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# SENSITISATION PATTERN OF HEALTHY VOLUNTEERS AND TUBERCULOSIS PATIENTS TO VARIOUS MYCOBACTERIAL ANTIGENS BY ELISA

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**Summary;** The sensitisation pattern of 39 tuberculosis patients and 21 healthy volunteers to 9 different mycobacterial antigen sonicates was estimated using ELISA. The antibody levels of patients and volunteers were high against *M. tuberculosis*-7219, *M. kanasii* and *M. scrofulaceurn* and low against *M. chelonei* and *M.fortuitum*. The tuberculosis patients showed a mean antibody level which was significantly different from that of volunteers to *M. tuber culosis*-7219, *M. kansasii, M. scrofulaceum, M. tuberculosis* S.I., *M. bovis* and PPD-S. With respect to three antigens, namely, *M. chelonei, M. fortuitum* and *M, avium intracellulare,* there was no significant difference between patients and volunteers.

## Introduction

Environmental mycobacteria are highly prevalent in most tropical areas and also exist in some subtropical areas (Nyboe, J., 1960). The majority of these are of either low or no virulence for men but many cause inapparent infections. Theoretically such infections could be expected to alter the immune status of the host not only against the causative organism but also against the classically pathogenic mycobacteria if the two are antigenically related. The most predominant species isolated from Chingleput District and the urban areas adjoining Madras are *M. avium intracellulare* followed by *M. terrae* and *M. scrofulaceum* (Paramasivan *et al,* 1985). The evidence for sensitisation of the individuals living in the endemic area has been established using skin test reactivity to PPD-B (Tuberculosis Prevention Trial, 1980). But this skin test using PPD-B does not indicate the organism to which the individual is exposed. In the present study ELISA was used to identify the sensitisation pattern of volunteers from this endemic area and tuberculosis patients to various mycobacterial antigens.

## Materials and Methods

*Patients*

A total of 39 pulmonary tuberculosis patients whose "sputum were positive by smear and culture for *M. tuberculosis* and who have had no previous chemotherapy were included in the study.

*Control:*

Some of the staff members of the Tuberculosis Research Centre and the volunteers attending a blood bank were included as controls. In all, there were 21 controls.

*Preparation of sonicate extract of mycobacteria:*

The mycobacteria were grown on the surface of solid Sauton's agar medium until confluent colonies were just apparent. The cultures were then harvested and sonicated in phosphate buffered saline (Dulbecco 'A') for 15 minutes (in 5 second bursts) using a Rinco model MP ultra sonicator at 70% maximum intensity. After ultracentrifugation of the lysates at 100,000 g for 1 hour the supernatants were sterilized by filtration (0.22 urn Millipore SLGV membrane) and adjusted to a standard protein content by Lowry's method (Lowry *et al,* 1951).

*Quantitation of specific IgG:*

The amount of specific anti-mycobacterial antibodies of class IgG in the sera of patients and controls to various mycobacterial antigens, viz., South Indian variant of *M. tuberculosis*-7219, British strain of *M. tuberculosis-Si, M. bovis,* PPD-S, *M. kansasii, M. scrofulaceum, M. avium intracellulare* serotype-8, *M.fortuitum* and *M. chelonei* were measured by Enzyme linked immuno sorbent assay /(ELISA). The assay was done according to the method of Voller *et al*., (1976) as slightly modified by *Narayanan et al* (1983).

Coating of the microtitre plate (96-well, U-bottom plates from Dynatech Laboratories Inc., VA, USA.) with antigen was carried out by dispensing 0.1 ml of the antigen (5 ug/ml) in bicarbonate buffer, 0.06 M pH 9.6, into

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each well and sealing the plate with nylon sealing tape. The plate was incubated at 37°C for 3 hours and then stored at 4°C until use.

Before using, the plate was washed with three changes of phosphate buffered salinc-Tween-20 (PBST). Next, 0.1 ml of 1/20 dilution of serum was dispensed into the first row of the 96-well plate and 50 ul of PBST was dispensed into the other wells. The serum was serially diluted starting from 1/40 dilution upto 1/2560. The plate was again washed as before and 0.1 ml of 1/1000 diluted antibody conjugate (Peroxidase conjugated goat anti-human IgG obtained from Cappel Laboratories Inc., Cochranville, PA, USA) was added per well. Incubation and washings were carried out as mentioned earlier. The substrate stock solution consisted of Ortho-Phenylene diamine (OPD) 2.5 mg in 5.0 ml of distilled water. 0.5 ml. of the above stock solution and 50 ul of 3% $H_2O_2$were added to 49.5ml of distilled water. To each well, 0.1ml of this diluted substrate was added. After 30 minutes of incubation at room.pa temperature in the dark, 50 ul of 8N/$H_2SO_4$ was added to each well to arrest the reaction.

The optical density (O.D) of the resultant colour was read at 492 nm, using a Titertek Multiscan vertical photometer (Flow Laboratories, VA, USA). All the serum samples were randomised and coded so their identity was unknown to the laboratory worker setting up the test.

**Results**

Table-1 shows the mean antibody levels of 39 patients and 21 volunteers against 9 different mycobacterial antigens at 1/40 dilution. The antibody litres in both patients and volunteers were highest against South Indian variant of *M. tuberculosis* strain-7219 followed by *M. scrofulaceum, M. kansasii,* British strain of *M. tuberculosis-SI, M. bovis, M. avium intracellulare* serotype-8, *M. chelonei,* PPD-S, and *M. fortuitum.* The patients had significantly higher antibody levels than the volunteers against *M. tuberculosis-1219, M. kansasii, M. scrofulaceum, M. bovis,* PPD-S and British variant SI; the antibody levels between volunteers and tuberculosis patients were not significantly different with respect to *M. chelonei, M. fortuitum* and *M. avium intracellulare* serotype-8. The mean antibody level of 39 palienls to *M. tuberculosis-7219* was significantly, higher than the mean antibody level to other mycobacterial antigens. But, the mean antibody level of volunteers to *M. tuberculosis-1219* and other mycobacterial antigens did not differ significantly.

The O.D. value in the individual patients and volunteers who showed reactivity at 1/160 dilution is represented in Fig.l & 2.

TABLE 1

*MeanLevelof Antibody Bind ing (O.D.) to various Antigens in Tuberculosis Patients and Healthy Volunteers*

| S. No. | Antigen | Patients (39)* | Volunteers (21) | P-value |
|---|---|---|---|---|
| 1. | *M. tuberculosis-1219* | 0.345 ± 0.099 | 0.247 ± 0.087 | 0.001 |
| 2. | *M. scrofulaceum* | 0.299 ± 0.074 | 0.237 ± 0.071 | 0.001 |
| 3. | *M. kansasii* | 0.290 ± 0.048 | 0.237 ± 0.052 | 0.001 |
| 4. | *M. tuberculosis-SI* | 0.254 ± 0.049 | 0.222 ± 0.049 | 0.020 |
| 5. | *M. bovis* | 0.243 ± 0.059 | 0.211 ± 0.055 | 0.050 |
| 6. | *M. avium intracetlulare-S8* | 0.161 ± 0.064 | 0.184 ± 0.069 | 0.2 (N.S.) |
| 7. | *M. chelonei* | 0.151 ± 0.083 | 0.122 ± 0.078 | 0.2 (N.S.) |
| 8. | PPD-S | 0.149 ± 0.047 | 0.113 ± 0.060 | 0.02 |
| 9. | *M. fortuitum* | 0.124 ± 0.059 | 0.153 ± 0.054 | 0.07 (N.S.) |

*No of tuberculosis patients.

Fig, 1

**Antibody Levels of Patients and Volunteers to various Mycobacterial Antigens**

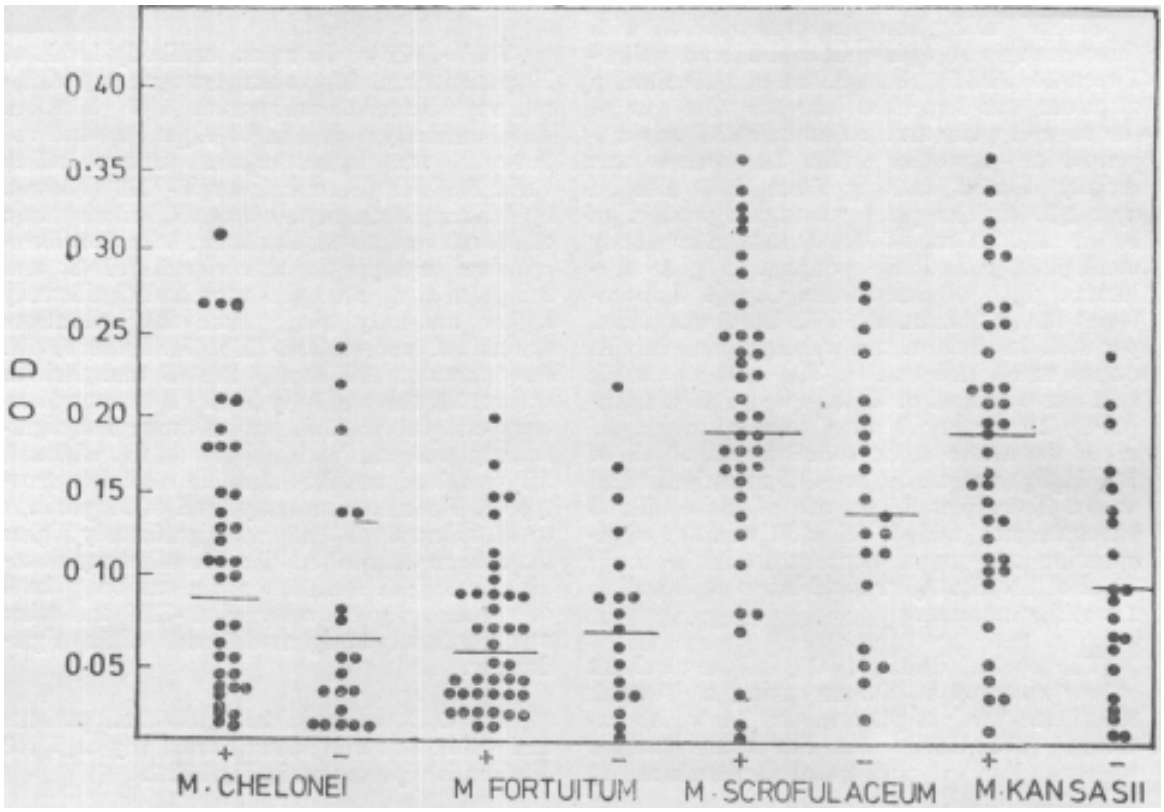
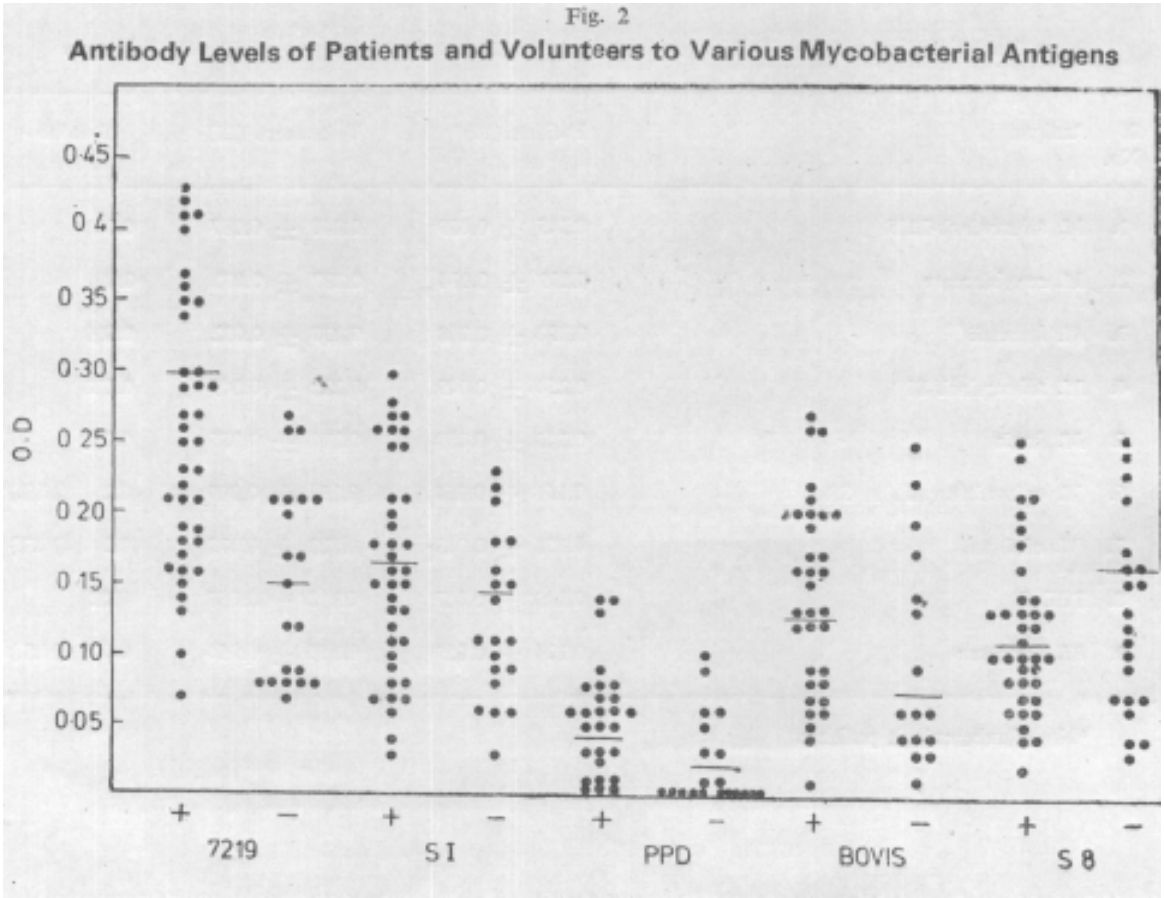



Fig. 2

**Antibody Levels of Patients and Volunteers to Various Mycobacterial Antigens**



There is a similarity in the antibody levels of patients against 7219, *M. kansasii* and *M. scrofulaceum. M. chelonei,* PPD-S and *M. fortuitum,* showed very low antibody levels in both patients and volunteers.

## Discussion

The mean antibody level of 39 patients to *M. tuberculosis-1219* was significantly higher than mean antibody levels to other mycobacterial antigens. But, the antibody levels of volunteers to *M. tuberculosis-1219* and other mycobacterial antigens did not differ significantly. This indicates the possibility that antibodies from patients recognise specific antigenic component along with other cross reacting components in *M. tuber culosis-7219* whereas the antibodies from volunteers lack this recognition capacity.

The antibody levels of the tuberculosis patients to the following antigens *M. tuberculosis-1219, M. kansasii, M. scrofulaceum,* British strain-SI and PPD-S were significantly higher than those of the control subjects. But the limitation of an extensive overlap between the control subjects and patients was observed with all the five antigens. The other mycobacterial antigenic extracts tested also showed binding, but to a lesser extent.

The high levels of antibody of patients and volunteers to *M. kansasii* and *M. scrofulaceum* indicate that these two species share more number of common cross reacting components with *M. tuberculosis-7219* which is the South Indian variant of *M. tuberculosis* or the exposure to these organisms is very common in the area. Previous study on DNA homology employing relative percent binding and thermal stability of bound DNA also showed that *M. kansasii* had high correlation with *M. tuberculosis* (Wendy and Lawrence, 1970).

In spite of the fact that crude sonicate extracts of mycobacteria were tested using a highly sensitive technique like ELISA, only low levels of antibody were consistently observed in patients with tuberculosis. The antibody levels do not seem to correlate with the severity of disease. It is not known whether the low levels of antibody are due to generalised or specific immunosuppression, or is genetically determined. However, the analysis of antibody deserves attention from the point of serodiagnosis though there has not been any convincing role for these antibodies in the protective immunity to tuberculosis.

Exposure to environmental mycobacteria. may account for some of the antibodies observed. The presence of such organisms in tap water, natural water and soil would facilitate such a continuous and universal exposure (Mollohan *et al,* 1961). Hence, it is likely that sensitisation with environmental mycobacteria could result in weak non-specific reactivity. Kulkarni and Kamath (1986) have shown cross-reactivity in the delayed hypersensitivity response in Swiss white mice immunised with live mycobacteria. All the mycobacterial strains tested gave cross-reactions and generally slow growers gave stronger cross-reactions with other slow growers than with rapid growers and vice versa (Kulkarni and Kamath, 1986).

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