Monocyte Lymphocyte Ratio as a Possible Prognostic Marker in Antituberculous Therapy

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Abstract

Background: To assess monocyte lymphocyte ratio (M/L ratio) as a biomarker to see the outcome of therapy and progress of tuberculosis.

Methods: In this descriptive study 45 newly diagnosed cases of tuberculosis and 45 age and sex matched healthy controls, were enrolled. The study was designed to determine the baseline, initiation phase and 2-months maintenance phase treatment values of M/L ratio in newly diagnosed active TB cases. Blood complete counts were performed in automated haematology analyzer. Manual differential leukocyte count was done by microscopy to determine M/L ratio.

Results: The pre-treatment lymphocyte and monocyte count in the study group were significantly different from the control group, while M/L ratio was similar in both the groups. The mean M/L ratio in cases and controls were 0.24 ± 0.14 and 0.24 ± 0.07 respectively. The M/L ratio significantly decreased after initiation phase and two months of maintenance phase of treatment from baseline 0.24 ± 0.14 to 0.20 ± 0.10 to 0.19 ± 0.10 with p value of 0.006.

Conclusions: Tuberculosis is associated with increased M/L ratio, which declines and returns to normal with antituberculous therapy.

Key Words: Monocyte Lymphocyte Ratio, Antituberculous Therapy

Introduction

Tuberculosis is a major health problem and is the second leading cause of death by infectious diseases after human immunodeficiency virus (HIV). It is much more common in low socioeconomic communities. Contrary to a global decline in active TB cases, Pakistan is still showing a rise despite adopting the directly observed treatment short course (DOTS) strategy on mass scale. According to WHO statistics, Pakistan is placed at 5th number in international ranking of tuberculosis with incidence rate of 175 per 100,000 population and shares almost half of the burden of the disease in East Mediterranean region covering 23 countries.1-3

Immune cells, both monocytes and lymphocytes have well defined role in innate as well as acquired immunity. Tuberculosis is considered to be one of the most imperative cause of monocytosis which then settles as the infection resolve.4 The results with lymphocyte count are still controversial, increasing in some cases while decreasing in others and returning to normal with therapy.4-6 In addition, with lymphocytosis IFN-γ levels are increased at the time of diagnosis which returns to normal with treatment. Lymphopenia is also another important marker of severe malnutrition along with blood albumin levels seen in malnourished individuals.7 Monocytes are the target cells for mycobacterial proliferation whereas lymphocytes provide resistance to the spread of infection causing mycobacterial clearance so it is reasonable to suggest that M/L ratio can also be used as a prognostic tool in TB.

M/L ratio has already been used as a prognostic marker in various malignances including colon cancer, non-Hodgkin lymphoma and multiple myeloma.8 In TB, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) has been commonly used for monitoring the treatment but later, Pakistan chest society guidelines revealed that ESR does not have any prognostic value.9 In tuberculous pleural effusion, neutrophilia is generally seen in its acute phase.10,11

Patients and methods

This cohort prospective study was conducted involving tuberculous patients and controls. Forty five newly diagnosed tuberculosis patients from Military hospital, Rawalpindi were enrolled via convenient non probability sampling and forty five age and sex matched healthy controls were taken from the community. Tuberculosis was diagnosed on either clinical evidence with chest X-ray or Gene Xpert studies or sputum smear and culture. Newly diagnosed cases with normal body mass index (BMI), and age between 18 to 65 years, were included.
Individuals with diabetes mellitus, hypertension, obesity and chronic infections were excluded. Blood counts were performed on automated haematology analyzer. Differential leucocyte count was done by making a thin film of blood on a glass slide, followed by fixing and staining the cells by Leishman’s stain. A total of 100 cells were visualized and out of which monocytes and lymphocytes were identified and calculated as percentages. M/L ratio was obtained by dividing monocyte count in percentage with lymphocyte count in percentage. The blood samples analysis was done thrice in tuberculous group, i.e. at the start of therapy and at the completion of initiation phase and end of 4th month of treatment (Table 1). Independent T-test and repeated measures analysis of variance (ANOVA) were done for statistical analysis between the groups. P value < 0.05 was considered significant.

Table 1: Experimental design

<table>
<thead>
<tr>
<th>Group</th>
<th>Controls (H)(n=45)</th>
<th>Study group (TB cases )(n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group description</td>
<td>Healthy</td>
<td>TB cases at start of treatment</td>
</tr>
</tbody>
</table>

Results

Forty five tuberculous patients with mean age of 38.42 ± 16.11 and BMI 19.95 ± 2.90 were compared with forty five healthy controls with mean age 38.27 ± 15.83 years and BMI 20.76 ± 3.21. Majority (75.6%) patients were diagnosed on clinical evaluation and chest radiography, whereas 20% patients were diagnosed on gene Xpert studies. Only 4.4% were smear positive. Samples were taken at the start of therapy (0 month), completion of initiation phase (at the end of 2nd month) and during maintenance phase (at the end of 4th month). In control group, sampling was done at 0 month (start of study). The mean monocyte counts in study group were 4.80 ± 2.35, 5.00 ± 2.24 and 5.97 ± 2.43 at the start, end of initiation phase and during maintenance phase respectively. In control the monocyte count was 7.20 ± 1.76. The mean lymphocyte counts at the commencement and completion of initiation phase and end of 4th month of treatment were 22.07 ± 7.97, 28.20 ± 8.93 and 32.57 ± 7.36 respectively whereas it was 30.58 ± 8.08 in the control group. M/L ratio in TB0, TB2 and TB4 group were 0.24 ± 0.14, 0.20 ± 0.10 and 0.19 ± 0.10 respectively. In controls it was 0.24 ± 0.07 (Table 2; Figure 1). Lymphopenia was seen in 23 out 45 cases while others showed normal lymphocyte count in study group at start of study (Table 3). Comparison of monocytes, lymphocytes and Monocyte Lymphocyte ratio (M/L) in controls and TB group at 0 month by

Table 2: Monocytes, lymphocytes and M/L ratio in controls and study (tuberculous) group

<table>
<thead>
<tr>
<th></th>
<th>Control (0month)</th>
<th>Study (TB) group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>Monocytes</td>
<td>7.20 ± 1.76</td>
<td>4.80 ± 2.35</td>
</tr>
<tr>
<td></td>
<td>5.00 ± 2.24</td>
<td>5.97 ± 2.43</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>30.58 ± 8.08</td>
<td>22.07 ± 7.97</td>
</tr>
<tr>
<td></td>
<td>28.20 ± 8.93</td>
<td>32.57 ± 7.36</td>
</tr>
<tr>
<td>M/L ratio</td>
<td>0.24 ± 0.14</td>
<td>0.20 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>0.19 ± 0.10</td>
<td></td>
</tr>
</tbody>
</table>

*p value <0.05 is significant

Figure 1: Box plot showing M/L ratio in control group, study group at 0 month, end of 2nd month and end of 4th month of treatment showing a significant decline

Mann-Whitney U test revealed significant difference in monocyte and lymphocyte count between controls and TB0. However, M/L ratio was same in both the groups (Table 4). There was significant difference in lymphocyte count and monocyte lymphocyte ratio.
between TB 0, TB2 and TB4 by Repeated Measures Analysis of Variance (Table 5). There was no significant difference in lymphocyte neutrophil (L/N) ratio in tuberculous patients with and without pleural effusion. Study group (TB0) with pleural effusion (n=15) had L/N ratio 0.34 ± 0.13 and without pleural effusion (n=30) have L/N ratio 0.38 ± 0.17 showing that there was no significant difference between these two groups (Table 6).

Table 5: Comparison of monocytes, lymphocytes and Monocyte Lymphocyte ratio in study group at 0, end of 2 months and end of 4 months

<table>
<thead>
<tr>
<th></th>
<th>TB0</th>
<th>TB2</th>
<th>TB4</th>
<th>Significance (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.80 ±</td>
<td>5.00 ±</td>
<td>5.97 ±</td>
<td>0.000*</td>
</tr>
<tr>
<td>Mean</td>
<td>2.35</td>
<td>2.24</td>
<td>2.43</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>22.07 ±</td>
<td>28.20 ±</td>
<td>32.57 ±</td>
<td>0.000*</td>
</tr>
<tr>
<td>Mean</td>
<td>0.24 ±</td>
<td>0.20 ±</td>
<td>0.19 ±</td>
<td>0.000*</td>
</tr>
<tr>
<td>M/L ratio</td>
<td>0.14</td>
<td>0.10</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

*p value is <0.05

Table 6: Lymphocyte neutrophil (L/N) ratio in tuberculous patients (TB0) with and without pleural effusion

<table>
<thead>
<tr>
<th></th>
<th>Pleural Effusion(+)</th>
<th>No Pleural Effusion (-)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>L/N ratio</td>
<td>0.34 ± 0.13</td>
<td>0.32 ± 0.17</td>
<td>0.492</td>
</tr>
</tbody>
</table>

*p value <0.05 is significant.

Discussion

The treatment of tuberculosis is long term and demands continuous monitoring and motivation on part of both patient and doctor. The prognostic markers commonly available to monitor the progress of disease include CRP, ESR while several other expensive and time consuming markers are Interleukin (IL) -10, IL-13, and other immune markers but a single specific marker for monitoring of tuberculosis is yet to be found. In present study M/L ratio was calculated in TB patients at commencement and completion of initiation phase of antituberculous treatment and after 4 months of ATT to identify its role in monitoring the effectiveness of treatment.

The specificity of chest X-ray in diagnosing TB is up to 67%. Sputum smear and culture is the gold standard but may give inconclusive results and is time consuming. Negative sputum smears do not exclude the diagnosis of tuberculosis. Gene Xpert studies is a newly introduced, expensive investigation and is less readily available for public sector. Monocytosis is commonly seen in tuberculosis. The microorganism after entering the body is engulfed by alveolar macrophages. Some microorganisms escape the defense mechanisms and succeed to endure, resulting in infection with production of chemoattractant substances which then invites other leukocytes and results in unopposed production of monocytes. Malnutrition leads to inappropriate lymphokines production which may then lead to altered monocyte production and differentiation. This altered immunological response might be the reason of monocytopenia in present study. This was supported by history of weight loss in TB group which found in 73% cases coinciding with the findings of Morris et al.

Lymphopenia was seen in 50% of our patients at the time of diagnosis which later improved with treatment. None of the patient showed lymphocytosis. Lymphopenia is considered to be due to accumulation of lymphocytes at the site of infection leading to decreased number in peripheral blood. There are different studies available mentioning lymphocyte count in TB and the effect of TB on lymphocyte count is still uncertain. Santiago and colleagues supported lymphocytosis while Okamura et al proved lymphopenia in their study.

Shijubo et al published a study in 1992 showing decreased CD8 count. Similarly Astekar et al found significantly lower CD4 count with decreased CD4/CD8 ratio in reactive cases of TB but CD8 lymphocytosis in unreactive TB. M. tuberculosis induces release of various cytokines including interferon and multiple interleukins that may influence the production of lymphocytes predominantly T cells.

M/L ratio increases with chronic inflammations including TB which then settles under the effect of antituberculous therapy. A progressive decrease in M/L ratio was observed in our study. This is supported by a research conducted by Stotz et al on colon cancer patients who showed that cases having lymphocyte monocyte ratio > 2.38 do not respond to chemotherapy. Another recent study by Naranbhai et al on 3 to 4 months old children suspecting TB showed that M/L ratio has a predictive role in TB, thus helps in early detection and prompt treatment of the disease.

In this study about one third patients presented with pleural effusion which in most cases was exudative lymphocytic which is characteristic of tuberculous effusion, supporting the findings of previous studies. Infrequently, pleural fluid has predominance of lymphocytes at the site of infection leading to decreased number in peripheral blood. There are different studies available mentioning lymphocyte count in TB and the effect of TB on lymphocyte count is still uncertain. Santiago and colleagues supported lymphocytosis while Okamura et al proved lymphopenia in their study.
polymorphs for less than two weeks followed by lymphocytic preeminence. L/N ratio is an expedient marker of inflammation for foreseeing bacterial infection. Patients with earlier tuberculosis pleuritis show elevated neutrophil count in their pleural fluids with elevated adenosine deaminase (ADA) levels. Kashinkunti and his co-workers showed a significant increase in L/N ratio in patients who developed pleural effusion due to tuberculosis.10

**Conclusion**

1. M/L ratio can be considered as an independent prognostic marker and predictor of anti tuberculosis treatment.
2. Tuberculosis, a disease showing a rising trend in our set up, requires to have markers which can be helpful in diagnosis and predicting disease course

**References**