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Levels of erythropoietin Iron and ferritin in anemic pulmonary tuberculosis: A comparative study

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ABSTRACT

Background: - Tuberculosis is the 10th leading cause of deaths worldwide. In chronic infectious diseases inflammation is common rule and pulmonary tuberculosis (PTB) is one of them. Innate and adaptive immune system of host activates immune system specially T-cells which released cytokines. Cytokines altered iron homeostasis and reduced erythropoietin production which play a vital role in Patho-physiology of anaemia.

Methods: The present study was an observational type of case control study. This study included 50 sputum Acid-Fast Bacilli (AFB) confirmed newly diagnosed pulmonary tuberculosis anemic PTB subjects as case and 50 sputum AFB confirmed newly diagnosed pulmonary tuberculosis non-anemic PTB subjects as controls. Serum EPO were analyzed by ELISA method and serum Iron were observed by ferrozine method in both groups.

Results: Significantly lower levels of EPO (47.28 ± 6.40) were observed in anemic PTB cases than that of non-anemic PTB cases ($p<0.001$). Iron profile was also found significantly lower in anemic PTB group (24.85 ± 9.32) than non-anemic PTB group.

Conclusion: The dynamic changes in iron status, Erythroferrone and EPO during resolution of acute inflammation of anemia caused by tuberculosis

Keywords: Cytokine, Anemia, Inflammation, Pulmonary tuberculosis and Erythropoietin

Introduction:

Pulmonary tuberculosis is an infectious disease, where inflammation is a common rule. But the level of inflammation and extent of infection will reflect the severity of the disease. According to WHO estimates, the highest incidence of tuberculosis is recorded in highly populous countries and the majority of these inhabit Asia including India, and, around 48% of new cases every year are reported from these countries [(1)] Tuberculosis has many co-morbidities and anemia is one of the common comorbiditie of tuberculosis. One of the main reasons for the development of anemia is malnutrition. One of the common types of anemia is iron deficiency anemia, as iron is irreplaceable in the transport of oxygen as part of hemoglobin and red blood cells. Previously it was estimated that from 32 to 86% of diagnosed tuberculosis cases have anemia as a comorbidity. Social factors, dietary and lifestyle habits may contribute to tuberculosis infection and also to the development of anemia. [(2)] In mycobacterium pathogenesis innate and adaptive immune system of host is activated while activated macrophages increased the production of cytokines which reduced the erythropoietin production [(3)] by bone marrow suppression [(4)] and free radical formation [(5)].

Globally in developed countries common nutritional deficiency is iron deficiency anemia (IDA) [(6)]. Causes of IDA could be poor iron absorption, increase blood volume, inflammation in infectious disease [(7)] and blood loses like tuberculosis [(8)]. Both inflammatory anemia and IDA may coexist in PTB patients. [(9)] The purpose of selecting iron parameters is by looking into the importance of iron for the host as well as mycobacterium tuberculosis, as this trace element influences both acquired and innate immune response and is required in the replication process of bacteria. [(10)] In inflammatory disease like PTB cytokines modified iron homeostasis by withholding the serum iron from microbes which in some way influence the erythropoiesis because erythropoiesis process required iron for the maturation of RBCs [(4,9)].

Materials and Methods

The present study was an observational case control study, has conducted in the Department of Biochemistry, SBKSMI & RC, Dhiraj Hospital, Sumandeep Vidyapeeth deemed to be University, Vadodara, Gujarat with collaboration of GS Medical College, Hapur, Uttar Pradesh in the duration of June 2019-July 2020 after obtaining an Ethical Approval from both institutional Ethical committee (GSIEC) (GSMCH/2019/IEC/Approval/019) and Sumandeep Vidyapeeth Institutional Ethical Committee(SVIEC/ON/MED/PhD/19029). In this study total 100 sputum AFB confirmed pulmonary tuberculosis subject of either gender was enrolled. Out of 100 subjects, 50 were anemic PTB subjects (case group) and 50 were non anemic PTB subjects (control group). Anemic and non-anemic status was confirmed on the basis of HB levels as per WHO guidelines [(11)]. A hemoglobin (HB) levels for females is ≤ 12 g/dL and for males ≤13 g/dL considered as anemic PTB group and hemoglobin (HB) levels for females ≥12 g/dL and for males ≥ 13 g/dl has considered as non-anemic PTB subjects. Subjects with previous history of TB, inflammatory disease, cardiovascular disease, HIV, cancer and pregnant women were excluded from this study.

Sample collection and processing: Under the aseptic precautions 6 ml venous blood was collected from total subjects, sample was separated into two parts: 3 ml in plain tube and 3ml in EDTA. Plain tube was separated by centrifugation process at 3000 RPM for 10 min to get serum

for analysis of erythropoietin (EPO) and serum Iron. EDTA was used for the HB estimation. Serum Iron were estimated by Ferrozine method by autoanalyzer and Serum EPO (Catalogue no-DE3646) was estimated by sandwich ELISA method and levels of hemoglobin was measured by flow cytometry method by automated cell counter Nihon Kohden (3-part cell counter) at Central Lab Dhiraj Hospital, Sumandeep Vidyapeeth deemed to be University Vadodara, Gujrat, India after ensuring quality control.

Statistical Analysis:

The collected data in the case requestion form (CRF) were analyzed with by using SPSS software version 20.0. version. Data was presented in the form of mean ± SD. Statistical significance of results evaluate by student independent sample t-test. The p-value <0.05 was considered as statistically significant.

Results-

In this study 50 newly diagnosed sputum AFB positive anemic PTB subjects and 50 were non-anemic PTB group of age group of 18-70 years were enrolled. The mean age was 42.0±14.5 year of anemic PTB and 50 42.98±14.21 non-anemic PTB group respectively. The mean level of Respiratory Rate was higher in anemic PTB cases (21.44±2.38) compared to non-anemic PTB controls (19.36± 2.8) (p<0.001) [Table-1]. The mean level of BMI was lower in anemic PTB cases (16.62 ± 2.29) compared to non-anemic PTB controls (20.87 ± 3.11) (p<0.001) [Table-1]. 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]. Serum EPO was observed to be significantly decreased (47.28 ± 6.40) in case group and higher (58.44 ± 14.97) in control group(p<0.001)

Table-1:

Parameter	Anemic PTB (n=50)	Non-Anemic PTB (n=50)	p= value
Systolic	109.19 ±10.18	113.52 ± 8.08	<0.01
Diastolic	78.10 ± 8.72	75.30 ± 7.85	<0.05
Respiratory Rate (beats/minute)	21.44±2.38	19.36± 2.8	<0.0001
BMI (Kg/m2)	17.62 ± 2.29	21.87 ± 3.11	<0.001
Hb(g/dl)	9.78±1.53	13.13± 0.63	<0.0001
EPO (mIU/ml)	49.28 ± 5.40	59.24 ± 12.87	<0.001
Iron(µg/dl)	23.85 ± 6.98	36.86 ± 13.59	<0.001
Ferritin (ng/dl)	443.41 ± 89.01	302.67 ± 98.00	<0.001
Erythroferrone (ng/ml)	25.1 ± 89.01	16.45 ± 89.01	<0.001

P < 0.0001 highly significant, P< 0.05* significant & P> 0.05 # not significant***

Discussion

With the advent of twentieth century, the health priorities of resourceful countries have been resolutely focused on dealing with killer infectious diseases, particularly such as tuberculosis (TB). Dealing with tuberculosis is a challenging task as its co-morbidities are more prevalent with communicable. [(12)] Macrophages as effector cells and lymphocytes (especially T cells) as the immuno-responsive cellular counterparts are behind cell mediated immunity. There is a plethora of diseases which are entirely controlled by cell mediated immunity. In this perspective, tuberculosis emerges as a disease of prime concern with its distribution at a global scale [(13)] 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. [(14)] Anemia is one of the most common complications associated with tuberculosis due to chronic inflammation [(15)]. Anemia is a common co-morbidity at tuberculosis diagnosis, with previous estimates ranging from 32% to 86%. [(16)]. Tuberculosis and malnutrition both are considerable magnitude in developed and developing countries like India. Malnutrition is mainly associated with an increased severity, frequency of diseases and fatality of infection such as tuberculosis. Malnutrition is the most widely preventable risk factors for tuberculosis [(17)]. In present study also BMI is lower in anemic PTB group while higher in non-anemic PTB group.

Out of total subjects there was higher percentage of both males and females of pulmonary tuberculosis subjects with anemia. There must be some social factors, dietary and lifestyle habits. In our study we have observed a significant difference (<0.001) between weight (kg) between pulmonary tuberculosis with anemia patients compared to non-anemic pulmonary tuberculosis patients.

Several researchers have established that malnutrition plays crucial role in the development of anemia leading to pulmonary tuberculosis, [(18,19)] In our study, Body Mass Index is much lower than the designated normal values in both the groups and between both the groups the patients of pulmonary tuberculosis patients with anemia the values are much lower.

Respiratory Rate (beats/minute) in non-anemic pulmonary tuberculosis and anemic pulmonary tuberculosis patients was 19.36+2.8 and 21.44+2.38 and difference is significant (<0.0001), higher RR in anemic PTB group clearly indicate the effect of presence of anemia in the patients of pulmonary tuberculosis as the pulmonary function is compromised in the patients of tuberculosis with anemia.

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 [(20)], loss of appetite and fever due to nutritional deficiency [(4)] blood loss due to hemoptysis [(20)]. Therefore, both anemia due to chronic inflammation and iron deficiency anemia may coexist in patients of PTB [(9)] We have analyzed serum iron; the serum iron levels are 24.85+9.32 ug/dl and 33.46+12.69 ug/dl in anemic pulmonary tuberculosis and non-anemic pulmonary tuberculosis patients respectively. Our results indicate there is a limitation in the availability of iron for bacteria in the pulmonary tuberculosis patients with anemia. Erythropoietin stimulates erythropoiesis by acting on the bone marrow. The mean serum levels of erythropoietin

were 47.28+6.40 and 58.44+ 14.97 (mIU/ml) in pulmonary tuberculosis subject anemia and non-anemic pulmonary tuberculosis subjects respectively. Failure to increase the amount of circulating erythropoietin in response to hypoxic stress can lead to anemia. It implies that insufficient endogenous erythropoietin production is one of the pathogenic mechanisms of the anemia of chronic disease.

Some modification in the metabolism of Iron because of reactive oxygen and free radical generation by increased cytokines levels, which affect the RBC production or erythropoiesis and altered the Iron homeostasis by iron regulatory proteins (IRPs) [(9)]. Even though cytokines withhold the iron from microbes [(9,21)] and relocate the iron form into ferritin incorporated form which might be a factor of decreased serum iron and increased serum ferritin in pulmonary tuberculosis. Erythroferrone (ERFE) is the main erythroid regulator of hepcidin, the homeostatic hormone controlling plasma iron levels and total body iron. Present study shows significantly higher levels in in anemic PTB then non-anemic PTB [(22)].

Conclusion-

our study documents the dynamic changes in iron status, Erythroferrone and EPO during resolution of anemia of inflammation caused by tuberculosis. Our findings may help inform evidence-based guidelines for rational iron treatment of tuberculosis-related anemia. However, further clinical trials are needed to determine the risks and benefits, and optimal timing of iron supplementation in tuberculosis patients with both anemia of inflammation and iron deficiency anemia.

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