Original Research Article

Immunoglobulin profile in pulmonary tuberculosis in endemically prevalent Kashmiri population, Kashmir, India

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ABSTRACT

Background: Kashmir valley is endemically prevalent destination for tuberculosis. The role of T cell mediated immunity is very well known in the pathogenesis of tuberculosis. However, the B cell immune response has been studied less often. Thus, it was very interesting to estimate the levels of serum immunoglobulins in pulmonary tuberculosis.

Methods: Serum levels of the immunoglobulins IgG, IgA and IgM were evaluated in 100 adult patients of untreated sputum positive cases of pulmonary tuberculosis in the age group of 20-65 years (36.42±8.95) and 50 controls of the same age group (34.26±9.46). The Immunoglobulins were estimated using single radial immunodiffusion method of Mancini et al.

Results: The mean IgG and IgA levels were observed to be significantly increased in the patient group when compared with the controls (P<0.001). However, no significant difference was observed in the IgM levels between the two groups. There was no significant difference in the immunoglobulin levels between the two sexes as also between the various defined age groups. The mean serum IgG levels showed significant correlation with the duration of illness (P<0.001-highly significant), while as the difference was insignificant in case of IgA and IgM. Further, the immunoglobulin levels did not show any significant correlation with the extent and the nature of lesion on chest radiography.

Conclusions: Serum IgG and IgA levels were significantly increased in patients of pulmonary tuberculosis as compared to controls. However, no significant difference was observed in serum IgM levels.

Keywords: Immunoglobulins, Pulmonary, Radiography, Single radial immunodiffusion method, Tuberculosis

INTRODUCTION

Tuberculosis is a widespread infectious disease caused by various strains of Mycobacteria, usually Mycobacterium tuberculosis and commonly occurring in the lungs as pulmonary tuberculosis. The disease is characterized by the formation of granulomas in the infected tissues and by cell mediated hypersensitivity.

The discovery of tuberculosis bacillus was the starting point for further advances and the most obvious practical advance was diagnostic. A definitive diagnosis of tuberculosis is made by identifying M. tuberculosis in a clinical sample e.g. Sputum, pus, tissue biopsy etc. The detection of serum immunoglobulin levels may help as supportive serological evidence. The various serological methods used for immunoglobulin estimation include single radial immunodiffusion (SRID), Radio Immuno-Assay (RIA), Enzyme Linked Immunosorbant assay (ELISA).

There is lot of diversity in various reports of immunoglobulin response in pulmonary tuberculosis as detected in various studies. There are reports of significant increase of IgG, IgA and IgM levels in pulmonary tuberculosis. However, some have
observed a significant increase of IgG and IgA, some of only IgG, some of only IgA and some have observed no increase of all the three studied immunoglobulins.\textsuperscript{11-21} In view of the varied reports, it seemed to be quite interesting to estimate the levels of serum IgG, IgA and IgM levels in pulmonary tuberculosis in the Kashmir Valley, where we observe many such cases across the various ethnic groups.

**METHODS**

The current study was evaluated in 100 untreated sputum positive patients of pulmonary tuberculosis in the age group 20-64 years (36.42±8.95). The control group comprised 50 apparently healthy individuals of the same age group (34.26±9.46), taken from amongst the medical students, resident doctors and other hospital staff, besides others. The patient group was evaluated to rule out any problem that would otherwise interfere with the immunoglobulin levels.

The diagnosis of pulmonary tuberculosis was based on clinical, radiological, bacteriological and other relevant laboratory evidence. Health of the controls was ascertained on the basis of freedom from complaints of acute or chronic diseases and absence of obvious abnormalities. The sputum examination for M. tuberculosis was performed, using Ziehl Neelson staining and only those patients who were confirmed sputum positive on three separate occasions were included in the study group. The patient group was divided on the basis of different parameters as under:

**Sex/Age**

The patient group comprised 58 males and 42 females. The age of patient group was arbitrarily divided into three sub-groups i.e. a) 20-34 years (30 patients), b) 35-49 years (42 patients) and c) 50-64 years (28 patients).

**Duration of illness**

The patients were divided into three sub-groups, which comprised a) <3 months (25 patients) b) 3-6 months (46 patients) and c) >6 months (29 patients).

**Extent of lesion**

Three sub-groups as per the definition by National Tuberculosis Association New York-1961, based on the extent of lesion on X-Ray Chest (PA view) included a) Minimal Lesion (ML)-28 patients b) Moderately Advanced Lesion (MAL)-46 patients and c) Far-Advanced Lesion (FAL) - 26 patients.\textsuperscript{22}

**Nature of lesion**

The patients were also distributed as per the nature of lesion on X-Ray Chest (PA view), which included a) Exudative (E)-52 patients b) Fibrocavitatory (FC)-19 patients and c) Fibroproductive (FP)-29 patients.

**Immunoglobulin estimation**

The immunoglobulins were estimated by single radial immunodiffusion method of Mancini at al, using commercially available tripartigen plates (separate for each immunoglobulin).\textsuperscript{23} Each plate contained a prepared solidified agar gel, into which H-Chain specific antiserum (produced by immunization of goats) to the respective immunoglobulins (i.e. IgG, IgA and IgM) was already incorporated. Each plate had 12 wells, made out of cutting into the solidified agar.

Five milli-litres of blood, drawn by venupuncture was allowed standing. The serum, thus separated, was stored in a refrigerator after adding 0.1% sodium azide. The serum samples were utilized for immunoglobulin estimation on the next day. In one of the 12 wells of the tripartigen plate, 5 micro litres of control standard serum (WHO reference serum No. 67/97, containing 770 gms IgG, 135 mgs IgA and 81 mgs IgM in 100 ml) was put, and rest of the wells were filled with 5 micro litres of the test sera. As instructed, the plates were closed and left to stand at room temperature till the diffusion was complete (50 hours in case of IgG and IgA and 80 hours in case of IgM). Precipitin ring diameters were measured using a specific calibrated scale. The immunoglobulin concentrations related to the measured diameters were read directly from the table of reference values.

**Statistical analysis**

Statistical comparisons were based on students “t” test, ANOVA (Analysis of Variance) test and Pearson’s correlation. A value of 0.05 or less was considered significant.

**RESULTS**

The increase in mean serum IgG and IgA levels in the patient group was highly significant (P<0.001), when compared with controls. However, the difference in case of IgM was not significant (P>0.10). The mean serum immunoglobulin levels (mainly IgG and IgM) were observed to be marginally increased in females as compared to those of males, but the difference was not significant P>0.20 (Table 1).

The mean serum IgG showed a marginal decrease with age, but the difference was not significant (P>0.20). Further, no significant difference was also observed in case of IgA and IgM among the defined age group (P>0.20). The mean serum IgG and IgA showed an increase and IgM a marginal decrease with the duration of illness, but the difference was highly significant (P<0.001) in case of IgG and not significant in case of
IgA and IgM-P>0.20 (Table 2). The mean serum IgG and IgA showed an increase with the extent of lesion (on X-Ray chest PA view), but the difference was not significant (P>0.05). Also, the difference in case of IgM was not significant (P>0.05). The mean serum immunoglobulin levels also varied with the nature of lesion on X-Ray chest PA view (i.e. Exudative> Fibrocatitatory> Fibroproductive in case of IgG and IgA and Fibroproductive >Exudative> Fibrocatitatory in case of IgM), but the difference was not significant-P>0.05 (Table 3).

**DISCUSSION**

Mycobacterium tuberculosis comprises at least 50 various antigenic components and the fractional estimation demonstrates only a fraction of the total humoral response. Instead, estimation of the total serum IgG, IgA and IGM gives a true reflection of the humoral response and therefore, the antigenic load in tuberculosis. A highly significant increase of mean serum IgG and IgA levels in the patient group (P<0.001) and an insignificant difference in case of IgM, as observed in our study, is in conformity with some other reports.11-17

However, some have observed a significant increase of all the three, some of only IgG, some of only IgA and some in none of the three studied immunoglobulins.6-10,18-20 We observed a more predominant rise of IgA than IgG, in conformity with some other observations.12-14
However, some have reported a more predominant rise of IgG than IgA. The immunoglobulin levels, as observed in our study, differ from those in other Indian and various racial communities. The possible reasons, as attributed are environmental, genetic and racial factors. The levels are expected to vary in different laboratories and according to the technique employed. Thus, it becomes imperative that each laboratory should establish its own range of normal values for the genuine assessment of any variation in disease.

The increase in IgG in the patient group suggests an immunoglobulin response, related to be due to purified protein derivative. As it is evident, the serotary surfaces remain exposed to a variety of infections all the time. Thus, a highly significant rise of serum IgA among the patient group is not surprising, as it is the most abundant, naturally occurring antibody responsible for the immune surveillance of the secretory surfaces.

Further, it has earlier been observed that the chronicity of disease results in waning of the initial IgM response, with subsequent rise of both IgG and IgA. This justifies our observation of increase in mean IgG and IGA (IgG significantly so) and decreases in IgM with the duration of illness, as 75% of our patients had an illness of more than 3 months duration at the time of estimation. There are reports indicating a direct correlation between the antibody levels and the duration of illness, while as some have observed no such correlation.

We did not observe any significant difference in the mean immunoglobulin levels between the two sexes in the patient group, which is in agreement with some other authors. However, some have noticed a significant rise of IgA in female patients. The mean serum IgG showed a marginal decrease with the age in the patient group, which was not significant. This is in conformity with some earlier reports. However, some have observed that IgM (in addition to IgG) decreases with age, while as, IgA is changed very little.

It has earlier been observed that the adult levels of serum IgG and IgM are attained by 16 years of age but IgA continues to raise throughout early adulthood. The increase in mean immunoglobulin levels with the extent of lesion was not significantly correlated. However, some have noticed significant increases of IgA and some of all the three immunoglobulins with the extent of lesion. We observed that the mean IgM in the MAL group was increased when compared with the other two groups, especially FAL. This is in conformity with some other authors. We believe that in FAL group, antibodies may be increasingly circulating as immune complexes.

Also, the disease in FAL group may be more chronic to explain the fall in IgM levels. No significant difference was observed in the mean immunoglobulin levels as per the nature of lesion, in agreement with some other reports. However, some have observed increased antibody litres in the fibrocavitatory lesions. Further, as inverse relationship between the mean IgM levels and the extent of cavitation has been observed by some authors. This is in conformity with our observation of decreased mean IgM level in Fibro cavitatory lesions, when compared with the other two groups.

CONCLUSION

Although the role of humoral immunity in tuberculosis remains unclear, yet the hyperhumoral response seen in pulmonary tuberculosis probably indicates bacterial loading. Further, an inverse relationship has been observed between the cell mediated and the humoral immunity in tuberculosis. It is, therefore, suggested that there is a definite need to boost up T cell mediated response and to control the over activity of B cells in these patients.

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