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Hemotological Study on Pulmonary Tuberculosis (PTB) Predominance in Quetta.

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Abstract

Pulmonary tuberculosis (PTB) is an infectious disease and a major health problem in the world, especially Pakistan and South Asian countries. In 1993, tuberculosis was declared a worldwide emergency by WHO. It is a respiratory compelling contagious infection caused by a bacillus (*Mycobacterium tuberculosis*). TB is a treatable infirmity. Tuberculosis is a disease that generally attacks the lungs yet can also ambush any other part of the body. It spreads from individual to individual through the air. The treatment of TB is commonly completed in 6 to 9 months. Inoculations are open for TB which is BCG counter acting agent. In most cases, specially in Balochistan, patients carelessly quit taking drugs after two months as the torment and hacking clear out. It is notable that pulmonary tuberculosis is a serious threat specially in this region, so it is necessary to aware the people regarding TB also their treatment & control. The present study was conducted in the TB Sanatorium Quetta. A total of 100 sputum samples were collected from PTB patients, 43.75% and 56.25% tuberculosis prevalence was found both in males and females respectively with age group of 28-54 years with highest prevalence rate. Later, all the patients were analyzed for hematological profile and statistically significant difference was observered in parameters; neutophils, platelets and hemoglobin but there was non significant difference observed between mean values of age groups and gander with pulmonary tuberculosis in PTB positive subjects.

Keywords: *Mycobacterium Tuberculosis, Prevalence, Pulmonary Tuberculosis, Anemia*

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INTRODUCTION

The long-lasting distressing disease tuberculosis which is produced by the *Mycobacterium tuberculosis* is present worldwide especially in the unindustrialized countries including Pakistan (1). Presently almost 2 billion people are infected by tuberculosis. Collectively almost 1.8 million deaths occur due to this disease and it is almost equal to 4,500 deaths per day and around 48% deaths occur in over populated countries which include Indonesia, Pakistan, Bangladesh and China (1, 2). Tuberculosis is considered as the infectious disease which mostly affect the lungs but other parts of the body like organs and tissues also get affected by it (3, 4).

By inhaling the droplet nuclei of *M. tuberculosis*, the TB infection is attained and nearly 10% of infected people suffer from this disease (5). In pulmonary tuberculosis and non-pulmonary infections biochemical and hematological abcalulatedities play a vital role in diagnosis like fluctuating amount of white blood cells along with neutrophil and lymphopenia indicate the infection within the body. The decreased amount of hemoglobin indicates the anemic situation in the person and the number of platelets decrease as compared to the control samples. Sometimes it becomes difficult to diagnose the pulmonary tuberculosis from the hematological parameters because of the similarities between the hematological anomalies and of TB patient and other infectious diseases. While it is observed that hematological Table headings are in Arial font using size 8 with table number abcalulatedities can also be varied due to the topographical and environmental positions (6).

For protection of the pulmonary tuberculosis the commonly available vaccine present in the market which is (Bacille Calmette Guerin) BCG fails to secure the adults from this dreadful disease (7).

Multiple Drug Resistance (MDR) patients of tuberculosis get their treatment in combination of 4 to 6 medications in the form of therapy which is known as the 2nd line drug therapy which is less effective, but much toxic and this therapy has been given up to 2 years. MDR TB drugs have low mortality rate as compared to that of drug susceptible tuberculosis even the MDR drugs are more effective (8). There are many anti-TB drugs available in the market to cure the tuberculosis but the use of anti-TB drugs have some side effects like vomiting, rashes, fever, allergic reactions, sudden weight loss and hepatocellular inflammation etc. (9). Ethambutol, rifampicin, pyrazinamide, isoniazid is the antituberculotic drugs presently used to control the tuberculosis (1,10).

The effector cell macrophages,considered as the most important cell of the immune system, are attacked by the tuberculosis as it provides suitable habitat to them. The hematological and immunological parameters are important to investigate at the bottom line of the anti-TB treatment to provide the effective treatment to the patients (1). The significant effector cells, macrophages, present in the immune system targeted by the *M. tuberculosis* as it provides the favored habitation. To effectively regulate the pathogens numerous

diverse types of t-cell population is essential. Long term perseverance of *M. tuberculosis* is due to this effective defense system (1, 3). Other factors like innate immunity or the deficiencies in cell mediated immunity are responsible for causing the disease. Many hematological distortions like Hodgkin's disease, chronic myelogenous leukemia and multiple myeloma are linked directly or indirectly with the tuberculosis (11).

About 44% of the total country area is covered by the Balochistan and it is the largest province of Pakistan. The population of Balochistan is mostly dispersed into the remote areas. Regardless of the directly observed treatments (DOTs) programmed planned in the province, in 2012, roughly 20,000 new cases of tuberculosis were noted. By considering the above increasing trend, this study was designed to observe the risk factors and the changes in the hematological profile in the TB patients after the completion of therapy (12).

Methodology of Study

Patients selected for this study were grouped into three categories according to the age (i.e. 18-35, 36-50 and 51-75 years respectively). Two sputum samples were received from each patient; one before breakfast (fasting) and one after breakfast. The patients were advised to cough deeply into a sterile container at a well-ventilated atmosphere and keep away from people. Then the samples were immediately registered and labelled with lab number, name, age and gender respectively and then stored in a dry and cool place.

A well cleaned, new slide free from grease was labeled with patients' lab number using a sterile wire loop. These samples were smeared on glass slides and then stained them using Ziel Nelson stain (ZN stain) and later examined the smears under microscope using 100 x objectives.

The RBCs, WBCs and platelets were measured by direct current (DC) detection method. Hemoglobin was measured by SLS-Hb (sodium lauryl sulfate-hemoglobin) method. Erythrocyte sedimentation rate (ESR) was performed for the screening of active disease (ESR was measured using Westergren Method) in mm/hr. Comparison of hematological profiles of TB patients and healthy subjects was also performed.

RESULTS AND DISCUSSION

A total of 100 (44 male and 56 female) samples were collected from the known cases of pulmonary tuberculosis visiting Fatima Jinnah General & Chest Hospital, Quetta to evaluate the hematological profile of tuberculosis (TB) and control (healthy) subjects in Quetta, Pakistan. However, patients suffering from hepatitis (any type) before the diagnosis of PTB, having any type of tuberculosis other than PTB, insufficient medical records for analysis, chronic renal failure clinical history, pregnancy, hemoglobinopathy, neoplastic diseases and collagen vascular disease were excluded from the study.

The patients included in the study were belonged to 5 major ethnic groups i.e. Punjabi (11%), Pashtoon (24%), Baloch (47%), Persians (10%) and others like Sindhi, Hindko, Sariki etc. (8%).

Among all age groups the study subjects (TB patients; n=100) were positive for acid fast bacilli while the control subjects (healthy; n=50) were negative for acid fast bacilli.

Range of hemoglobin according to gender

The mean hemoglobin level was 10.18g/dl the mean hemoglobin level in males was 11.38 g/dl (maximum range was 15.43g/dl and minimum was 7.43g/dm). In female the mean hemoglobin level was 9.9g/dl (maximum range was 13.9g/dl and minimum was 6.8g/dl. In 30% patients level of Hb was < 9.9 gm/dl. The overall anemia was observed in 71% patients. (Table I). In case of hematocrit, the mean value was 29.5% (maximum range was 40.8 and minimum was 30.1) The mean value was 33.6 in males and 31.7 in females. In 29% cases hematocrit value was less than 31%. Thus, both values (hemoglobin and hematocrit) were correlated almost in 31% of cases in PTB. Comparison of hematological profiles of TB patients and healthy subjects was also performed (Table II).

Table I. Gender wise distribution of hemoglobin

Hemoglobin (gm/dL)	Male		Female		Total	
	No.	%	No.	%	No.	%
<11	5	17.2	22	52.4	27	38
11-17	21	72.4	16	38.1	37	52
>17	3	10.3	4	9.5	7	9.8
Total	29	100	42	100	71	10

According to the WHO recommendations for anemia, concentration of hemoglobin less than 12.5 g/dl and 13.5 g/l in females and males respectively was used in the present study. Anemia of different types was observed in 69 subjects (the types of the anemia were based on smear findings of MCH, MCV and peripheral).

The patients suffering from pulmonary tuberculosis show less information about hematologic abnormalities in Asian population. Therefore, the researchers are trying to study hematological profile in pulmonary tuberculosis. Our results correlate with the previous studies where the occurrence of anemia was normocytic normochromic, the most common form (16-18). Blunted erythropoietic response of bone marrow was observed by Singh et al., 2001 while studies bone marrow (16). Other authors also reported the same type of anemia in untreated tuberculosis. These studies showed that activated monocytes release cytokines and tumor necrosis factor-a (TNF-a) which suppress the production of erythropoietin which further leads to anemia (16). The most common type normocytic normochromic anemia was reported in this study, however macrocytic and microcytic types of anemia were also found. The results showing Macrocytic blood picture was as similar to the studies of Morris et al. (18). In other studies, elevated serum ferritin was found in all patients, because of its performance as an acute phase reactant (18).

Leucocyte response may differ from leukocytosis to pancytopenia. Mild leukocytosis was reported in few patients suffering from pulmonary tuberculosis (19). The percentage of leucocytes currently present is similar to the percentage reported in Singh KJ et al, studies. Neutrophilia was present in all the patients with leukocytosis and monocytosis in just three patients. Even though, changes have been reported in relative number of lymphocytes, monocyte and polymorphonuclear leucocytes, these had not proved useful either as clinical or prognostic value (20). Leucopenia percentage on pulmonary tuberculosis is 1-4%, our results are not in agreement of these studies. The major finding in these patients was neutropenia. Numerous pathophysiological mechanisms associated with neutropenia are not well identified. Though, it is a consequence of the combined effect of hypersplenism, excessive margination of neutrophil or marrow granulopoietic failure mediated by the T- lymphocyte showing granulopoietic inhibitor activity. Several authors stated lymphocytopenia in pulmonary tuberculosis patients. Still, the exact reason for lymphocytopenia development has not been explained. However, it was suggested to study the function of cytokine including TNF in the pathogenesis of lymphocytopenia (16). Some previous studies reported pancytopenia in miliary tuberculosis patients and rare

in pulmonary tuberculosis patients (21). In present study no case of pancytopenia was observed. Another study a case of pancytopenia was reported in patient with tuberculosis (22). Normal maturation and normocellular was detected of all three series of bone marrow cells and a major development in all parameters after anti-tuberculous treatment was observed (22).

Table II. Comparison of hematological profiles of TB patients and Healthy subjects.

Hematological parameter	PTB Mean ± SD	Control Mean ± SD	*P* value
WBC x 10^3/ µl	6.9 ± 2.8	6.9 ± 3.2	0.529
RBC x 10^6/µl	5.1 ± 0.72	4.12 ± 0.72	0 .108
Hb (g/dl)	11.0 ± 2.9	10.8 ± 2.4	0.049
HCT (%)	38.3 ± 8.0	36.1 ± 6.1	0.114
MCV (fl)	91.7 ± 5	90.7 ± 4.3	0.056
MCH (pg)	29.3 ± 4.6	29.8 ± 5.5	0.612
MCHC (%)	34.2 ± 3.8	33.5 ± 3.2	0.282
Platelets x 10^3/µl	401 ± 149	257 ± 132	0.007
Lymphocyte	2.0 ± 0.2	1.6 ± 0.4	0.273
Neutrophil	5.1 ± 0.5	3.9 ± 0.5	<0.001
ESR (mm/hr)	69.2 ± 29.9	66.7 ± 28.6	0.827

*Independent t-test was applied to derive the *P* values (<0.05 was significant difference); significant difference was observed in neutophils, platelets and hemoglobin.

Splenomegaly in tuberculosis patients having pancytopenia (16). Immune mediated bone marrow suppression, a decreased bone marrow reserve and splenic sequestration are the mechanisms for pancytopenia. The identification of pancytopenia or thrombocytopenia with tuberculosis might be increases the chances of drug toxicity or another underlying process which entails further evaluation. Morris et al, observed thrombocytosis in half of the patients with military tuberculosis. Singh KJ et al, found more than 1/4 patients with thrombocytosis and 26% was reported in recent studies.

In tuberculosis, many cytokines, mediators and inflammatory cells are responsible for the development of granulomatous lesions. Interleukin-6 (IL-6) helps in the production of platelets (23). Recently, as platelets have been considered as immune cell, the characteristic morphologic features of platelets with higher PDW and MPV values in tuberculosis may reflect an activated platelet as observed for other immune cells.

The Erythrocyte sedimentation rate (ESR) is a type of blood test which indirectly measures the infection in a body (24). This test is not very useful because it rarely shows disease in asymptomatic patients. The disease is detected when ESR increases, a physical examination and history of patient discloses the main cause of the disease. Various studies reported that ESR plays an important role in pulmonary tuberculosis as it provides exact and reliable information about the development and regression of tuberculous lesion. Previously, it was reported that the patients with negative sputum have decreased level of elevated ESR and 99% patients have increased ESR level. Children with symptomatic tuberculosis had higher ESR values than asymptomatic children and similarly, children with positive culture for tuberculosis had significantly higher ESR values than children with negative tuberculosis cultures (25). However, there was a large range of individual values with considerable overlap, making it difficult to see how individual patient values could be useful in either diagnosing or excluding tuberculosis. In conclusion, Al-Marri MR and Kirkpatrick MB stated that the ESR value is to be expected less or no diagnostic value in the diagnosis of childhood tuberculosis (25).

Table III. Percentage of paients lying in different age, ethinic and literacy groups.

Characters Age Groups (Years)	Total n=100 Patient with %
18-35	23%
36-50	49%
51-75	28%
>75	Nil
Gender	
Male	43%
Female	57%
Marital status	
Married	73%
Unmarried	27%
Ethnicity	
Punjabi	11%
Pashtoon	24%
Baloch	47%
Persians	10%
Others	8%
Education	
Literate	29%
Illiterate	71%
Employment	
Employed	51%
Unemployed	49%
Income per month	
>25000	81%
<25000	19%
Socioeconomical status	
High	1%
Middle	48%
Low	51%
Crowding index	
>5 person/ house	43%
1-5 person /house	57%
Smoking habit	67%
Anemic patients	
Male	29%
Female	42%

CONCLUSION

The decreased amount of hemoglobin indicates the anemic situation in the person and the number of platelets decrease as compared to the control samples. Sometimes it become difficult to diagnose the pulmonary tuberculosis from the hematological parameters because of the similarities between the hematological anomalies and of TB patient and other infectious diseases.

Various hematological abnormalities has been reported in current study which observed in one hundred patients suffering from pulmonary tuberculosis. A comprehensive hemogram was performed in sputum smear positive patients for AFB using automated cell counter. Anemia was a common complecation in the patients having pulmonary tuberculosis (n=71), while the most common type of anemia was normocytic normochromic. Leucocytosis was seen in 31%. Leucopenia with neutropenia and lymphocytopenia was occured in 4%. Thrombocytosis was observed in 23%. Most of the

findings were persistent with published studies and support the fact that they could be important tools in observing such as anemia and increased ESR. Moreover, statistically significant difference was observered in parameters; neutophils, platelets and hemoglobin, whereas no significant differences were observed among the parameters WBC, RBC, lymphocytes, thrombocytes, monocytes and ESR by comparing hematological profiles of TB patients and healthy subjects. Other findings such as thrombocytosis and pancytopenia recommend the prime need for further studies.

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