



INTERPLAY OF SERUM ERYTHROPOIETIN AND INFLAMMATORY CYTOKINE IN ANEMIC TUBERCULOSIS PATIENTS

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ABSTRACT

Introduction- As tuberculosis is a chronic infectious disease the anemia of inflammation may contribute significantly in pathophysiology of anemic tuberculosis patients so this study was carried out to find out the role of Tumor Necrosis Factor alpha (TNF α) and serum erythropoietin (EPO) in anemic tuberculosis Patients.

Material & Methods - The study comprised of sixty-eight anemic pulmonary tuberculosis patients. Levels of Hemoglobin (Hb), serum Iron, serum erythropoietin & serum TNF α were analyzed in all of them.

Result- The inflammatory parameter TNF α along with serum erythropoietin was found to be increased in anemic tuberculosis patient and levels of Hb, iron was decreased. Hemoglobin showed fair degree of linear relationship with TNF levels ($r = -0.273$). Hemoglobin and erythropoietin did not show linear or inverse correlation with each other ($r = 0.161$). Iron showed negative but non linear correlation with TNF & EPO levels ($r = -.128$ & -0.042). TNF and erythropoietin showed inverse but non linear correlation with each other ($r = -0.100$).

Conclusion - Insufficient response of erythropoietin to the degree of anemia was found and an increased level of tumor necrosis factor was probably the contributing factor in induction of anemia in tuberculosis patients.

Key word- Anemia of chronic diseases, erythropoietin, Tumor Necrosis Factor alpha

INTRODUCTION

Active tuberculosis infection is generally associated with major abnormalities in hemopoiesis.¹ Anemia is a common complication of pulmonary tuberculosis, the reported prevalence ranging from 16 to 76% in different studies.² The precise mechanism of anemia in pulmonary tuberculosis patients (PTB) is not clearly known however, anemia due to inflammation & iron deficiency have been implicated.^{3,4} As tuberculosis is a chronic infectious disease,

anemia of inflammation may contribute significantly in pathophysiology.⁵

The pathologic processes involved in Anemia of inflammation /Chronic Diseases(ACD) are, shortened erythrocyte survival, failure of the bone marrow to increase red blood cell (RBC) production and impaired release of iron from the reticuloendothelial system.⁶ The supply of erythropoietin (EPO) to the marrow might be the rate-limiting factor in the impaired marrow response to ACD.⁴ Erythropoietin is a 30.4 kD glycoprotein. The level of EPO

in the plasma ultimately influences the rate of production of new erythrocytes by the bone marrow. Failure to increase the amount of circulating EPO in response to hypoxic stress can lead to anemia.⁷ The insufficient endogenous erythropoietin (EPO) production is probably one of the pathogenic mechanisms of the anemia of chronic disease (ACD). Cytokine Tumor Necrosis Factor alpha (TNF α) is believed to play multiple roles in the immune and pathological responses in tuberculosis.⁸ The elevated plasma levels of inflammatory cytokines, particularly TNF- α , are implicated in the EPO resistance process and have been shown to interfere with the peripheral actions of EPO.⁹

Tuberculosis is one of the major chronic diseases that are prevalent in India. Mild to moderate anemia is associated with tuberculosis, however, iron and other haematonic supplements can initiate an initial improvement in some haematological indices in tuberculosis patients but ultimate recovery from anemia occurs only after complete cure from tuberculosis. Thus, the present study was carried out with an objective to find out the effect of inflammatory cytokine Tumor Necrosis Factor alpha on the serum erythropoietin levels in anemic tuberculosis patients.

MATERIAL & METHOD

This Study was designed to estimate the serum levels of tumor necrosis factor alpha, erythropoietin, iron along with haemogram of each anemic tuberculosis patient and to evaluate their correlation with each other. The study included newly diagnosed sputum positive anemic tuberculosis patients that were coming during the study period of one year. Patients (68) who were registered during the study period and who were fulfilling the inclusion criteria were selected in the study. Patients of pulmonary tuberculosis were taken from Manorama Raje Tuberculosis Hospital & DOTS Centre, Indore. Pulmonary tuberculosis patient aged between 18-55 yrs & those who have hemoglobin (Hb) less than <13 g/dL for Men & HB <12 g/dL (for non-

pregnant) Women were included. Thirty-two healthy controls were selected from staff members & volunteers Tuberculosis patient with other disease or disorder and patients with incomplete data records, Patients with history of prior anti-TB treatment and moderate to severe injury or surgery during the previous month were excluded. Patient with extra pulmonary involvement, blood donors, subjects with history of smoking and who had taken iron supplementation were excluded. The study was approved by the Institutional & Human Ethical Committee of the Sri Aurobindo Institute of Medical sciences, @ Indore. Informed consent was taken from each subject, and then they were enrolled in the study.

Fasting venous blood samples (5ml) were drawn from subjects, each sample was divided into two parts: the first part was collected in EDTA while the second sample was collected in a plain tube and left to clot. Serum was obtained after centrifuge. The serum samples were aliquoted and stored according to the instructions in the kits, until the various assays described below were performed

Haemogram was estimated by automated cell counter. (Sysmex KX-21). Serum iron was estimated on an autoanalyser from Rocche diagnostics using commercial test kits from Randox Laboratories, UK. Serum TNF α was estimated by using a commercially available immunoassay Kit from DIACLONE, United Kingdom. Serum erythropoietin was estimated by two-site immunoenzymatic determination by using kit acquired from DRG International Inc. USA

SPSS Version 10 was used for statistical assessments to evaluate mean levels of variables between study groups and healthy controls by Unpaired t-test. Correlations were calculated by Pearson's correlation coefficients (two-tailed). P value \leq 0.05 was used as a threshold of significance.

RESULT

Table 1: Mean \pm SD values for hemoglobin, iron, inflammatory cytokine TNF & erythropoietin in anemic tuberculosis patient group

Parameter	Control (n= 32) (Mean \pm SD)	Anemic TB patient (n=68) (Mean \pm SD)	P value
Hb (g/dl)	14.0 \pm 1.53	9.08 \pm 1.34	p<0.001
RBC (millions/ μ L)	5.14 \pm 0.59	4.07 \pm 0.62	p<0.001
PCV (%)	41.1 \pm 3.08	28.1 \pm 4.16	p<0.001
MCH (Pg/cell)	27.6 \pm 3.65	22.9 \pm 5.54	p<0.001
MCV (fl)	80.5 \pm 6.65	69.6 \pm 9.43	p<0.001
MCHC (g/dl)	35.0 \pm 2.53	32.9 \pm 6.55	P< 0.05
Iron (μ mol/l)	16.12 \pm 4.47	7.38 \pm 1.79	p<0.001
EPO Mu/ml	24.40 \pm 9.72	34.25 \pm 11.4	p<0.001
TNF α (pg/ml)	12.46 \pm 7.03	57.55 \pm 14.7	p<0.001

p<0.001 & p>0.05= significant

The mean level of hematological values (Hemoglobin, PCV, RBC, MCV, MCH and MCHC) and serum iron in anemic tuberculosis patients were found to be lower when compared to controls. The levels of TNF and erythropoietin were increased in anemic tuberculosis patients than healthy controls. (Table 1)

Table 2: Correlations between the Parameters

Parameters	IRON r value	TNF r value	EPO r value
Hb (g/dl)	0.179	-0.273 *	0.161
IRON ($\mu\text{mol/l}$)	-	-0.128	-0.042
EPO	-	-0.100	-

r is correlation coefficient . r * fair degree of linear relationship

Table 2 shows that Hemoglobin showed fair degree of linear relationship with TNF levels and this correlation was negative. Haemoglobin and erythropoietin did not show linear and inverse correlation with each other. Iron showed negative but not linear correlation with TNF & EPO levels. TNF and erythropoietin showed inverse but not linear correlation.

DISCUSSION

Pulmonary tuberculosis is occurring predominantly in socio-economically deprived populations. Therefore, both anemias of inflammation as well as of iron deficiency may coexist in pulmonary tuberculosis.⁴ Hematological abnormalities are associated with tuberculosis.^{10,11} The one of the possible mechanisms may be due to the cytokine-mediated blunted response of erythropoietin and found an inadequate erythropoietin formation for the degree of anemia in patients with anemia of chronic diseases.¹² The levels of erythropoietin in present study also found to be insufficient in comparison to anemic status of patients. Tuberculosis itself is a chronic disease with increased level of Tumor necrosis factor which might be associated with anemia; it may be one of the factors along with nutritional deficiency that was responsible for subnormal hematological values. Increased levels of TNF α & decreased Hb and serum iron found in present study & their correlation with each other was in accordance with the above finding. Cytokine-mediated defense against microbial pathogens caused effectively withholding of iron from microbes, which incidentally also deprive erythroid precursors of their iron supply.¹³ The development of hypoferrremia thus results in the development of iron-restricted erythropoiesis and anemia.⁷

Endogenous TNF α response during inflammation is a sufficient proximal stimulus to mediate an associated anemia with decreased RBC survival & impaired erythropoiesis.¹⁴ EPO controls bone marrow erythroid cell proliferation, differentiation, and survival through its binding to an erythroid progenitor cell surface (EPOR). The burst-forming unit erythroid (BFU-E), & colony-forming unit erythroid (CFU-E) requires EPO for survival and proliferation.¹⁵ The cytokines interleukin-1 and TNF α directly inhibit erythropoietin expression in vitro, which is probably due to cytokine-mediated formation of reactive oxygen species, which in turn affects the binding affinities of erythropoietin- inducing transcription factors and also damages erythropoietin-producing cells.¹⁶ Inflammatory cytokines are mainly responsible for causing anemia through various roots like hypoferrmia and blunted erythropoietin response etc. Though the correlation between decreased erythropoietin and increased TNF levels was not significant in present study the blunted response to erythropoietin, levels were found and it might be due to hypoferrremia and underlined diseased condition.

CONCLUSION

Insufficient response of erythropoietin to the degree of anemia was found and an increased level of tumor necrosis factor is probably the contributing factor in induction of anemia in tuberculosis patients.

Limitation and Recommendation of the study

As there were constraints regarding time and budget (cost of kits) the number of tuberculosis patients and healthy individuals taken, as controls were restricted. More prospective studies with a larger sample size are needed to determine the role of inflammatory cytokine on erythropoietin levels.

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