Assessment of cytokine (α-TNF) with Erythropoietin and their correlation in Pulmonary Tuberculosis with Anaemia

ABSTRACT

Globally tuberculosis is the 9th leading cause of death worldwide. As pulmonary tuberculosis (PTB) is a chronic disease, anaemia of inflammation due to bacterial burden play a vital role in pathophysiology of anaemia. Inflammation interferes with erythropoietin (EPO) function.**Methods:**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. The PTB was confirmed by microscopic examination of sputum specimen for the detection of Acid-Fast Bacilli (AFB). Both cases and controls were subjected to hematological analysis by automated cell counter and serum α-TNF and EPO by ELISA method. **Results:**Statistically significant difference was observed in levels of both α-TNF and EPO in anemic and non-anemic PTB groups($p<0.001$). α-TNF (214.56±82.30) levels were found to be significantly higher in anaemic PTB group while EPO level (58.44 ±14.97) were found to be significantly higher in non anaemic PTB group. Significant inverse correlation (r1=cases, r2=controls) was observed between α-TNF and EPO ($r=-0.257$, $p<0.05$) and α-TNF and Hb($r=-0.202$, $p<0.05$) in both the groups. **Conclusion:**Increased α-TNF with decreased EPO and hemoglobin infers that inflammation interferes with normal functioning of EPO and probably contributes in induction of anemia in tuberculosis patients.

Keywords: Cytokine, Anemia, Inflammation and Pulmonary tuberculosis.

INTRODUCTION:

Pulmonary tuberculosis is one of the major chronic infectious disease caused by mycobacterium tuberculosis pathogen and key cause of mortality and morbidity in the world.[1,2,3]Globally tuberculosis is the 9th leading cause of death worldwide.[2,3]Mycobacterium tuberculosis pathogen has the ability to live in latent state in humans and approximately 95% humans build up latent infection and can develop tuberculosis in their life.[3,4]It means that these latent infectious people cannot spread MTB to other person but they can developed active TB in future.[5] India's TB incidence was 27 lakh and 21.5 lakh in 2017 and 2018 respectively as reported by RNTCP.[3]PTB is a known inflammatory condition of chronic type and these types of chronic inflammatory diseases are mostly associated with anaemia.[6,7] In various studies prevalence of mild to moderate anemia was shown to be ranging from 16-76% in chronic inflammatory disease like PTB.[8-12] The precise mechanism of anemia in chronic inflammatory disease like PTB is not clearly known[8,13] however, anemia due to iron deficiency and inflammation have been implicated in these types of conditions.[8,11]

PTB is known to be associated with complications and anemia is a commonly associated one withmajor risk factor for mortality.[1]Generally pulmonary tuberculosis is coupled with major abnormality in heamopoiesis.[8,14]In chronic PTB abnormal hemoglobin levelsisconsidered to be multifactorial and attributable to the fundamental chronic inflammation. Decrease red blood cell survival, disturbance in iron metabolism and reduced EPO action have been implicated in the

pathogenesis of PTB anemia.[15]In some infectious disease and chronic inflammatory disorders EPO act as anti-inflammatory cytokine.[16]Erythropoietin primarily act as a regulator of erythropoiesis or red blood cell production.[16-20]EPO affect the production of red blood cells by stimulatingdifferentiation and proliferation of erythroid cells in bone marrow.[21-24]Previous studiesmentioned EPO activity is affected by cytokine mediated proinflammatory signaling.[6]

Inflammation in PTB is mainly due to bacterial burden leading to increased production of proinflammatory cytokines like TNF-α,[25] which is known to contribute to anemia by decreased erythropoietin production,[7,26] suppressed action of bone marrow to erythropoietin and its interference with metabolism of iron.[1,25]

Looking into above aspects, the present study was taken up to assess the effect of extent of chronic inflammation on Haemoglobin and Erythropoietin.

Materials and Methods:

The present study was an analytical type of case control study conducted at Smt. BK Shah Medical Institute and Research Center and Dhiraj General Hospital (DGH) Sumandeep Vidhyapeeth, Piperia, Vadodara, Gujrat, India from April 2019 to April 2020 year.The study enclosed 150 newly diagnosed PTB subjectswhichwere classified as 100 newly diagnosed anemic PTB cases and age and sex matched 50 newly diagnosed non anemic PTB controls. PTB clinical diagnosis of cases and control was diagnosed by pulmonologist and microscopic examination of sputum for of Acid-Fast Bacilli (AFB)detection and by CBNAAT. The study protocol was approved by Institutional Ethics Committee (SVIEC/ON/MED/PhD/19023). After describing the study in details, the written consent was taken from all the patients before enrolment.

Patients' selection:

Newly diagnosed anaemicPulmonary Tuberculosis subject of either gender having age group of 18-70years who were sputum smear positive for Acid Fast Bacilli (Z-N staining) and CBNAAT positive with following Hb levels (acc to WHO)[27]; Males: ≤13 g/dL and Females: ≤12 g/dL

Newly diagnosed non anaemic Pulmonary Tuberculosis subjects of either gender having age group of 18-70years who were sputum smear positive for Acid Fast Bacilli (Z-N staining) and CBNAAT positive with following Hb levels (acc to WHO)[27]; Males:≥13 g/dL and Females: ≥12 g/dL.

Exclusion criteria: The patients with extra-pulmonary TB and/or patients requiring surgical intervention, patients with history of prior anti TB treatment, pregnant women, patients with HIV, patients with chronic kidney disease, heart disease, Cancer were excluded from the study.

Sample collection and processing:

5 ml blood was collected from each study group individual under all aseptic precautions. Out of which one part of blood (2 ml) was transferred in EDTA vial for CBC count using automated cell counter Nihon Kohden (3-part cell counter) by flow cytometry method. Remaining blood (3 ml) was transferred in plain vial and left to clot for 30 min and then centrifuged at 3000 RPM. Separated serum samples were stored at -20°C in research lab for erythropoietin and α-TNF analysis. The patient confidentiality was maintained at each and every level.Serumα-TNF and EPO were estimated by sandwich ELISA method using commercially available kits (Fine test and Demeditec, respectively) according to manufacturers' instructions.

Statistical Analysis:

Data were analyzed with the help of software SPSS version 20. The mean values are represented as mean and SD. The statistical difference between cases and control were determined by

Student independent sample t-test. Relationship betweenα-TNF, EPO and Hb were determined by Pearson's correlation analysis. The p-value <0.05 were considered as statistically significant.

Results:

In present 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 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, Packed Cell Volume (PCV), RBC, Mean Corpuscular Hemoglobin (MCH) Mean Corpuscular Volume (MCV), and Mean Corpuscular Hemoglobin Concentration (MCHC) were lower in anemic PTB cases compared to non-anemic PTB controls while WBC values and platelets values were higher in anemic PTB case group compared to non-anemic PTB controls group. EPO (47.28± 6.40) was found to besignificantly lower in anemic PTB cases and observed higher (58.44 ±14.97) innon-anemic PTB controls ($p<0.05$) as illustrated in [Table-1].α-TNF (214.56±82.30) levels were found to be significantly higher in anaemic PTB group as compared to controls (55.32±24.18).Further,with respect to Hb, Hb was found to be significantly lower in cases as compared to controlsas shown in [Table-1].

Correlations betweenα-TNF, EPO and Hb were measured in anemic and non-anemic PTB cases and controls [Table-2].It was observed that statistically significant inverse correlation between serum α- TNF and Hb level ($r=-0.202$, $p<0.05$) in anemic PTB cases[Fig-1] and ($r=-0.288$, $p<0.05$) in non-anemic PTB controls. Also,statistically significant inverse correlation was

observed between serum levels of TNF-α and EPO (r=-0.257, p<0.05) in PTB cases[Fig-2] and (r=-0.358, p<0.05) in non-anemic PTB controls.Furthermore, we observed statistically insignificant correlation between serum EPO and Hb level (r=0.106, p>0.05) in PTB cases and (r=0.027, p>0.05) in non-anemic PTB controls [Fig-3].

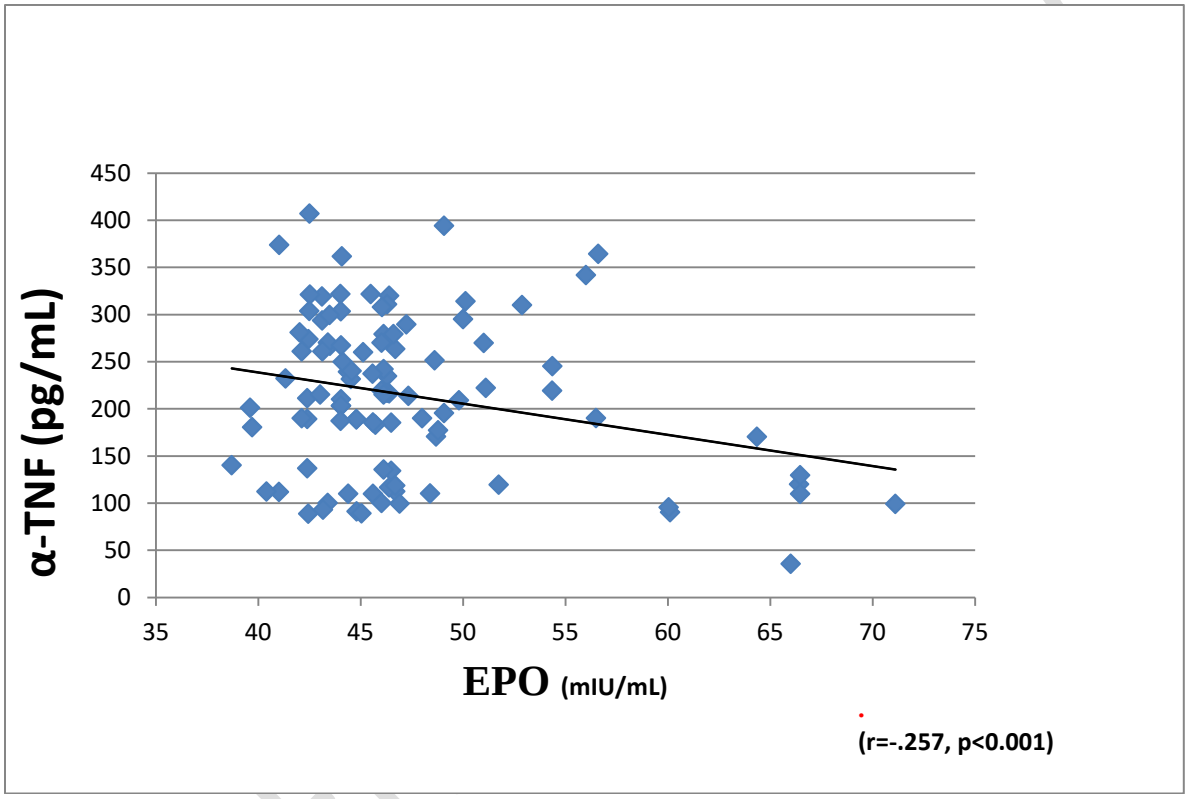
Table-1: Comparison between mean of different parameters in PTB cases and controls.

Parameter	Non-Anemic PTB Control (n= 50) (Mean ± SD)	Anemic PTB patient (n=100) (Mean ± SD)	p-value
Hb (g/dl)	13.13± **0**.63	9.78±1.53	0.000 *
RBC (millions/μL)	4.59±0.85	3.95±1.02	0.000 *
PCV (%)	39.44±2.19	29.70±4.52	0.000 *
MCH (Pg/cell)	27.99±9.99	24.83±3.23	0.005*
MCV (fl)	88.17±6.74	80.28±8.57	0.017 *
MCHC (g/dl)	32.24±1.09	29.93±3.13	0.000*
WBC(Cells/cumm)	9758.00±3115.53	11137.70±3171.49	0.013 *
Platelets (lakhscells)	2.38±0.56	2.7818±0.82	0.003*
EPO(mIU/mL)	58.44 ±14.97	47.28± 6.40	0.002*
α-TNF(pg/mL)	55.32±24.18	214.56±82.30	0.000*
*p<0.05 is significant			

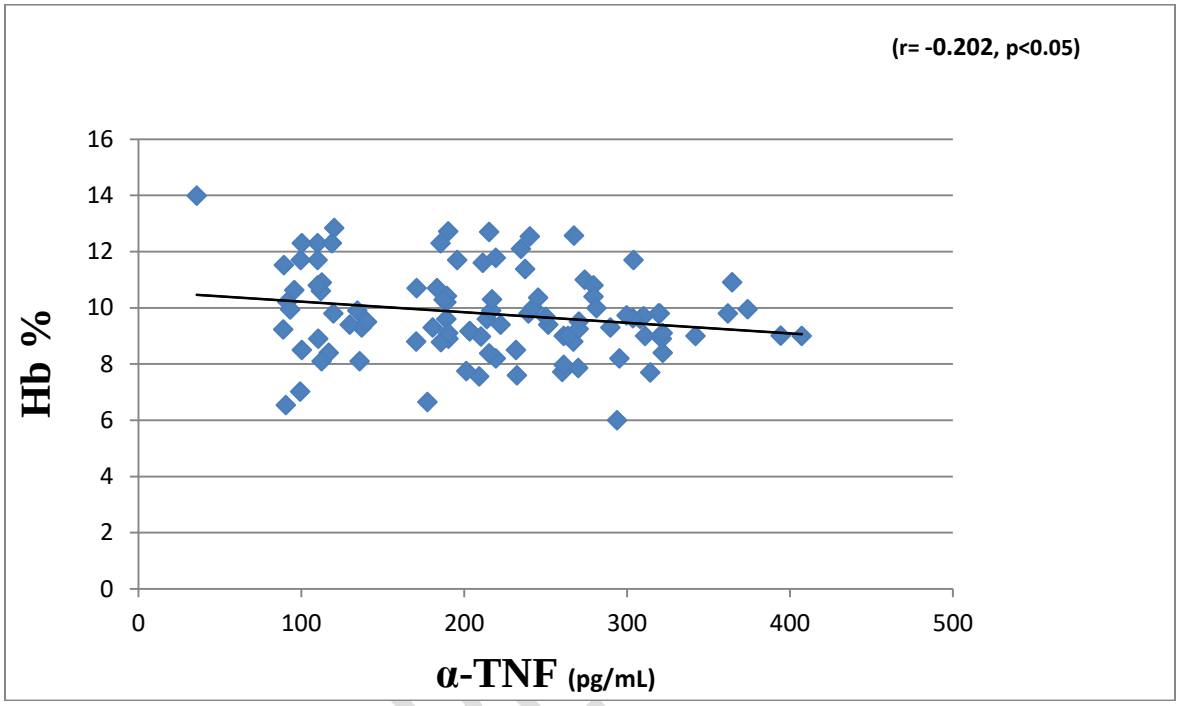
Table-2: Correlations between the Parameters in both groups

PARAMETERS		α-TNF r value	Hb r- value	EPO r-value
α-TNF (pg/mL)	Case	-		-0.257**
	Control	-	-	-0.358*

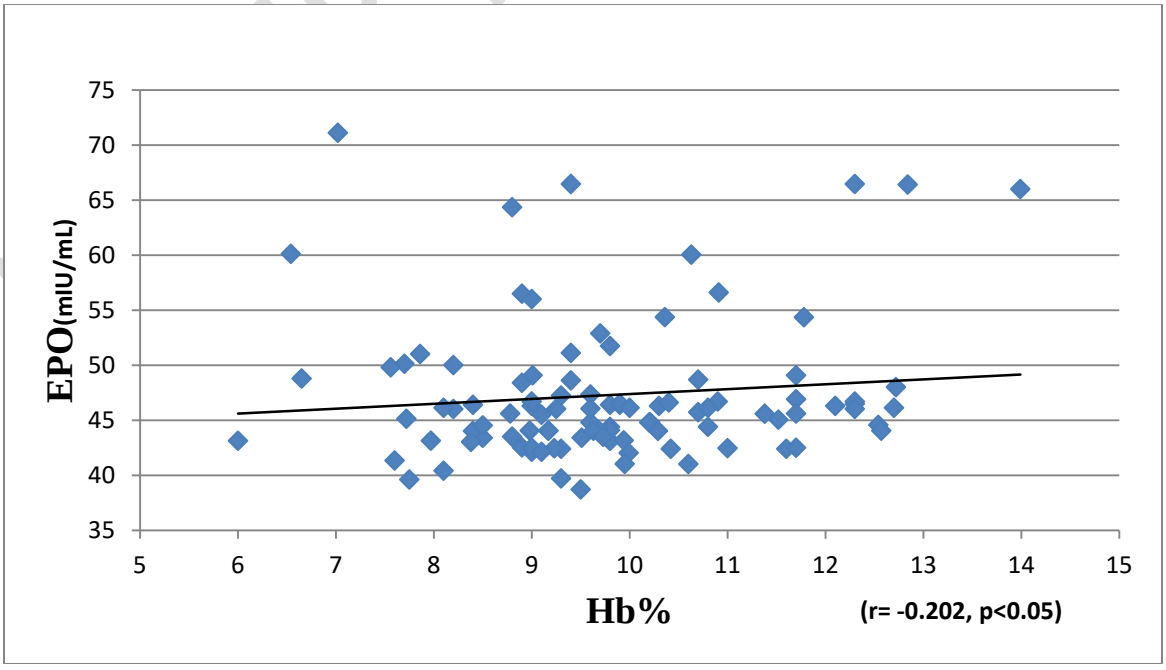
Hb(g/dl**)**	Case	-.202*	-	_
	Control	-0.288*	-	_
EPO (mIU/mL)	Case	-	0.106	-
	Control	-	0.027	-
* p<0.05, **p<0.001				



[Fig-1]: Correlation between α-TNF and EPO in anemic PTB group



[Fig-2]: Correlation between α-TNF and Hb in anemic PTB group



[Fig-3]: Correlation between EPO and Hb in anemic PTB group.

Discussion-

Anemia is one of the most common abnormality seen in TB patients caused by poor nutritional [10] status and increased cytokines levels due to inflammation.[11,28]In this study, we aimed to assess levels of α-TNF and EPO and their correlation in anemic and non-anemic pulmonary tuberculosis with same age matched criteria.

Present study showed statistically significant differences in α-TNF and EPO between both the groups($p<0.05$) shown in (Table-1). It was observed that α-TNF and EPO levels were higher in both the groups as compared to normal range in healthy individuals[2,8] also α-TNF levels were significantly higher in anemic PTB group as compared to control group ($p<0.001$, Table-1). PTB is a chronic disease which is correlated with increased cytokines such as α-TNF, which might be allied with anemia of chronic diseases. It may also be due to increased bacterial burden in case group compared to control group that α-TNF levels are significantly increased in cases.[2]

Chronic inflammatory diseases have also been associated with decreased EPO levels due to various mechanisms.[29] Chronic inflammation leads to increased expression of inflammatory cytokines like interleukin (IL-1) and α-TNF.[29] In chronic inflammatory disease like PTB, invading bacteria induces the production of TNF-α together with other inflammatory cytokines during the activation of T- lymphocytes and monocytes.[2] α-TNF inhibits EPO synthesis and interfers with differentiation and proliferation of erythroid progenitor cells.[2,30,31]α-TNF also induces the production of ROS[32,33] which again interferswith EPO producing cells and affecting

the binding affinity of EPO inducing transcription factors thus suppressing bonemarrow response to EPO.[8] Inverse correlation between TNF-α and EPO observed in this study in case group ($p<0.001$, Fig-2) can be explained by above reasons. Other authors like Kulkarni RA et al., and Bhat H et al., also observed similar relation between α-TNF with EPO in PTB cases.

In cases EPO levels were significantly lower as compared to control group in this study ($p<0.05$, Table-1). When compared with other studies containing healthy control group, it was observed that EPO levels were higher in both the groups of this study as compared to healthy controls of other studies.[2,8,15] Studies had also shown that in PTB, EPO levels generally increases as compared to healthy controls. As PTB is considered as chronic inflammatory disease, therefore increased EPO levels in both cases and controls can be attributed to chronic inflammation.[8,29] Also lower levels of EPO in case group as compared to control group of this study can be attributed to increased cytokine levels and bacterial burden in these subjects.[2]Bruno CM et al.[37] had observed increased EPO levels in anaemic as compared to non anaemic chronic inflammatory disease condition, but they have not explained any mechanism for such an observation.

Hemoglobin levels were taken as grouping variable in this study, hence statistical difference was observed among the groups. Decreased mean Hb levels in anemic PTB group because according to inclusion criteria Hb (males and female cases) and normal mean Hb levels in control group as compared to case group ($p<0.001$, Table-1) because of non anaemic nature(inclusion criteria of controls) of subjects.

TNF-α along with other cytokines also affects hemoglobin concentration in PTB cases.[8]Hemoglobin concentration is affected by iron concentration. TNF-α meddles with iron metabolism by efficiently withholding Iron from microbes as a protection against invading

microbial pathogens and stimulate the iron storage which reduced the iron supply for erythroid cells, leading to decreased hemoglobin concentration.[2,9] The inverse correlation between TNF-α and Hb concentration observed in both the groups ($p<0.05$, Table-2) in this study can be because of bacterial burden[2]and meddling effect of TNF-α with iron metabolism.[9]

EPO is a hormone responsible for erythropoesis[18]In normal healthy individuals[34] and iron deficiency anaemia[35] significant negative correlation is present between EPO and Hb concentration. In chronic inflammatory conditions, this correlation is disrupted and neither negative nor positive relation is seen.[2,8]In studies having PTB cases, no significant correlation is observed between the two because of the effect of cytokines and bacterial burden. In conjunction with other studies, present study also observed that there is no significant correlation between EPO and Hb ($p<0.05$, Fig-3). Thus, in this study we observed the subnormal Hb associated with the severity of cytokine α-TNF and their action of suppression of EPO which might be linked to underlying inflammatory mechanism and caused anemia in PTB.

Conclusion-The present study shows that anaemic or non anaemic PTB, both are associated with increased TNF- and decreased EPO and Hb, but the extent of inflammation i.e., TNF- levels are responsible for the development of anaemia. Thus, we conclude that though anaemia in PTB is a complication, but this complication can be reduced by reducing inflammation rather than focusing on treatment of anaemia, which will further increase the cost of treatment in PTB cases.

Limitation- Further comprehensive large sample size study would be needed to achieve firm conclusion.Restricted budget (cost of kits) and time, number of PTB subjects were restricted. In this study, we have not done follow-up of anemic and non-anemic PTB patients which could have been more enlightening in evaluating predictive applications of inflammatory cytokine and EPO.

Abbreviation- PTB: Pulmonary tuberculosis; Hb: Haemoglobin; RBC: Red blood cell; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; WBC: White blood cell; α -TNF: Tumour necrosis factor-alpha; EPO: Erythropoietin.

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