis can increase following an inflammatory process, irrespective of iron status [24]. Usually values of TIBC are high in IDA (iron deficiency anemia) and low in inflammatory anemia (25). In the present study TIBC concentration found to be normal. This may be attributed to newly diagnosed PTB subjects taken in the study. But kulkarni R et al(14) found decreased TIBC level in non newly diagnosed PTB due to an acute phase response (ferritin) and Mishra S et.al found increased TIBC level in PTB but mechanism was not explained for such as observation.(26). Our study subjects were different from subjects of both of these studies as we have considered only new diagnosed PTB cases.

In the present study we have observed significantly inverse correlation of iron with α-TNF ($p<0.05$) in case group but with ferritin($p<0.05$) in both groups. While we found significant positive correction between α-TNF and ferritin in both groups ($p<0.05$). However correlation of these with each other were accordance with the observation that α-TNF induces hypoferraemia via mechanism like altered iron metabolism (20,21) and induce ferritin production similar observation was seen by Kulkarni R et al (14) for inflammation, ferritin and Iron. Alteration of iron metabolism is due to generation of free radicals and reactive oxygen species (ROS)

which is stimulated by increase in cytokines level, results decrease in RBC production(27) and short lived radicals like nitric oxide(NO) which modify iron homeostasis post transcriptionally through iron regulatory proteins(IRPs) [14]. Also ROS interfere with erythropoiesis (20)In anemic pulmonary tuberculosis, cytokines by withholding of iron from microbes (20,14) and shifting of iron from transferring bound position to ferritin incorporated storage position might be a cause of disturbance in iron metabolism.

Conclusion

Increased α-TNF might be associated with increased bacterial burden and relatively decreased Iron. Cytokine-mediated defence against microbial pathogen causes effective withholding of iron within macrophages which affects erythropoiesis and disturb the iron metabolism, which might be involved in pathogenesis of anemia.

Limitation

Budgetary constraints for Kits purchases, availability of less time and less number of PTB subjects were some of the limitations of this study. We have not done follow-up of anemic and non-anemic PTB patients which could have been more helpful in evaluation of inflammatory cytokines, serum iron and Ferritin.

Abbreviation

PTB: Pulmonary tuberculosis; Hb: Haemoglobin; PCV: Packed cell volume; WBC: White blood cell; α -TNF: Tumour necrosis factor-alpha; EPO: Erythropoietin, TIBC: Total iron binding capacity.

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