

## ORIGINAL ARTICLE

## HAEMATOLOGICAL PARAMETERS ALTERED IN TUBERCULOSIS

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**Background:** Tuberculosis is a chronic granulomatous inflammation highly prevalent in developing countries resulting in high mortality rate. The study was conducted to investigate the change in haematological parameters with effective anti-tuberculosis therapy. **Methods:** A total of 45 active tuberculous patients and 45 age and sex matched healthy controls were enrolled for study by convenient non-probability sampling. Patients were taken from Military Hospital, Rawalpindi, whereas controls were taken from community. All the subjects underwent blood sampling for complete blood picture. The samples were taken in ethylene diamine tetraacetic acid (EDTA) treated tube and were analysed in KX-21 Sysmex<sup>®</sup> automated haematology analyser. Differential leukocyte count was done manually by microscopy of Leishman's stained blood films. Statistical analysis was done and  $p < 0.05$  was considered significant. **Results:** Haemoglobin and lymphocyte were significantly different in cases and controls at time of diagnosis. Haemoglobin concentration, neutrophil, monocyte and lymphocyte count were significantly different, whereas total leukocyte counts were not significantly different. Change in platelet count among cases and controls were statistically significant. Monocyte Lymphocyte (M/L) ratio and Lymphocyte Neutrophil (L/N) ratio were also significantly different. **Conclusion:** Haemoglobin concentration, platelet count, lymphocyte count, and neutrophil count alter with chronic inflammatory process and revert to normal with effective therapy. These parameters can be used as indicators in the assessment of response to chemotherapy.

**Keywords:** Anti-tuberculosis therapy, Monocyte-Lymphocyte ratio, Lymphocyte-Neutrophil ratio

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## INTRODUCTION

Tuberculosis (TB) is one of the earliest and fatal ailments, a major health risk, and socioeconomic burden in both developing and industrialised countries that kill approximately 2 million people annually. World Health Organization global report of TB estimated the incidence and prevalence rate of 125 and 170 cases per 100,000 population respectively, with 1.5 million deaths reported in 2011.<sup>1</sup> It is primarily a disease of low socioeconomic community. Several risk factors that promote the development of TB include impaired immune response, addiction, alcoholism, malnutrition and several systemic disorders including diabetes, hypertension and renal diseases.<sup>2</sup> In addition to the mentioned risk factors immigration to endemic areas also contributes substantially to causation and spread of the disease.

To label the disease curable, timely diagnosis and prompt compliant therapy is needed. Diagnosis of TB can be made on chest X-ray supported by positive clinical findings but sputum smear and culture remains the key investigation.

Complete blood picture is the routine investigation done for every patient irrespective of the type of infection that provides much needed information for making decision of treatment. In tuberculosis various haematological derangements are seen including low haemoglobin, decreased lymphocyte count with its subsets, neutrophilia, monocytosis, monocytopenia, thrombocytopenia and thrombocytosis in few cases.<sup>3</sup>

Anaemia is one of the commonest findings seen in TB patients and considered to be responsible for poor prognosis.<sup>4</sup> Most commonly seen anaemia is iron deficiency anaemia that decreases hosts capacity in defending against foreign antigen resulting in impaired immune response.<sup>5</sup> Regarding lymphocyte count the results are found controversial; a group of scientists stated lymphopenia<sup>6</sup> a marked finding of tuberculosis, whereas others found lymphocytosis<sup>7</sup>. The first line of defence against any foreign organism is innate immunity characterised by phagocytosis mediated by neutrophils and macrophages. Tuberculosis is characterised by granuloma formation hence neutrophils play a vital role in its formation. Release of tumour necrosis factor alpha (TNF- $\alpha$ ) from polymorphs efficiently kills the mycobacterium.<sup>8</sup> Platelet count also has a significant role in immune functions and thrombocytosis is generally seen in chronic inflammation stated as 'reactive thrombocytosis'.<sup>9</sup>

The aim of current study was to investigate blood cell counts during anti-tuberculosis therapy and to find out the association of various cell counts, lymphocyte neutrophil ratio (L/N) and monocyte lymphocyte ratio (M/L) ratio with constant anti-tuberculosis therapy.

## MATERIAL AND METHODS

This was a prospective cohort study conducted at Military Hospital Rawalpindi in collaboration with Army Medical College. Formal approval from Ethical Review Committee of Army Medical College,

Rawalpindi was obtained before commencement of the study. Written informed consent was taken from all the participants of the study. Forty-five patients diagnosed with pulmonary tuberculosis having normal body mass index (BMI), and aged 18–65 years were included whereas individuals with diabetes mellitus, hypertension, obesity and chronic infections were excluded. Forty-five age and sex matched healthy controls were also included for comparison. All subjects were enrolled via convenient non-probability sampling. Tuberculosis was diagnosed on either clinical evidence with chest X-ray, Gene Xpert studies, or sputum smear and culture.

Blood samples from both, the cases and controls were taken by venipuncture from antecubital vein, following strict aseptic measures; 2.5 mL of blood was drawn and placed in ethylene diamine tetraacetic acid (EDTA). Samples were transported in cold box from Military hospital to Pathology Laboratories, Army Medical College, Rawalpindi where samples were analysed using KX-21 Sysmex® automated haematology analyser and printed reports of complete blood count were obtained. Differential leukocyte counts were also determined using the standard methods and lymphocyte/neutrophil and monocyte/lymphocyte ratios were calculated.

The blood samples analysis was done and analysed three times for tuberculous group, i.e., at the start of therapy, at the completion of initiation phase, and 4 months of treatment. In control group sampling was done once only.

Data were analysed using SPSS-22. Mean and standard deviation were calculated for continuous variables whereas frequency and percentage for the qualitative variables. The alpha value was kept at 0.05 for significance.

## RESULTS

Age of cases and controls was 38.42±16.11 and 38.27±15.83 years respectively. The BMI was 19.95±2.90 among cases, whereas in controls it was 20.76±3.21.

At the time of diagnosis (0 month) 51% TB cases presented with lymphopenia. Differences in haemoglobin concentration, neutrophil, lymphocyte and monocyte count between controls and cases were statistically significant. M/L and L/N ratio in both cases and controls were significantly different. Seventy-eight percent patients presented with anaemia at the time of diagnosis (Table-1).

**Table-1: Comparison of haematological and biochemical parameters among controls and cases**

Variables	Control (at the start of study)	Cases			p
		TB0	TB2	TB4	
Hb (g/dL)	12.69±1.00	12.67±1.54	13.00±1.98	12.38±2.06	0.046*
TLC ( $\times 10^3/\mu\text{L}$ )	7.95±1.81	7.64±2.27	7.17±4.17	8.08±1.98	0.34
Platelets ( $\times 10^3/\mu\text{L}$ )	251.76±75.25	228.73±122.07	224.91±92.64	261.95±105.57	0.008*
Neutrophils (%)	59.88±8.66	68.22±12.8	63.27±13.31	57.73±11.2	0.000*
Monocytes (%)	7.20±1.76	4.80±2.35	5.00±2.24	5.97±2.43	0.000*
Lymphocytes (%)	30.58±8.08	22.07±7.97	28.20±8.93	32.57±7.36	0.000*
M/L ratio	0.24±0.07	0.24±0.14	0.20±0.10	0.19±0.10	0.006*
L/N ratio	0.52±0.22	0.32±0.16	0.46±0.19	0.55±0.19	0.000*

TB0= at start of treatment, TB2= at 2<sup>nd</sup> of treatment, TB4=at 4<sup>th</sup> of treatment, \*Significant

## DISCUSSION

Various haematological abnormalities were evident in tuberculous patients like anaemia, and lymphopenia. More than three-fourths (¾) of the patients presented with anaemia, i.e., either normocytic normochromic or iron deficiency anaemia coinciding with the results of Lee *et al*<sup>10</sup> who reported suppression of erythropoietin secretion under the effect of inflammatory mediators. Malabsorption and nutritional deficiency are also major contributing factors in the development of anaemia. Iron is a growth factor required by mycobacterium for growth and survival which prevents the release of iron from reticulo-endothelial system. Due to non-availability of iron to bone marrow, there is reduced erythropoiesis resulting in anaemia. With effective therapy anaemia improved in our patients after the completion of initiation phase. However, after two months the haemoglobin status altered again which

might be due to side effects of folic acid inhibitors given in treatment that resulted in maturation failure of red blood cells<sup>11</sup> or malnutrition resulting in nutritional anaemia.

Total leukocyte count was found within normal range in our study coinciding various studies. Brahmhatt *et al*<sup>12</sup> showed that leukocyte count was significantly raised in slow responders whereas it was slightly raised in fast responder of anti-tuberculosis therapy (ATT). In contrast to our findings, Venestra *et al*<sup>13</sup> found a significant increase in total leukocyte count that resulted from immune reaction taking place in response to foreign antigen Mycobacterium tuberculosis, that also resulted in increased cytokines levels. These cytokines are IFN- $\gamma$ , IL-1 $\beta$ , and IL-18 which in turn causes further proliferation of white blood cells.

Neutrophilia was a significant finding seen in our study that improved with successful treatment. This

elevated neutrophil count seemed to be due to innate immune response of body against the antigen<sup>3</sup>. In contrast Bozoki *et al*<sup>14</sup> found neutropenia in their patients that might be due to decreased production either because of bone marrow invasion or malnutrition in their patients leading to folate deficiency resulting in decreased haemopoiesis.

Lymphopenia was seen in half of our patients at the time of diagnosis which later improved with initial phase of treatment. None of the patients showed lymphocytosis. Lymphopenia might be due to accumulation of lymphocytes at the site of infection leading to decrease number in peripheral blood.<sup>16</sup> There are different studies available mentioning lymphocyte count in TB but the effect of TB on lymphocyte count is still uncertain. Ashtekar *et al*<sup>7</sup> reported lymphocytosis and Okamura *et al*<sup>6</sup> showed lymphopenia in their studies.

Similarly Astekar *et al*<sup>7</sup> found significantly lower CD4 count with decreased CD4/CD8 ratio in reactive cases of TB but CD8 lymphocytosis in unreactive TB. Mycobacterium tuberculosis induces release of various cytokines including interferon and multiple interleukins that may influence the production of lymphocytes predominantly T cells and its subsets.<sup>17</sup>

Monocytosis is commonly seen in tuberculosis.<sup>13</sup> The microorganism after entering the body is engulfed by alveolar macrophages. Some microorganisms escape the defence mechanisms and succeed to endure, resulting in infection with production of chemoattractant substances which then invite other leukocytes and result in unopposed production of monocytes. We found decreased monocyte count in TB group compared with the control healthy group. TB is a disease of low socioeconomic society and prevails more in poor and malnourished individuals. Malnutrition leads to inappropriate lymphokines production which may then lead to altered monocyte production and differentiation.<sup>18</sup> This altered immunological response might be the reason of decreased monocyte count in our study. Our findings of weight loss in tuberculous group are supported by Morris *et al*<sup>3</sup>. Immune deficiency is strongly linked with malnourishment thus making individual more prone to infections. An immune reaction starts with antigen presenting cells which are affected by malnutrition. In addition malnutrition also affects cytokine production and proliferation of memory cells.<sup>18</sup> Sakai *et al*<sup>19</sup> explained that malnourished individuals have altered T-cell immunity but not B-cell immunity. Another study<sup>21</sup> done in malnourished children showed that protein deficiency decreases T-cell proliferation that in turn decreases IFN- $\gamma$  and IL-2 production.

Platelet count was decreased at time of diagnosis till the completion of initiation phase when compared with controls. Decreased platelet count in TB patient may be due to release of IL-1 that act as a

procoagulant on endothelial cells.<sup>21</sup> Thrombocytopenia continued even during initiation phase of ATT.<sup>22</sup> Among ATT, rifampicin is considered as a major drug that decreases platelet count. Thrombocytopenia is caused by formation of antibodies that bind to platelets and undergo complement cascade resulting in lysis of these cells. These antibodies also suppress the production of platelets.<sup>23</sup> During continuation phase the normal platelet count was resumed. Another study by Morris *et al*<sup>3</sup> also found thrombocytopenia in sputum smear of negative patients undergoing ATT which was considered mainly because of rifampicin and isoniazid.

A study conducted in Fatima Jinnah General and Chest Hospital Quetta, partially supported our findings of anaemia, lymphopenia and thrombocytopenia but varied in opinion in neutrophil count showing marked neutropenia. This difference may be due to variation in selection criteria, i.e., they selected patients with late disease in contrast to freshly diagnosed patients in our study.

L/N ratio is an expedient marker of inflammation for foreseeing bacterial infection. Patients with earlier tuberculous pleuritis show elevated neutrophil count in their pleural fluids with elevated ADA levels. Kashinkunti<sup>27</sup> showed a significant increase in L/N ratio in patients who developed pleural effusion due to tuberculosis. However our results showed that L/N ratio was similar in patients with and without TB pleural effusion. Our study also showed a significant change in L/N ratio in patients undergoing treatment with reduced inflammatory response of body. Neutrophilia and lymphopenia improve with settlement of inflammation and appropriate treatment. Like tuberculosis various malignancies also exhibit chronic inflammatory response mediated by a number of inflammatory products secreted by neutrophils and other leukocytes. Chua *et al*<sup>28</sup> reported that L/N ratio was a consistent prognostic marker to quantify the regression of tumour and survival chances by evaluating the chronic inflammatory response of disease.

## CONCLUSION

Various haematological parameters are influenced by pulmonary tuberculosis and are improved with effective ATT. Anaemia, lymphopenia, and thrombocytopenia were the key findings that revert with treatment. However L/N ratio was increased in new diagnosed patients and M/L ratio was unchanged.

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