To Compare the Competency of Prognostic Markers- ESR, CRP against ZN Staining in Determining Pulmonary Tuberculosis Cases

Rashmi Chandragouda Meti*, Anand Nagalikar

ABSTRACT

Background: Tuberculosis (TB) is a highly prevalent chronic infectious disease caused by Mycobacterium tuberculosis an aerobic intracellular binding bacterium (bacillus). Active TB disease can be fatal if left untreated. The disease today is considered curable and preventable. This retrospective study was undertaken to compare and analyzed the relationship between acute phase reactant proteins CRP (quantitative) and ESR in sputum smear positive for AFB patients and to evaluate their diagnostic and prognostic significance. Methods: The present prospective study was carried on a randomly selected study group comprising of 180 cases of pulmonary TB patients who were sputum smear positive for AFB and 25 normal healthy (sputum smear negative for AFB) individual were taken as control. The cases of PTB group was further categorized on the basis of recommendation of RNTCP grading system of AFB in Sputum ZN staining into four subgroups AFB – 3+, AFB – 2+, AFB – 1+ and scanty. The control groups were negative for the Acid Fast bacilli in sputum. Results: In our study showed that the maximum patients seen in grade III (36.11%) followed by grade II (31.66%), grade I (19.44%) and 12.77% scanty. The distribution of mean values of CRP, ESR in different grades of ZN staining of sputum in AFB positive patients as well as controls. CRP mean value was 55.4, 40.8 & 22.9 in grade III, grade II & grade I respectively and ESR was 48.1, 38.8 & 23 respectively in different grading significantly higher (p vol < 0.0001) in group II there were no serious adverse reactions in either group. Conclusions: Our study concluded that during the compression of values of CRP and ESR along with the grading of sputum AFB positive patients, the CRP raised more significantly as compare to ESR; with markedly raised values in all the grades of sputum AFB positive patients.

Key words: Pulmonary tuberculosis, AFB positive, CRP, ESR, ZN staining

INTRODUCTION

Global propagation of TB more than any other disease is affected by social and economic factors. The persistence of TB is compounded by the fact that resources to combat TB in the affected countries are very scarce. With the emergence of HIV infection has made the situation worse.[1] TB remains at an epidemic level affecting about two billion people in the world i.e. one in every third person, is infected with Mycobacterium tuberculosis.[2] 95% of all cases and 99% of deaths due to tuberculosis (TB) occur in the developing countries, with the greatest burden in South-East Asia and Sub-Saharan African countries.[3] India accounts for one fifth of the total global TB burden. According to eleventh five-year plan of RNTCP estimated annual prevalence per lakh population was 250 and percentages annual risk of TB infection was 1.1%, in India.[4] Active TB disease can be fatal if left untreated. The disease today is considered curable and preventable. Progress in the scaling up of tuberculosis diagnostic, treatment, and control efforts worldwide is associated with improvement in diagnostic techniques and TB control measures. Microscopic examination of sputum specimens has been the basis of TB case detection for over 100 years and still, in resource-limited settings, the diagnosis of TB relies on Ziehl-Neelsen smear microscopy with the light microscope.[5] Although microscopic examination is rapid, simple and economical, it is relatively

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insensitive, requiring more than 10,000 bacilli per milliliter to detect AFB.[6]
Though culture based techniques is still the “Gold standard” for the diagnosis and follow up on PTB.[1] This retrospective study was undertaken to compare and analyzed the relationship between acute phase reactant proteins CRP (quantitative) and ESR in sputum smear positive for AFB patients and to evaluate their diagnostic and prognostic significance.

METHODS
A retrospective study was conducted in department of Microbiology. The patient population included in the study were sputum positive for the presence of acid fast bacilli by ZN staining technique.

Inclusion Criteria: -
- At least one positive smear for AFB along with chest X ray consistent with PTB and decision by physician to treat.
- At least one sputum ZN positive plus sputum culture positive for AFB.
- Patient were randomly selected suffering from pulmonary tuberculosis.

Exclusion Criteria: -
- Extrapulmonary tuberculosis patients were excluded.
- Patients having primary hematological malignancy like leukemia or other Myeloproliferative disorders.
- Patients who are on immunosuppressive drugs or immune-compromised as in AIDS.
- Patients having liver diseases, renal diseases and endocrine disorder.
- Repeated sample from the same patient.

Procedure
1. Smear was prepared from yellow purulent or blood tinged portion of the sputum specimen using a stick in the pre-marked area over the new glass slide,
2. The smear could air dry for 15-30 min
3. The smear was fixed by passing the slide over the flame 3-4 times.
4. Carbol fuschin was poured over the smear and heated gently till fumes appear; keeping precaution to avoid overheating.
5. Carbol fuschin could act for 5 minutes, and then washed off with tap water.
6. Acid-alcohol decolorizer was poured and kept for one minute. This step was repeated, until the slide appears light pink in color. At the end slide was washed with tap water.
7. Counter stain i.e. Methylene blue was poured, and kept for two minutes; and washed with tap water
8. Slide was air dried; examined under oil immersion objective to observe for Acid fast bacilli (bright red) against blue background of pus cells and epithelial cells.[7]

RESULTS
In our study showed that the maximum patients seen in grade III (36.11%) followed by grade II (31.66%), Grade I (19.44%) and 12.77% scanty according to RNTCP grading system of ZN staining of sputum for acid fast bacilli (Table 1). The distribution of mean values of CRP, ESR in different grades of ZN staining of sputum in AFB positive patients as well as controls. CRP mean value was 55.4, 40.8 & 22.9 in grade III, grade II & grade I respectively and ESR was 48.1, 38.8 & 23 respectively in different grading (Table 2).

Table 1: Distribution of mean values of CRP, ESR in different grades of ZN staining of sputum in AFB positive patients as well as controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (25)</th>
<th>AFB positive patients as per RNTCP grading system (n=180)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/l)</td>
<td>4.5 (1.5-9.1)</td>
<td>55.4 (24.2-82.2)</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>11.9 (2.0-19.3)</td>
<td>40.8 (12.2-58.2)</td>
</tr>
</tbody>
</table>

Graph 1: Distribution of patients according to RNTCP grading system

DISCUSSION
The present prospective study was carried on a randomly selected study group comprising of 180 cases of pulmonary TB patients who were sputum smear positive for AFB and 25 normal healthy (sputum smear negative for AFB) individual were taken as control. The cases of PTB group was further categorized based on recommendation of RNTCP grading system of AFB in Sputum ZN staining into four subgroups AFB – 1+, AFB – 2+, AFB – 3+ and scanty. The control groups were negative for the Acid-Fast bacillus in sputum.
In the present study, 180 patients positive for Acid fast bacilli in their sputum were grouped into different grades of AFB positivity as per RNTCP grading system and that’s showed that 65(36.1%) patients were of 3+ grade, 58(31.7%) were of AFB 2+, and 34 (19.5%) were of AFB-1+, while 23(12.8%) were included in scanty group. The possible explanation to the gradual increase in the number of patients with increase in the grading in RNTCP grading system which might be due to the unawareness of patients regarding the diseases in initial stages, or avoidance of treatment, unavailability of hospital facilities at their native places and / or poor education level. It might also be possible that fear of hospitalization may be important cause of enhancing the stage of the disease. Mohammed Shameem et al (2012) also reported the maximum number of patients with AFB – 3+. [8]
The highest mean CRP value of the study was found to be 55.7 mg/l obtained in AFB3+ and the level of CRP value was found to be gradually decreasing with the grades of AFB positive patients with the lowest of 15.2 mg/l in scanty group. The CRP value was found to be directly proportional to the severity of disease. Similar result had reported by Mohammed et al (2012)[9] and Bing Sun et al (2012).[10] The increase in CRP level in tuberculosis as compared to that of normal control might be due to the activity of inflammation in PTB. The reduced lung function may be responsible for systemic inflammation. Like hepatocytes, inflammatory lung or pulmonary epithelial cells, have been shown to express CRP and IL-6. High levels of cytokines and acute phase reactants in peripheral circulation maybe a cause rather than a consequence of poor lung function and they also suggested that the magnitude of increased of serum CRP concentration over the normal level reflected the extent of tissue injury and might be predicted the course of enduring illness. The determination of serum CRP concentration has been employed as an aid to diagnosis and also acts as marker of the severity of the disease. Thus, the detection of CRP is more sensitive and more reliable marker in the inflammation process.[4,10,11]

The increased ESR was might be due to acute and chronic infection and systemic inflammation in pulmonary tuberculosis. The highest mean ESR value of the study was found to be 48.1 mm/hr obtained in AFB3+ and the level of ESR value was found to be gradually decreasing with the grades of AFB positive patients with the lowest of 15.7 mm/hr in scanty group. The ESR value was found to be directly proportional to the severity of disease. These data suggested that ESR increased with the increase of severity of the disease. Sarkar K et at (2004) and Kanfer and Nicof et al (1997) also reported that increase of ESR from moderate to extreme elevated level in moderate to acute phase of tubercular patients.[11,12]

CONCLUSION

Our study concluded that during the compression of values of CRP and ESR along with the grading of sputum AFB positive patients, the CRP raised more significantly as compare to ESR; with markedly raised values in all the grades of sputum AFB positive patients. Estimation of serum CRP before and after treatment in PTB can be helpful to evaluate the prognosis and also aids in objective assessment of the efficiency of chemotherapy used in the treatment.

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