Effect of antituberculosis drugs on serum immunoglobulins of pulmonary tuberculosis patients

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ABSTRACT
The levels of five immunoglobulins (IgG, IgA, IgM, Ig Kappa and Ig Lambda) were measured by “IMMAG” Immunochemistry System based on nephelometric principle, in sera of pulmonary tuberculosis patients. Samples were collected on zero day, 15 day, 1 month, 2 months, 3 months, 4 months and 6 months post treatment. Significant increase have been observed in IgA and IgG levels in zero day patients which declined up to 6 month post treatment period. A non-significant increase in IgM, Ig Kappa and Ig Lambda has been observed which also decreases from 15 days to 6 months post treatment periods. The results suggest that these changes of plasma immunoglobulins in pulmonary tuberculosis patients may be due to humoral responses against tubercular antigens and due to the antituberculosis drugs.

Key words : Anti-tuberculosis drugs, Immunoglobulins, IgG, IgM, Ig Kappa, Ig Lambda.

INTRODUCTION
Tuberculosis (TB) remains one of the leading causes of morbidity and mortality worldwide with approximately 8 million new cases and nearly 2 million deaths reported in 2006 by the World Health Organization (WHO, 2008). The mainstay for the control of TB is the rapid and accurate identification of infected individuals. The rapid and widely used method is the detection of acid-fast bacilli by microscopy (WHO; 2008).

However, 40% to 60% of patients with pulmonary disease and approximately 75% of patients with extrapulmonary disease are smear negative, and in this situation, even contemporary culture methods take several weeks to become positive (American Thoracic Society, 2000). Additionally, other pulmonary non-TB diseases such as bronchitis, cancer and pneumonia can mimic both the clinical and the radiographic symptoms of pulmonary TB patients (Ormenod, 1994). Therefore, a rapid diagnostic tool with both high sensitivity and specificity is needed to improve the conventional diagnostic methods, especially to enhance the early recognition of paucibacillary and smear negative TB cases (Wald-Ben-Selma et al., 2010). These workers have performed the study to evaluate the clinical usefulness of detection of immunoglobulins IgG, IgM and IgA antibodies raised against the mycobacterial A60 antigen for the diagnosis of active TB patients. In the present study we have attempted to find out the changes in IgA, IgG, IgM, Ig Kappa and Ig Lambda in anti-tuberculosis patients in comparison with non-treated, treated patients and healthy controls.

MATERIAL AND METHODS
The study was conducted on out-door patients of one of the Semi Govt. Hospitals of Mumbai. 50 Patients were examined in respiratory Medicine Unit. Patients of either sex between age group 15 to 60 year with pulmonary tuberculosis were studied for serum immunoglobulin pattern electrophoretically with respect to duration of treatment.

Diagnosis of tuberculosis (TB) was based on detection of Acid Fast Bacilli (AFB) and direct smear examination by Ziehl Nelson staining and also on culture for AFB (Warke and Khan, 2004). Serum analysis for immunoglobulins electrophoresis was carried out in Tata Memorial Hospital, Mumbai by “IMMAGE Immunoche-mistry System manufactured by Beckman Coulter” USA.

Patients were treated with standard antituberculosis drugs. All the drugs were given thrice a week through out for 6 months under the direct supervision of clinical staff. Patients were treated with the following drugs: Cap. Rifampacin (RIF) on empty stomach 1 Cap. (450 mg), Tab. Isoniazid (INH) 2 Tab. (300 mg), Tab. Ethambutol (ETH) 2 Tab. (600 mg), Tab. Pyrazinamide (PZA) 2 Tab. (750 mg).

All the above drugs were given for two months after which ethambutal and Pyrazinamide were ceased but...
treatment continued with Rifampacin and Isoniazid were given for a period of further four months. Blood samples of all patients were collected on zero day (before start of treatment), 15 days, 1 month, 2 months, 3 months, 4 months and 6 months post treatment period. Approval from Institutional Ethics committee for research work was obtained for the study. Venous blood samples were collected in plain bulb and serum was separated following standard methods.

Patients were taken of either sex in the age group of 15-60 years. New cases of Pulmonary T.B. were diagnosed by using Revised National Tuberculosis Control Programme (RNTCP) diagnostic algorithm.

Exclusion Criteria: Patients were excluded in the study for retreatment cases of pulmonary T.B., relapse, failure, treatment after defaulter with cagetory II, sputum smear negative for pulmonary T.B., and patients suffering from active liver disease, patients suffering from severe renal cardiac disease patients suffering from other associated pulmonary diseases, Pregnant and lactating females and patients suffering from HIV.

Selection of Control was done keeping in few: Blood of healthy 5 male volunteers in the age group of 15-60 years were used on the basis, and following lab investigations were carried out: Hb / CBC, ESR, LFT and AST, ALT, S. Bilirubin, BUN, Serum creatinin. Serum Immunoglobulin estimation was done by “IMMAGE” Immunochrometry Systems by using specific reagents and Kits for IgA, IgG, IgM, Ig Kappa and Ig Lambda respectively from BECKMAN COULTER Company, USA. All reagents were prepared for use as per the directions given in leaflet (Chemistry Information Sheet, 2010) in Kit (Chemistry information Sheet AUGUST 2010 IgA 988633, IgG 988636, IgM 988637, Ig Kappa 988640, Ig Lambda 988641 Procedure was followed as per instructions)

RESULTS AND DISCUSSION
Table 1 shows the effect of antituberculosis drugs on IgA, IgM, IgG, Ig Kappa and Ig Lambda, which are markers of tuberculosis. In healthy subjects of control group IgA concentration was found to be mean 185 mg/dl which was significantly increased in pretreated T.B Patients to 457.71 from 105.70 mg/dl (Zero days) as compared to the control group. The value decreased on post treatment with T.B drugs. A significantly gradual decreased value was observed on 15 days, 1 month, 2 months, 3 months, 4 months & 6 months respectively post treatment period of T.B drugs.

Table 1 :- Effect of Anti Tuberculosis drugs with respect to dose duration on plasma Immunoglobulins in T. B. Patients.

<table>
<thead>
<tr>
<th>Days</th>
<th>IgA</th>
<th>IgM</th>
<th>IgG</th>
<th>IgKappa</th>
<th>Ig Lambda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>185.5</td>
<td>153.6</td>
<td>1460</td>
<td>1250</td>
<td>673.5</td>
</tr>
<tr>
<td>Zero days</td>
<td>457.71</td>
<td>± 105.70</td>
<td>± 52.93</td>
<td>± 336.32</td>
<td>± 1911.42</td>
</tr>
<tr>
<td>15 days</td>
<td>426.71</td>
<td>± 96.72</td>
<td>± 52.33</td>
<td>± 274.28</td>
<td>± 213.85</td>
</tr>
<tr>
<td>1 Month</td>
<td>370.85</td>
<td>± 79.78</td>
<td>± 58.19</td>
<td>± 257.71</td>
<td>± 209.00</td>
</tr>
<tr>
<td>2 Month</td>
<td>337.42</td>
<td>± 80.01</td>
<td>± 57.21</td>
<td>± 283.75</td>
<td>± 1861.42</td>
</tr>
<tr>
<td>3 Month</td>
<td>303.37</td>
<td>± 164.79</td>
<td>± 51.05</td>
<td>± 568.38</td>
<td>± 445.88</td>
</tr>
<tr>
<td>4 Month</td>
<td>298.66</td>
<td>± 82.15</td>
<td>± 59.12</td>
<td>± 208.33</td>
<td>± 1648.00</td>
</tr>
<tr>
<td>6 Month</td>
<td>292.14</td>
<td>± 70.33</td>
<td>± 69.13</td>
<td>± 1901.42</td>
<td>± 401.08</td>
</tr>
</tbody>
</table>

P values: P = 0.584 Not Significant
P = 0.059 Not Significant
P = 0.078 Not Significant

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It was 426.71  96.72, 370.85  79.78,  337.42  80.01, 303.37  164.79, 298.66  82.15, 292.14  70.33 mg/dl respectively, which is higher than the control group, 185.5 mg/dl on 6th month the value was 292.14 and 70.33 mg/dl which is higher than control group.

IgM : - Similar pattern has been observed in IgM also. In control group the mean IgM Concentration was 153.6 mg/dl which increased to 190.42 from 52.93 mg/dl in pretreated T.B patients which is higher at zero days as compared to the control group. After treatment of T.B drugs the values gradually decreased on post treatment with T.B drugs. Gradual decrease in values was observed on 15 day 1 month, 2 months, 3 months, 4 months and 6 months, post treatment of T.B drugs. The values were 178.42  52.32 mg/dl, 166.71  58.19 mg/dl, 157.28  57.21 mg/dl, 137.64  51.05 mg/dl, 138.68  59.12, 161.18  69.13 mg/dl. The 6th month value was 161.18  69.13 ml/dl respectively.

IgG : - Significant changes have been observed in IgG values. In Control group IgG Concentration was 1460 mg/dl which got increased to 2575.71 from 336.39 mg/dl in pretreated T.B patients i.e. Zero days Compared to the control group. After treatment of T.B drugs the values gradually decreased on post treatment with T.B drugs. IgG concentration reached to highest value on 15th day post treatment which was 2744.28 from 481.0 mg/dl as compared to Zero day group. After one month treatment of T.B drugs till 6 months values significantly decreased from 2575.71, to 2322.85, 2085.28, 2028.33 1901.42 mg/dl. the 6 months value was 1901.42 which was still higher than control group.

Ig Kappa, Ig lambda :- Similar pattern has been observed in Ig Kappa, Ig lambda, in control group Ig Kappa mean was 1250 mg/dl while Ig Lambda mean was 673.05 mg/dl, which increased to 1991.42 and 1190.28 respectively in Ig Kappa and Ig lambda.

After treatment of T.B drugs the Ig Kappa concentration reached highest value on 15th day post treatment which was 2132.85  297.52, this was higher than Zero days Ig Kappa. A gradual decrease in value was observed on 15th day, post treatment which was 1181.42 (178.17) which is lower than zero days of Ig Lambda. A significant gradual decrease in values was observed in Ig Kappa and Ig Lambda after 1 month, 2 months, 3 months, 4 months & 6 months respectively being 2090  560.50, 1063.14  248.67, 1861.42  445.88, 1001.14  268.51, 1648  507.13, 904.57  390.35, 1664.66  410.08  841.33 183.92. On the 6th month the values of Ig Kappa and Ig Lambda were 1521.57   329.01, 845.42  188.46 respectively which are quite higher than control group.

Diagnosis of TB can be difficult and combines clinical presentation, radiologic results, acid-fast staining, and culture methods. In fact, smear staining of sputum has a sensitivity of only 50% to 60%, and mycobacterial culture usually requires 6 to 8 weeks to be interpretable (Aber et al., 1980; American Thoracic Society, 2000). Thus in this situation clinicians are often confronted with patients suspected of mycobacterial infection and the necessity to introduce or not an antituberculous therapy, which is associated with moderate to severe side effects, including polyneuropathy and hepatitis (McNeill et al., 2003).

It is therefore important to correctly diagnose TB before the onset of therapy. New serologic tests for the diagnosis of TB infection have become commercially available. These tests detect IgG, IgM, and IgA raised against mycobacterial A60 antigen. The A60 antigen complex is present in the cytosol of typical and atypical mycobacteria (Cocido, 1991). It contains about 89 epitopes recognized by different antibodies. It is part of the group of antigenic complexes called the thermostable macromolecular antigens, which are
highly conserved within the mycobacteria. Significant changes in the levels of several serum proteins have been observed to be associated with tuberculosis. However, though they may not be of any diagnostic or aetiological significance, they do indicate a humoral response of the body to infection or against tubercular antigens (Chong and Nilmani, 1989 and CIS, 2010).

These tests may not be or may be of diagnostic value, they do, however, indicate the response of the body to the infection and recovery after treatment. In particular, the raised values of IgA, IgG, Ig Kappa and Ig Lambda in T.B. infected patients may be due to humoral response against tubercular antigens. In the present study IgG was significantly higher at zero months as compared to the control population and also IgA was significantly higher at zero months.

IgM had no significant difference but slightly higher value was observed at zero months as compared to control population. Ig kappa and Ig Lambda were also significantly higher from the control. In general, IgG levels have been reported to be increased in pulmonary tuberculosis (Faulkner et al., 1967; Buckley and Dorsey 1970; Alarcon-segovia and Fishbein, 1971; Grange et al., 1980; Gatner et al., 1982; Grange et al., 1984; Papiha et al., 1985; Caplin et al., 1989).

IgA levels have also been reported to be increased in several studies (Faulkner et al., 1967; Alarcon-segovia and Fishbein, 1971; Grange et al., 1980; Gatner et al., 1982; Grange et al., 1984; Caplin et al., 1989; Bhatnagar et al., 1977).

As for IgM levels, they have also been reported to be increased by Alarcon-segovia and Fishbein, (1971) and Grange et al., (1984) while several studies revealed no significant change (Faulkner et al., 1967; Buckley and Dorsey 1970; Grange et al., 1980; Gatner et al., 1982; Papiha et al., 1985; Bhatnagar et al., 1977) it is well the serum IgG levels increase in pulmonary tuberculosis which is in agreement with most of the published reports as for IgA levels have been reported to be increased in several studies.

While for IgM levels only two of these papers have reported increase levels, while the rest reported no change. It is likely that the presently observed rise in IgG and IgA levels is due to the increased production of these antibodies in response to the presence of the mycobacterial Antigens. The normal response of the body to any antigen is an initial increased production of IgA antibodies which normally precedes IgG production. IgA had been reported to be increased (Faulkner et al., 1967; Alarcon-segovia and Fishbein, 1971; Grange et al., 1980; Gatner et al., 1982; Grange et al., 1984; Papiha et al., 1985; Bhatnagar et al., 1977; Sela et al., 1977) or not changed (Buckley and Dorsey 1970; Emmett et al., 1987 and Walid et al., 2010).

It would be of interest to measure the level of IgA in pulmonary secretion of these patients. IgG concentration was significantly lower at 6 months from zero months but was still significantly higher than that in the control series. IgM concentration at 6 month was not significantly different from zero months or control values. Ig Kappa and Ig Lambda concentration was significantly lower at 6 months than at zero days but was still significantly higher than that in the control series. In general trend is that as the condition of the patients improved the levels of all five immunoglobulins decreased towards control values. IgG and Ig Kappa concentration increased at 15 days but decreased gradually in 6 months and got significantly lowered only at 6 months as compared to that of zero months.

In conclusion, significant changes in the five immunoglobulins have been observed to be associated with tuberculosis. Though they may not be of diagnostic value, they do, however, indicate the response of the body to the infection and recovery after treatments. In particular the decreasing levels of IgG, IgA, IgM, Ig Kappa and Ig Lambda with treatment may reflect response to medication in the patients.

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Chemistry Information Sheet (2010) IgA 988633 AE, IgG 988636, IgM 988637, Ig Kappa 988640, Ig Lambda 988641.


