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 abcalculatedities 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 abcalculatedities 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 persistence 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 notified. 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 in the study. Two sputum samples were collected from each TB patient; one before breakfast (fasting) and one after breakfast. The patients were advised to cough deeply into a well-ventilated container 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.

Hemoglobin, Leukocytes, Hematocrit, and Hematocrit Hemoglobin level was 9.9 g/dl (maximum range was 13.9 g/dl and minimum was 6.8 g/dl). In 30% patients level of Hb was < 9.9 g/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 |
|---------------------|------|--------|-------|
| <11                 | 5    | 22     | 27    |
| 11-17               | 21   | 18     | 39    |
| >17                 | 3    | 4      | 7     |
| Total               | 29   | 100    | 129   |

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 macrophages release cytokines and tumor necrosis factor-a (TNF-α) 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 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 military tuberculosis patients and rare

Range of hemoglobin according to gender

The mean hemoglobin level was 10.18 g/dl the mean hemoglobin level in males was 11.38 g/dl (maximum range was 15.43 g/dl and minimum was 7.43 g/dl). In female the mean hemoglobin level was 9.9 g/dl (maximum range was 13.9 g/dl and minimum was 6.8 g/dl). In 30% patients level of Hb was < 9.9 g/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.
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          | Control       | P value |
|-------------------------|--------------|---------------|---------|
| WBC x 10^9/l           | 6.9 ± 2.8    | 6.9 ± 3.2     | 0.529   |
| RBC x 10^9/l           | 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  | 68.7 ± 28.6   | 0.827   |

*Independent t-test was applied to derive the P values (<0.05 was significant difference); significant difference was observed in neutrophils, 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 in half of the patients with military tuberculosis. Thrombocytosis was observed in 23%. Most of 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 patients lying in different age, ethnic and literacy groups.

| Characters Age Groups (Years) | Total n=100 Patient with % |
|------------------------------|---------------------------|
| Age Groups                   |                           |
| >18                          | 50%                       |
| >30                          | 45%                       |
| >40                          | 40%                       |
| >50                          | 35%                       |
| >60                          | 30%                       |
| >70                          | 25%                       |
| >80                          | 20%                       |
| Gender                       |                           |
| Male                         | 45%                       |
| Female                       | 55%                       |
| Marital status               |                           |
| Married                      | 75%                       |
| Unmarried                    | 25%                       |
| Ethnicity                    |                           |
| Punjabi                      | 15%                       |
| Pashtoon                     | 25%                       |
| Baloch                       | 45%                       |
| Persians                     | 10%                       |
| Others                       | 5%                        |
| Socioeconomical status       |                           |
| High                         | 10%                       |
| Middle                       | 30%                       |
| Low                          | 50%                       |
| Crowding index               |                           |
| >5 person/ house             | 45%                       |
| 1-5 person/ house            | 55%                       |
| Smoking habit                | 60%                       |
| Anemic patients              |                           |
| Male                         | 30%                       |
| Female                       | 70%                       |

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 complication 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 lymphopenia 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 observed in parameters; neutrophils, 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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