EVALUATION OF ANTI-TUBERCULOUS ANTIBODIES IN HEALTHY CONTACT AND NON-CONTACTS PERSONS

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This study was conducted to see the presence of the antimycobacterial antibodies in healthy household contacts of tuberculous patients and healthy normal subjects who have never been in contact with tuberculous patients. A total of 200 subjects, 120 with history of household contact and 80 without such history were included in the study. Routine Haematological investigations were performed and all the sera of 200 subjects were tested for IgM, IgG and IgA anti tuberculous antibodies using ELISA technique. There was no difference in the average age of the household contacts and non-contacts. The complaints of pyrexia, night sweats and loss of weight was more in house hold contacts as compared to non-contacts. The awareness about BCG vaccination was equal among the household contacts and non-contacts. The combined serological positivity of the household contacts was 65.8% and the combined serological positivity for non-contacts was 34.1%. There was no statistically significant difference in the presence of IgM among household contacts as compared to non-contacts. However both IgG and IgA were present in significantly higher number of household contacts as compared to non-contacts. This study concludes that the persons living in the house with a patient suffering from active pulmonary tuberculosis (household contact) have more chances of being infected with Mycobacterium tuberculosis as compared to the healthy non-contacts.

Tuberculosis is an ancient and common communicable disease that is known to have existed in prehistoric time.[1] It is a chronic infectious disease of worldwide importance, and according to the latest survey conducted by this organization TB still exists at an alarming level with about one third of the world's population infected with Mycobacterium tuberculosis, eight million people developing the disease, and two million to three million people dying of TB each year.[2,3] It affects both sexes and all ages due to poverty, overcrowding, low socio-economic status, multiple pregnancies, smoking, smoke exposure, lack of health education, under-nutrition, poor housing environment etc.[4-7] Tuberculosis has been neglected as an important public health issue for many years which remains the major cause of death from a single infectious agent among adults in developing countries. Tuberculosis morbidity and mortality continues to rise because of deterioration of public health system.[8]

There is an estimation of 8 million new cases of tuberculosis, each year leading to 3 million deaths.[9]

The incidence of tuberculosis in Pakistan was 234/100,000 in 1995, and has been estimated to rise to 269/100,000 by the year 2005. One of the maxims of tuberculosis control has been inadequate therapy, which is worse than no therapy at all.[10]

Mycobacterium is rich in lipids. The lipids are fatty acid (Mycolic Acid), waxes and Phospholids. They are bound to proteins, polysaccharides and phosphatides. The fatty acids induce the formation of granulomas and phosphatides cause caseation necrosis. Lipids are responsible for acid fastness of the mycobacterium. The proteins behave as an active antigen and elicit the formation of a variety of antibodies. The polysaccharides, induce the immediate type hypersensitivity and can serve as antigen in reactions with sera of infected persons. They bring about immediate accumula-tion of polymorph nuclear leukocyte at the site of lesion. Their role in pathogenesis of tuberculosis is uncertain.[13]

Epidemiological data suggest that BCG vaccination imparts greater and more consistent protection against systemic disease, in particular milliary tuberculosis and tuberculous meningitis in children, than against pulmonary disease.[14]

An ideal serological test should be able to differentiate between the antibodies arising in response to tuberculous infection of disease, on one hand, from antibody arising in response to BCG vaccination on the other hand.[15,16,17]

Enzyme linked immunosorbent assay has been shown to be a rapid, reliable, and relatively simple test, which could be performed easily in developing countries, as a highly specialized equipment is not required. This test has excellent sensitivity and specificity. It has been shown to be of great value in developing countries, where its sensitivity is likely to be higher than in developed countries because tuberculosis is expectedly more severe in developing countries.[18,19,21-24]

This study is designed to compare the presence of the antimycobacterial antibodies in healthy household contacts of tuberculous patients and healthy normal subjects who have never been in contact with tuberculous patients.

MATERIALS AND METHODS

The study included 200 persons selected among the family members suffering from active pulmonary tuberculosis and normal healthy persons who did not have any history of contact with the patients of pulmonary tuberculosis. Different categories of subjects irrespective of age and sex were as Group-I, 49 persons living in the same house as patients of active pulmonary tuberculosis. These persons (49) had positive Mantoux test (table 1, 2).

In group II, 71 Persons living in the same house as patients of active pulmonary tuberculosis. They shows negative Mantoux tests. In group III, 80, normal healthy persons without any history of contact with patients of active pulmonary tuberculosis. These persons had Mantoux test negative.

Three (3) ml of blood was collected and transferred to the vial containing anticoagulant for routine haematological investigations like haemoglobin, total and differential lucocyte counts etc. Three ml of the blood was delivered into a sterilized centrifuge tube. The serum was stored at -20 C until it was tested by ELISA for the presence of IgM, IgG and IgA antibodies against tuberculosis.

The chest Radiography were taken. The mantaux test was performed on every subject:

IgG, IgA and IgM antimycobacterial antibodies were detected by enzyme linked immunosorbent assay utilizing microtitration plates coated with A-60 antigen extracted and purified from mycobacterium bovis provided by ANDA biological S.A.

Student's' t test and Chi-Square tests were applied for evaluation of our observations.

RESULTS AND OBSERVATIONS

Our study included 200 subjects, out of these 120 were the persons who were apparently healthy, but living in the same house in which patients are suffering from active pulmonary tuberculosis (contact). Eighty subjects were selected among the persons who were healthy and did not have any known contact with patients suffering from pulmonary tuberculosis (non-contact), as control.

The females were slightly greater in members than males in both the contact and contact groups. The mean age of the household contacts included in the study was 24.70 ± 17.24 years and that of non-contacts was 26.40 ± 15.5 years. There is no statistical difference in the mean age of the household contacts and non-contacts ($P>0.05$). The maximum number of household contacts & non-contacts was in the age group 10-19 years (Table 1-2).

Twenty-six household contacts (21.66%) gave history of cough and 12 (10%) household contacts gave history of pyrexia and night sweats. There was almost no history of cough (1.21%) among the non-contacts. Significantly higher number of household contacts showed history of cough ($P<0.05$), presence of pyrexia ($P<0.05$), history of night sweats ($P<0.05$), weight loss ($P<0.05$) and haemoptysis ($P<0.05$) as compared to non-contacts. Forty household contacts (33.33%) showed findings in the respiratory system on physical examination. No positive finding was seen in systemic examination of non-contacts.

A total of 51 household contacts (53.12%) had a positive history of BCG vaccination as compared to 45 non-contacts (46.88%). A statistical analysis shows that there is no difference of BCG vaccination among the household contacts and non-contacts ($P>0.05$). Table 3 shows distribution of household contacts and non-contacts according to the presence of BCG scar, 43 (35.8%) household

Table 1: *Sex distribution of household contacts and non contacts.*

Sex	Household Contacts (120)	Non-Contacts (80)	Total
Male	52	40	92
Female	68	40	108
Total	120	80	200

Statistical Analysis: $P>0.05$ (Non-significant)

Table 2: *Distribution of household contacts and non contacts according to age groups.*

Age groups	Household Contacts (120)	Non-Contacts (80)	P-Value
0-9 Years	27	8	<0.05 (S)
10-19 Years	29	32	>0.05 (NS)
20-29 Years	21	8	<0.05 (S)
30-39 Years	22	11	0.1<p>0.05 (APRO-S)
40-49 Years	7	12	>0.05 (NS)
50-Years and above	14	9	<0.05 (S)

Key: S = Significant, NS = Non-significant, APRO-S = Approaching Significant

contacts showed BCG scar as compared to 38 (47.5%) non-contacts. The statistical analysis reveals that there is no difference in the presence of BCG scar among the household contacts as compared to non-contacts (P>0.05). The Mantoux test was positive in 49 (48.8%) of contacts. In contrast only 3 (3.75%) non-contacts showed a positive mantoux test. There is statistically highly significant difference between the household contacts and non-contacts, (P<0.01).

The mean ± SD values of haemoglobin in household contacts were 11.7 ± 2.4 gm/dl and it was 11.9 ± 2.2 gm/dl in non-contacts. Statistically there is no significant difference (P>0.05). The mean ± SD values of ESR in mm after first hour for the household contacts was 20.25 ± 18.5 and it were 16.37 ± 16.6 in non-contacts. The mean values of total leukocyte count (TLC) were 8.05 ± 1.4x10^9/L in the household contacts and 7.5 ± 1.06x10^9/L in the non-contacts. The household contacts showed significantly higher TLC as compared to the non-contacts (P<0.05).

IgA was present in significantly higher number of household contacts as compared to non-contacts (P<0.05). Table 3 shows the distribution of IgG in household contacts and non-contacts. There is significant difference in the presence of IgG between household contacts and non-contacts. It is present in significantly higher number of household contacts as compared to non-contacts (P<0.05). There is no statistically significant difference of presence of IgM in household contacts as compared to non-contacts (P>0.05). The positivity of IgA in household contacts vaccinated with BCG is higher as compared to positivity of IgA in household contacts not vaccinated with BCG (P<0.1 and >0.05 approaching significant).

There is no statistical difference in (P>0.05) the results of IgG and IgM in household contacts with reference to the presence or absence of BCG scar (BCG vaccination). The positivity of IgM in non-contacts vaccinated with BCG is higher as compared to positivity of IgM in non-contacts not vaccinated with BCG (P<0.1 and >0.05, approaching significance) (Table 3-4).

Table 3: *Specificity of immunoglobulins in household contacts.*

Immunoglobulins	Specificity
IgM	94.16%
IgG	35.83%
IgA	60%

Table 4: *Specificity of immunoglobulins in non-contacts.*

Immunoglobulins	Specificity
IgM	95.12%
IgG	79.26%
IgA	89.02%

DISCUSSION

Tuberculosis is included in the top health problems of Pakistan. Similarly its diagnosis and surveillance is even bigger problem in this country due to inadequate health facilities, poverty, illiteracy and ignorance. Therefore, a study was designed to evaluate the disease process in its initial stage by evaluating IgM; IgG and IgA antibodies against Mycobacterium tuber-culosis in people exposed to disease (study group) and compare the results with the levels of these immunoglobulins in the control group selected from unexposed people.

Though the females were more in number among the household contacts and non-contacts this female preponderance was not statistically significant (P>0.05) (Table 3). Crampin et al also found same type of ratio in there study.[6] The

average age of household contacts was 27.4±17.24 years and the average age of non-contacts was 26.4±15.50. Considering household contacts and non-contacts separately, again no statistical difference was found (p>0.05 Table 3.2). This finding is consistent with the finding of Crampin et al.[6]

There was no significant difference in the number of household contacts and non-contacts in the age group 10-19 years and age group 40-49 years; however, in the age group 0-9 years the household contacts were significantly higher in number as compared to non-contacts (P<0.05). The same was true for the age group 20-29 years and the age group 50 years and above (P<0.05). For the age group 30-39 years, household contacts were more as compared to non-contacts. Statistically only in this range (30-39 years) the difference was found to be significant. (0.1> P>0.05) (table 3). It was found that the complaints of pyrexia, night sweats and loss of weight were more in household contacts as compared to non-contacts (P<0.05) (table 3). The abnormal findings in the respiratory system and cardio vascular system of the household contacts were more than the non-contacts (P<0.05) (Table 3).

Forty-nine household contacts (40.8 %) showed positive Mantoux test and only 3 non-contacts (3.7%) were Mantoux test positive. Significantly higher number of household contacts showed positive Mantoux test as compared to non-contacts P<0.05 (Table 3.8). This reveals that higher percentage of the household contacts were exposed to infection with Mycobacterium tuberculosis. Bothamley et al[25] performed Mantoux test in 39 hospital staff members and 36 factory employed personnel. He showed that the hospital staff had more chances of exposure with Mycobacterium tuberculosis as was revealed by positivity of Mantoux test than the factory workers. These results are in complete agreement with those of the present study. Hussain et al[26] reported that 87% of their household contacts were Mantoux positive and 56% of their endemic controls were positive for Mantoux test (comparable to non-contacts). Seven (5.8%) household contacts were positive for all the three immunoglobulins, 57 (47.5%) were positive for IgG, IgA & 15(12.5%) were positive for only IgG. The combined serological positivity of the household contacts was 79 (65.8%).

Malati et al[27] evaluated antibodies against antigen 60 in pulmonary tuberculous patients and non-tuberculous patients along with healthy persons not exposed to tuberculous patients and healthy persons exposed to tuberculous patients i.e. staff working in wards of tuberculosis hospital for one to thirty years. The combined positivity for anti-tuberculous antibodies (IgM, IgG, IgA) for non-exposed group and exposed group in Malati et al study, were 5.4% and 14.8% respectively. The combined positivity in the present study for comparable group is 34.1% and 65.8% respectively. The figures in this study are on the higher side.

Bothamely et al[25] showed that level of anti-tubercular antibodies in hospital staff was more as compared to the factory workers (these two groups are almost comparable to our household contacts and non-contacts respectively). These results are almost in agreement with those of our study.

Fada et al[21] evaluated the presence of IgG antibodies in-patient suffering from active secondary pulmonary tuberculosis, patient with no tuberculous pulmonary disease, healthy persons with no pulmonary disease. They could not detect any IgG antibodies in patients with non-tuberculous pulmonary pathology and in normal healthy controls. The present study showed the presence of IgG antibodies in 77(64%) of household contacts and 15 (18.75%) of non-contacts (table 3). As such the findings of Fada et al are totally different from those of the present study. The study conducted by Baelden et al to evaluate immune response in patients suffering from pulmonary tuberculosis and healthy controls had shown that most of the healthy controls were negative for IgM and IgG. These results are not in agreement with the findings in non-contacts of the present study (table 3).

Gevaudan et al[22] carried out study to evaluate immune response to Mycobacterium tuberculosis (serodiagnosis) in patients suffering from tuberculosis. The control subjects of their study were selected among the members of the hospital staff (clinicians, nurses, technicians and students) and among the non-tuberculous patients. They showed that none of the healthy persons was positive for IgM and only 10(5%) were positive for IgG. This study was only partially comparable with either of the present study groups (household contacts and non contacts). The positivity of IgM (0%) and IgG (5%) are quite lower than the positivity for IgM (6%) and IgG (64%) in the present study household contacts and positivity for IgM (2.5%) and IgG (18.75%) in the present study non-contacts (Table 3) moreover IgA positivity 48 (40%) in household contacts and 7 (8.7%) in non-contacts (P<0.05) (Table 3). Cocito and El-Barrawy et al[29] have shown that antibodies against Mycobacterium tuberculosis existed in 6.4% - 25.7% of the healthy persons.[24] These figures are comparable with present study.

Out of 120 house-hold contacts 50 of the present study were vaccinated with BCG as revealed by the presence of BCG scar. There is no statistical difference in the presence of IgM anti-

bodies with reference to vaccination status (P>0.05) (Table 3). Thirty-two (64%) of the vaccinated household contacts were positive for IgG, 49 (70%) of the non-vaccinated household contacts were positive for IgG. There is no statistical difference in the presence of IgG antibodies with reference to vaccination status. (P>0.05) (Table 3). Fifteen (30%) of the vaccinated household contacts and 33 (47%) of the non-vaccinated household contacts showed the presence of IgA antibodies. The positivity of IgA in vaccinated household contacts is lower as compared to non-vaccinated household contacts (0.1> P>0.05) (Table 3).

In our control group 43 were found to be vaccinated and 37 were found to be non vaccinated. Three (9%) of the vaccinated non-contacts and 0(0%) of the non-vaccinated non-contacts showed the presence of IgM antibodies. The positivity for IgM in vaccinated non-contacts is higher as compared to the positivity for IgM of non-vaccinated non-contacts (0.1> P>0.05) (Table 3), 7 (18%) of the vaccinated non-contacts and 08(23%) of the non-vaccinated non-contacts were positive for IgG, 2 (4.8%) of the vaccinated non-contacts and 5(12.8%) of the non-vaccinated non-contacts were positive for IgA. There is no statistical difference in the presence of IgG and IgA antibodies in vaccinated non-contacts as compared to the corresponding antibodies in non-vaccinated non-contacts (P> 0.05.) (Tables 3). The present study showed that chances of a person becoming IgM positive increase with BCG vaccination (P<0.05) (Table 3).

It is concluded, thus, the persons living in the house with a tuberculous patient is much more likely to be infected by Myco. tuberculosis as compared to those who do not have any source of contact with patients.

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