Original Article

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Pathology Section

Clinico-haematological Profile in Acid Fast Bacilli Positive Pulmonary Tuberculosis Patients at Tertiary Care Centre in Raichur, Karnataka

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ABSTRACT

Introduction: Tuberculosis (TB) is a major public health problem in India and it remains an epidemic, affecting one third of the population. It manifests with diversified haematological manifestations of varying severity like anaemia, leukocytosis, thrombocytosis and raised erythrocyte sedimentation rate levels. Therefore, monitoring clinical and haematological alterations in sputum positive Pulmonary Tuberculosis (PTB) patients necessitates the need for deliberation of supportive care and varied treatment options that would amplify the treatment outcome. This study was carried out to explore the early changes and the relationship between the social risk factors like smoking, alcohol intake and the haematological profile of TB patients which will help in early identification of altered haematological parameters and in minimising the risk of transmission among susceptible population.

Aim: To do analysis of various haematological manifestations in sputum positive PTB patients and to evaluate diagnostic and prognostic significance.

Materials and Methods: A prospective study was conducted over a period of six months between November 2019 to April 2020 on 50 PTB patients referred by Department of Respiratory Medicine, Navodaya Medical College, Raichur. Patients who were on Anti-Tubercular Treatment (ATT), extra-PTB patients, pregnant women and those with any other major co-morbid condition were excluded from the study. All patients were thoroughly examined clinically and investigated with plain chest x-ray, complete blood count; ESR, PS examination and sputum for acid-fast bacilli stained with Ziehl-Neelsen (Z-N) stain and AFB grading charts were used according to Revised National Tuberculosis Control Programme (RNTCP). Data collected was analysed by SPSS software version 16.0 and Chi-square test was used to calculate the sample size.

Results: Of total 50 cases of PTB, there were 30 males and 20 females and mean age of patient was 44 years. Commonest haematological parameters raised were ESR (48 cases; 96%) followed by anaemia (45 cases; 90%), neutrophilia (36 cases; 72%), leukocytosis (24 cases; 48%) and thrombocytosis (12 cases; 24%). The frequent peripheral smear picture was Microcytic Hypochromic Anaemia (MHA) (27 cases; 54%) followed by normocytic normochromic anaemia (21 cases; 42%) and least common was macrocytic anaemia (2 cases; 4%). There was significant improvement in all haematological parameters after three months of treatment, with sputum conversion of PTB cases (75%).

Conclusion: TB can lead to heterogenous and diversified haematological abnormalities like anaemia, leukocytosis, neutrophilia, thrombocytosis and raised ESR. These haematological parameters can be a guide to assess the improvement of TB patients after ATT which indirectly speaks about the improvement in immune response.

Keywords: Anaemia, Leucocytosis, Sputum positive pulmonary tuberculosis, Thrombocytosis

INTRODUCTION

Tuberculosis (TB) is one of the world's most dreaded disease and globally remains an epidemic affecting one third of world population [1]. There are 12 million active cases of which around 3 million are infectious and every year 1 million cases get added to the existing statistics [2].

TB is the ninth leading cause of death worldwide and single largest infectious disease causing high mortality in humans [3]. About 1.8 million deaths occur due to this disease world wide, while 48% of them occur in the highly populated countries like India, Pakistan, China, Indonesia and Bangladesh [4]. In India, the condition is getting worse as one third of Indian population are infected with *Mycobacterium Tuberculosis* [2].

TB apart from affecting lungs, bone marrow, also causes significant alterations of haematological parameters involving myeloid and lymphoid cell lines and plasma components [5]. Apart from anaemia, leucocytosis, thrombocytosis, monocytosis and lymphocytosis are frequently reported haematological abnormalities [3].

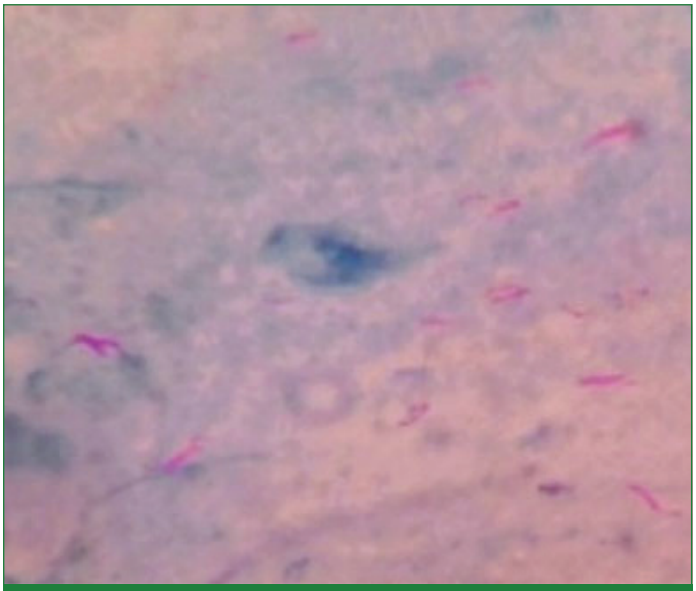
Activation of immune system in affected individual mediates the systemic inflammatory response leading to an increase in the ESR levels. ESR is considered as a sensitive indicator of the disease activity and its return to normal values from initially elevated levels may indicate a good therapeutic response. Hence, it is used in the diagnosis and follow-up of patients affected by TB [6,7].

However, there is scant literature about the existence of alterations in the haematological parameters and also the changes in those parameters after ATT in the Indian scenario. Haematological abnormalities serve as good diagnostic and prognostic aids for proper follow-up of the patients during anti-tubercular therapy [8]. Hence, this study was conducted to evaluate changes in the haematological parameters of sputum positive PTB patients before and after treatment, to identify and minimise the risk of transmission among the vulnerable groups.

MATERIALS AND METHODS

The present study was a prospective and observational study done at NMCHRC, Raichur, over a period of six months between November 2019 to April 2020. Approval was obtained from the Institutional Ethical Committee of NMCHRC, Raichur prior to commencement of the study. Sample size was calculated in following steps: Prevalence of PTB was 196.6/100,000 population

[9]; 11.5% precision and 5% level of significance was taken. Sample size was calculated by using the formula: n=(4PQ/e^2). After the calculation, the sample size was 50. Sputum samples from all 50 cases suspected of PTB were taken in sterile container; smears were prepared and stained with Z-N stain by using standard protocol [Table/Fig-1].



[Table/Fig-1]: Acid Fast Bacilli Positive Tuberculosis (TB) Bacilli stained with Z-N Stain 100x.

Comparative AFB grading charts were used according to RNTCP lab guidelines [Table/Fig-2] [10].

If the Slide has:	Result	Grading	No. of fields to be examined
More than 10 AFB per oil immersion field	Positive	3+	20
1-10 AFB per oil immersion field	Positive	2+	50
10-99 AFB per 100 oil immersion field	Positive	1+	100
1-9 AFB per 100 oil immersion fields	Positive	Scant (to record exact number seen)	100
No AFB in 100 oil immersion fields	Negative		100

[Table/Fig-2]: Comparative AFB grading chart according to RNTCP guidelines [10].
RNTCP: Revised national tuberculosis control programme

CBC, ESR and peripheral smear examination was carried out, compared and correlated as per WHO standards and the same protocol was carried out at the end of three months of ATT treatment to look for changes and treatment response in the haematological parameters.

Selection Criteria

Inclusion criteria: All patients of 15-75 years age group and all sputum positive PTB cases attending the OPD of respiratory medicine of NMCHRC were included in the study.

Exclusion criteria: Patients with extra-PTB, renal/liver disease, underlying malignancy, pregnant women, HIV positive patients, old TB patients with multidrug resistance andpatients on ATT were excluded from the study.

Data and sample collection: Each participant was explained about the study and informed consent was taken prior to data collection. A detailed history was obtained of the presenting illness in the form of questionnaires, general and local examination along with Chest x-ray was done. Under aseptic precautions 5 mL of venous blood was collected in EDTA vacutainers, 2 mL was used for haematological analysis (by SYSMEX XP-100) and remaining 3 mL for ESR estimation (by Westergren tube method).

STATISTICAL ANALYSIS

Data of 50 patients was collected and generated in an Excel sheet on the Computer, analysed by SPSS software version 16.0. Before and after ATT treatment analysis was done by using chi-square test and p-value (<0.05) was considered to be significant.

RESULTS

Demographic Characteristics of Cases

Of the 50 PTB cases included in the study, males (60%) were more than the females (40%) with M:F ratio being 3:2, with a mean age of 44.08 years ,while mean age for males was 45.9 years and for females 41.35 years [Table/Fig-3].

Parameter		Number of cases	Percentage%
Gender	Male	30	60
	Female	20	40
	Total	50	100
Age (Years)	15-25	9	18
	26-35	12	24
	36-45	13	26
	46-55	8	16
	56-65	4	8
	65-75	4	8
	Total	50	100

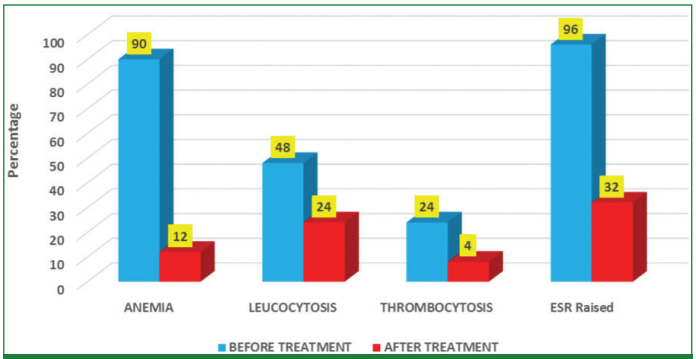
[Table/Fig-3]: Gender and age-wise distribution of cases.

Among total cases, those graded as AFB 2+(20 cases) and AFB 3+(20 cases) were more common, compared to AFB 1+(10 cases) [Table/Fig-4]. After ATT 75% cases tested negative for AFB on sputum examination.

Haematological alterations among PTB patients [Table/Fig-5,6]:

AFB status	Number of cases	Percentage%
1+	10	20
2+	20	40
3+	20	40
Total	50	100

[Table/Fig-4]: Distribution of cases according to AFB status.



[Table/Fig-5]: Haematological alterations among PTB patients before and after treatment.

Haemoglobin levels: The severity of anaemic cases was determined by Haemoglobin levels and categorised as mild (10-11 g/dL), moderate (7-10 g/dL) and severe (4-7 g/dL). Cases of mild anaemia (27 cases; 54%) was most common followed by moderate anaemia (16 cases; 32%) and the least common was severe anaemia (2 cases; 4%).

WBC count: There were equal number of cases of normal WBC count (48%) and leukocytosis (48%) and least cases of leucopenia (4%).

Neutrophil count: Cases with neutrophilia (72%) were frequent compared to cases with normal neutrophil count (28%).

		Before treatment		After treatment		Chi-square	p-value
		Cases	%	Cases	%		
HB Levels	Mild anaemia	27	54	4	8	60.994	0.001**
	Moderate anaemia	16	32	2	4		
	Severe anaemia	2	4	0	0		
	Normal cases	5	10	44	88		
TLC	Normal	24	48	38	76	9.161	0.010*
	Leucocytosis	24	48	12	24		
	Leucopenia	2	4	0	0		
Neutrophil	Normal	14	28	49	98	14.492	0.001**
	Neutrophilia	36	72	1	2		
Lymphocyte	Normal	26	52	44	88	6.199	0.045*
	Lymphocytosis	5	10	1	2		
	Lymphopenia	19	38	5	10		
Eosinophil	Eosinophilia	4	8	1	2	4.167	0.041*
	Normal	46	92	49	98		
Monocyte	Monocytosis	5	10	0	0	5.263	0.022*
	Normal	45	90	50	100		
ESR	Raised	48	96	16	32	44.444	0.001**
	Normal	2	4	34	68		
ESR (Normal)	0-20 mm/hr	2	4	34	68	45.275	0.001**
ESR (Raised)	20-40 mm/hr	7	14	3	6		
	40-60 mm/hr	10	20	5	10		
	60-80 mm/hr	31	62	8	16		
Peripheral smear	Microcytic hypochromic anaemia	27	54	8	16	46.439	0.001**
	Normocytic normochromic anaemia	21	42	11	22		
	Macrocytic anaemia	2	4	0	0		
Platelets	Normal	33	66	47	94	12.260	0.002**
	Thrombocytosis	12	24	2	4		
	Thrombocytopenia	5	10	1	2		

[Table/Fig-6]: Comparison of haematological parameters among PTB patients before and after treatment.
*Statistically significant; **Statistically with high significance (Using chi-square test); PTB: Pulmonary tuberculosis

Lymphocyte count: Cases with normal lymphocyte count (52%) were more common followed by lymphopenia (38%) and lymphocytosis (10%).

Eosinophil count: Cases with normal eosinophil count (92%) were frequent followed by cases with eosinophilia (8%).

Monocyte count: Cases with normal monocyte count (90%) were more common followed by cases with monocytosis (10%).

Platelet count: Cases with normal platelet count (72%) were frequent, followed by cases with thrombocytosis (24%) and the least common was thrombocytopenia (4%).

Peripheral smear examination: MHA was most common (54%) followed by Normocytic Normochromic Anaemia (NNA) (42%) and macrocytic anaemia (4%).

ESR levels: Majority of the cases had elevated ESR (96%), while few cases (4%) had normal levels (32%).

DISCUSSION

TB is the most common communicable disease in the world and is a major health problem in India. In 1993, WHO declared TB as a global emergency. It is estimated that 40% of Indian population is infected by TB bacteria. In 2014, TB was the main cause of death due to infectious diseases worldwide. Haematological abnormality is a common finding among TB patients and anti-TB treatment has its own spectrum of effects on tuberculosis patients [11]. The current study evaluated the haematological parameters of TB patients before and after ATT.

Maximum number of cases (75%) tested negative for AFB on sputum examination, which almost correlated to the results given by Kotresh N et al., (95%) [2]. There was close correlation between sputum conversion of PTB patients and corresponding improvement in all the haematological parameters, ESR and peripheral smear findings after ATT. These findings were identical to the results found in studies conducted by Kotresh N et al., and Abdelkareem Y et al., [2,8].

Sputum smear positive patients most commonly manifest with cavitatory lesions in the lung and serve to be an important source of infection as they produce infectious droplets so much as 2000-3000 droplet nuclei after coughing. These droplets stay in the air for longer period of time and hence the disease infectivity is more in sputum positive PTB patients as compared to extra-pulmonary TB. The haematological alterations like anaemia, leukocytosis, neutrophilia and thrombocytosis are more observed in case of sputum smear positive patients as it is an active disease from which there is an easy spread of pulmonary TB [11].

In the present study, the higher incidence of TB occurred between the age 15-55 years, the mean age of patients was 44 years and the lowest prevalence in extremes of age group (<15 years and >60 years). This finding correlated with the studies conducted by Abdelkareem Y et al., and Yaranal P et al., where, the incidence of TB was 20-40 years and 20-50 years, while the mean age of the patients was 46±17.8 years and 41 years [8,12]. There was a male preponderance in this study with M:F ratio 3:2 which was in accordance with study conducted by Kotresh N et al., (3:1) and Yaranal P et al., (2.4:1) [2,12].

Most of the patients in the study had habits of smoking and alcohol intake (30%) while some were either only smokers (25%) or alcoholics (27%). Similar findings were observed in the study of Kotresh N et al., and the literature suggest that smoking and alcoholism would reduce the immunity and may lead to increase risk of infection [2]. In the present study, the symbiotic relationship between these above mentioned social risk factors and the deranged haematological parameters in sputum positive PTB patients showed a thorough understanding of the prevention and governance of the percolation of the haematological risk factors which will curtail down the risk of transmission among the susceptible group of patients especially in the immunocompromised individuals.

And, hence a thorough and successful implementation of TB control programme can be achievable with rigorous and prompt surveillance, identification and prevention of risk factors.

Among the haematological abnormalities in this study anaemia was more common (90%) followed by leukocytosis (48%), thrombocytosis (24%), thrombocytopenia (4%). In these patients, mild anaemia was more frequent manifestation (54%) compared to moderate anaemia (32%) and severe anaemia (4%). This finding was similar in the studies conducted by Kotresh N et al., Abdelkareem Y et al., and Alamlih L et al., [2,8,13]. On evaluation of peripheral smear, MHA (54%) was predominant followed by normocytic normochromic anaemia (42%) and macrocytic anaemia (4%) and the same was in accordance with the studies of Abdelkareem Y et al., and Bala J et al., [8,14]. In the present study there was a significant improvement in haemoglobin levels (88%) and in peripheral smear findings after treatment where normocytic normochromic blood picture was most common compared to earlier (10%), with p-value (<0.001) being statistically with high significance. These findings were in accordance with the studies conducted by Kotresh N et al., and Abdelkareem Y et al., [2,8].

The mechanism behind the occurrence of anaemia in PTB patients was explained by Means RT and Nemeth E et al., in their studies

where they stated that the invasion of bacteria leads to activation of T-lymphocytes and macrophages, which induce the production of the cytokine like Interferon gamma (INF-gamma), Tumour Necrosis Factor-alpha (TNF-alpha), Interleukin-1(IL-1) and Interleukin-6 (IL-6) which with their products will cause diversion of iron into iron stores in the reticuloendothelial system resulting in decreased iron concentration in the plasma [15,16]. This limits the availability of iron to red cells for haemoglobin synthesis, inhibition of erythroid progenitor cell proliferation, its inappropriate production and inhibition of activity of erythropoietin, the former cause leads to anaemia and the latter two, results in suboptimal response of the bone marrow to anaemia [15,16]. The present study reported leukocytosis in 48% cases before treatment. Studies conducted by Abdelkareem Y et al., and Sulochana S et al., reported leucocytosis in 49% and 45.3% cases [8,11], while Kotresh N et al., documented 20% [2]. The above finding correlated with Abdelkareem Y et al., and Sulochana S et al., [8,11] and was in contrast with the study of Kotresh N et al., [2]. There was significant decrease in number of patients with leukocytosis aftertreatment (24%) in this study with p-value (<0.010) being statistically significant, similar findings were reported by Kotresh N et al., and Abdelkareem Y et al., [2,8]. To combat the invading bacterial population, body's immune defence mechanism plays a vital role. Neutrophils migrate early to the site of infection followed by monocytes which differentiate into macrophages. The macrophage present processed *Mycobacterium Tuberculosis* antigen to T-lymphocytes. Thus, PTB patients have raised WBC counts with neutrophilia, lymphocytosis and monocytosis. These all host responses are responsible for clinical manifestation of TB [17]. In the differential count, in the decreasing order of frequency, neutrophilia (72%) was most common followed by lymphocytosis (10%), monocytosis (10%) and eosinophilia (8%) before treatment. Cases with neutrophilia (72%) and lymphocytosis (10%) corroborated with the study of Yaranal P et al., (neutrophilia: 76.9%, lymphocytosis: 11.53%) [12], whereas, it was lower when compared to Abdelkareem Y et al., (neutrophilia: 57%, lymphocytosis: 6%) and Bala J et al., (neutrophilia: 52%, lymphocytosis: 3.75%) [8,14]. The cases with eosinophilia (8%) and monocytosis (10%) of this study were almost agreeable to the study of Sulochana S et al., with respect to the former (eosinophilia: 10%) [11], whereas it was lower in the case of latter (monocytosis: 4.6%). The same when compared with the study of Kotresh N et al., it was lower in cases of eosinophilia (4%) and equal with those presenting with monocytosis (10%) [2]. There was significant alteration in the differential count after treatment of all the cells {(neutrophilia-2%); (lymphocytosis-2%); (lymphopenia-10%); (eosinophilia-2%); (monocytosis-0%)} and p-value being statistically significant (<0.001). Similar findings were noted in the studies conducted by Kotresh N et al., (1%) Abdelkareem Y et al., (4%) and and Kamate S et al., (3%) with respect to neutrophilia [2,8,18], while with lymphocytosis and lymphopenia, results of this study corroborated with that of Kamate S et al., (lymphocytosis-1%; lymphopenia-10%) [18] and Sheetal MRM et al., (lymphocytosis-2.5%; lymphopenia-7.5%) [18,19]. The findings of eosinophilia and monocytosis with p-value (<0.041 and <0.022, respectively) of this study was statistically significant and when compared these findings were closely related with study of Kotresh N et al., {(eosinophils: 1% and monocytosis: 0%)} [2]. Among the parameters of platelets observed in the present study, thrombocytosis was more frequent (24%) compared to thrombocytopenia (5%) and the same were almost similar to the studies conducted by Sulochana S et al., and Yaranal P et al., (thrombocytosis-24% in the former and 33% in the latter; thrombocytopenia-9% in the former and 3% in the latter) [11,12]. Thrombocytosis was significantly declined in cases (4%) after treatment with p-value (<0.027) being statistically significant, which was agreeable with study done by Kotresh N et al., (2%) [2]. Diverse theories like drugs, immune mechanism, bone marrow fibrosis, granulomatous involvement of bone marrow and hypersplenism have been postulated as causal factors for thrombocytopenia. Whereas, various chemical mediators like IL-6 and others are responsible for thrombocytosis and play an active role in platelet production and due to thrombopoietic factors as an inflammatory response [20]. Elevated ESR levels in the current study (96%) were analogous with studies done by Abdelkareem Y et al., Yaranal P et al., and Banerjee M et al., (97%, 98% and 99%), respectively [5,12,20]. Whereas, there was significant decrease in cases with raised ESR (32%) after treatment with p-value (<0.001) being high significant statistically, which corresponded with studies done by Kotresh N et al., (30%) and Abdelkareem Y et al., (43%) [2,8]. In TB at the beginning of the inflammatory process there is strong pro-inflammatory cytokine activity (interferon-gamma and tumour Necrotic factor-α) which stimulated expression of acute phase proteins which includes alpha 1-glycoprotein acid, mucoprotein, alpha 1-globulin, alpha 2-globulin and gammaglobulin. These proteins influence ESR, a non-specific test for inflammatory and infectious processes used to evaluate activity level, response to treatment and the prognosis [1].

Limitation(s)

There were certain limitations of study. Other WBC findings like eosinophilia, monocytosis and lymphopenia needed further explanations for which there is necessity of ancillary study techniques in this field and larger number of sample size can also be considered to have more precise findings which can be conclusive based on the above parameters. Besides these haematological alterations, changes in supplementary parameters like RBC indices (MCV, MCH, MCHC) and raised C-Reactive Proteins (CRP) levels can be studied further in detail for the future prospective of the study.

CONCLUSION(S)

Mycobacterium Tuberculosis demonstrated a wide spectrum of alterations in haematological parameters in this study like MHA followed by normocytic normochromic anaemia, leukocytosis, neutrophilia and thrombocytosis. Raised ESR was noted in 96% cases. These findings can be set-off relevant guiding tools for observing the prognostic outcome of sputum positive PTB patients. They also can be used to indicate the response to the ATT and thus considerable reduction in the morbidity and mortality. However, a detailed research on this subject with larger number of cases needed to be carried out keeping in mind the present scenario of PTB worldwide.

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