RISK TO HOUSEHOLD CONTACTS OF TUBERCULOUS PATIENTS BASED ON MANTOUX TEST AND ANTIBODY TITRE

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Background: Tuberculosis, being an infectious disease, carries a risk of infection to contacts attending tuberculous patients. This study was conducted to evaluate the risk for household contacts of tuberculous patients as compared to non-contacts. The study was conducted at PGMI, Gulab Devi Hospital and Defence Housing Authority Lahore. The study included 120 household contacts and 80 non-contacts. **Methods:** A Cross sectional study for evaluation of antituberculous antibodies levels by ELISA method in two groups; Mantoux positive household contacts 49, Mantoux negative household contacts 71 and normal healthy persons 'non contacts' 80. Routine Haematological investigations like HB, TLC and ESR were done by conventional methods and all the sera of 200 subjects included in the study were tested for IgM, IgG and IgA anti tuberculous antibodies by enzyme linked immunosorbant assay (ELISA).Purified protein derivative 0.1 ml containing 5 TU was injected intradermally. The test was read after 72 hours by measuring the induration around injection site of forearm. **Results:** There was no difference in the average age of the household contacts and non-contacts. The complaints of pyrexia, night sweats and weight loss were more in house hold contacts as compared to non-contacts. The awareness about BCG vaccination was equal in both. There were 49 contacts with positive Mantoux test while negative Mantoux test was found in 71 contacts. There were only three Mantoux positive among eighty non-contacts. There was no 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 compared to non-contacts. **Conclusion:** household contacts of patients suffering from active pulmonary tuberculosis have more chances of being infected with Mycobacterium tuberculosis as compared to the healthy non-contact, as shown by the higher levels of antituberculous antibodies & positivity of Mantoux test.

Keywords: Tuberculosis, Mycobacterium tuberculosis, ELISA, Mantoux, BCG

INTRODUCTION

Tuberculosis is a chronic infectious disease of worldwide importance and TB still exists at an alarming level with about 1/3 of the world's population infected with Mycobacterium tuberculosis, Eight million people developing the disease, and two to three million people die of TB each year.[1,2]

Mycobatcterium tuberculosis is generalized and widespread in Pakistan. It is fourth leading cause of death in our country and estimated 68000 people die of this disease annually. A number of surveys have been carried out in Pakistan to asses the prevalence of tuberculosis. Reports reveal that about 54% of entire population of Pakistan are infected with tuberculosis. It has been predicted by the WHO that 30 million people could die with tuberculosis (WHO 2001). [3]

It affects both sexes and all ages due to poverty, overcrowding, low socioeconomic status, multiple pregnancies, active & passive smoking, lack of health education, under-nutrition, poor housing etc.[4,5,6]

Tuberculosis has been neglected as a public health problem for many years and remains a 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).[7] There is an estimation of 8 million new cases of tuberculosis, each year leading to 3 million deaths.[8] The incidence of tuberculosis in Pakistan was 234/100,000 in 1995, and was estimated to rise to 269/100,000 by the year 2005. One of the maxims of tuberculosis upsurge has been inadequate therapy, which is worse than no therapy at all.[9]

The disease produced by tuberculous bacilli is characterized by granulomas that typically undergo central necrosis.[10] These caseating granulomas are the histological hallmarks of tuberculosis. The disease usually affects the lungs but may produce lesions in any organ or tissue of human body.[11]

The usual method of tuberculin skin reaction measurement of induration is palpation. Unfortunately, even skilled examiners may have considerable difficulty in identifying or measuring induration by this method. The measurement of tuberculin skin reaction by the pen method has been suggested as a superior alternative, since it can be used by less experienced observers.[12] Positive Mantoux test was present in 52% of patients. Negative Mantoux test does not rule out tuberculosis.

Various factors like overwhelming disease may be responsible for negative results. [3]

Diagnosis of Mycobacterium tuberculosis in the human host and the stage of its relationship (infection, primary tuberculosis and secondary tuberculosis) are very important and crucial to prevent the spread of the disease in the community.[13] There are many laboratory methods including detection of Mycobacterium tuberculosis after staining by Ziehl-Neelsen method and fluorochrome dyes, and Culture of the micro-organism have been widely used for the diagnosis of tuberculosis.[13,14]

Tuberculosis is included in the top list of health hazards in Pakistan. Its diagnosis and surveillance is even bigger problem in this country due to inappropriate health facilities, poverty, illiteracy and ignorance. This study was designed to evaluate the disease process in its most initial stage by evaluating IgM, IgG and IgA antibodies against Mycobacterium tuberculosis in people exposed openly to disease (study group) and compare the results with the levels of these immunoglobulins in the controls group selected from unexposed people. Enzyme linked immunosorbent assay (ELISA) was used to detect exposure to Tuberculosis by estimating IgM, IgG and IgA antibodies

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 including 49 persons living in the same house of patients of active pulmonary Tuberculosis diagnosed on the basis of clinical features, positivity of sputum for acid-fast bacilli and radiological evidence of pulmonary tuberculosis. In addition these persons had positive Mantoux test, 71 Persons living in the same house as patients of active pulmonary tuberculosis diagnosed on the same basis , these persons had negative Mantoux test and Group 2 healthy normal persons no contact history of Tuberculous patients.

Three ml of blood was collected and transferred to the vial containing anticoagulant for routine haematological investigations like Hb, TLC and DLC etc. Another 3 ml of the blood was delivered into a sterilized centrifuge tube and allowed to clot. The clotted sample was allowed to stand at room temperature for one hour and then was centrifuged at 3000 rpm for 15 minutes to extract the serum, which was stored in three vials in almost equal quantity. The vials were properly labelled with permanent marker and put into a deep freezer at -20 °C having uninterrupted power supply. The serum was kept frozen until ELISA test carried out for IgM, IgG and IgA antibodies against tuberculosis.

To carry out Mantoux test, the volar surface of the left forearm was cleaned with 70% ethanol, 0.1 ml of purified protein derivative containing 5 TU was injected intradermally with the help of a disposable tuberculin syringe fitted with a 26 bore needle, and the area around the injection was encircled with a marker. The test was read after 72 hours by measuring the induration around the injection site transversely to long axis of the forearm. Induration of 5 mm or more was taken as positive tuberculin test.

In this study BCG positive and BCG negative also mentioned on the basis of history and scar presence along with Mantoux readings in all the 200 subjects and controls. The other infections/vaccination other than BCG in recent past to effect immunoglobulins levels were excluded from the study.

IgG, IgA and IgM antituberculous antibodies were detected by enzyme linked immunosorbent assay (ELISA) utilizing microtitration plates coated with A-60 antigen extracted and purified from mycobacterium bovis (provided by ANDA biological SA) were pipetting of reagents and sera, read by ELISA reader. False positive and false negative results in immunoglobulins were excluded. Tests were carried out according to manufacture instructions

Data was analysed using Epi-Info 3.4.3. Numeric variables were described in terms of Mean±SD, Chi-Square test was used for observation and significance testing in the case categorical variables with 5% significance level.

RESULTS

Our study included 200 subjects, out of these 120 subjects 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 results of the present study are shown below in the tabulated form.

Table-1: Distribution of Positive Mantoux Test in Household Contacts and Non contacts

	Mantoux Test			
	Positive		Negative	
Subjects	**No.**	**%**	**No.**	**%**
House hold Contacts (n=120)	49	40.8	71	59.2
Non contacts (n=80)	3	3.75	77	96.25
Total (n=200)	**52**	**26**	**148**	**74**

$p<0.01$ (Highly Significant)

Table-2: Distribution of IgM according to Mantoux test in household contacts

Mantoux test	IgM Positive	IgM Negative	Total
Negative (Group-I)	5 (7%)	66 (93%)	71
Positive (Group-II)	2 (4%)	47 (96%)	49

p=0.496 (not significant)

Table-3: Distribution of IgG according to Mantoux test in household contacts

Mantoux test	IgG Positive	IgG Negative	Total
Negative (Group-I)	42 (59%)	29 (41%)	71
Positive (Group-II)	34 (69%)	15 (31%)	49

p=0.25 (NS)

Table-4: Distribution of IgA according to Mantoux test in household contacts

Mantoux test	IgA Positive	IgA Negative	Total
Negative	26 (37%)	45 (63%)	71
Positive	21 (43%)	28 (57%)	49

p=0.49 (NS)

Table-5: Distribution of IgM according to Mantoux test in non-contacts (Group-III)

Mantoux test	IgM Positive	IgM Negative	Total
Negative	4 (5%)	73 (95%)	77
Positive	0 (0%)	3 (100%)	3

p=0.685 (NS)

Table-6: Distribution of IgG according to Mantoux test in non-contacts (Group-III)

Mantoux test	IgG Positive	IgG Negative	Total
Negative	14 (18%)	63 (82%)	77
Positive	0 (0%)	3 (100%)	3

p=0.509 (NS)

Table-7: Distribution of IgA according to Mantoux test in non-contacts (Group-III)

Mantoux test	IgA Positive	IgA Negative	Total
Negative	8 (10%)	69 (90%)	77
Positive	0 (0%)	3 (100%)	3

p=0.21 (NS)

DISCUSSION

One hundred and twenty household contacts and 80 controls were subjected to Mantoux test and their sera examined for immunoglobulin titre. Forty-nine household contacts showed positive Mantoux test and only 3 non-contacts were Mantoux test positive. Significantly higher number of household contacts showed positive Mantoux test as compared to non-contacts. This reveals that higher percentage of the household contacts were exposed to infection with Mycobacterium tuberculosis. Bothamley *et al* performed Mantoux test in 39 hospital staff members and 36 factory employed personnel. They observed that the hospital staff had more chances of infection 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* reported that 87% of their household contacts (comparable to household contacts in this study) 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 65.8%.

Malati *et al* (1995) evaluated antibodies against (antigen 60) in pulmonary tuberculosis patients and neurotuberculosis patients along with healthy persons not exposed to tuberculosis 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-tuberculosis antibodies (IgM, IgG, IgA) for non-exposed group and exposed group in Malati *et al* were 5.4% and 14.8% respectively. The combined positivity in the present study comparable group is 34.1% and 65.8% respectively. The figures in this study are on the higher side and as such are not in complete agreement to those in Malati *et al*. Tuberculosis is more endemic and exposure is more due to poverty and over crowding.

Bothamely *et al* showed that level of antitubercular 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 our study.

Fada *et al* evaluated the presence of IgG antibodies in-patient suffering from active pulmonary tuberculosis, patient with no tuberculous pulmonary disease, healthy persons with no pulmonary disease. They applied enzyme linked immunosorbent assay based on Antigen 60 (The enzyme linked immunosorbent assay based on Antigen 60 was applied in the present study as well). Fada *et al* 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. As such the findings of Fada *et al* are totally different from those of the present study.

Gevaudan *et al* 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. It was found that none of the healthy persons was positive for IgM and only 10 (5%) were positive for IgG. This study only partially compared either of the present study groups (household

contacts and non contacts). The positivity of IgM (0%) and IgG (5%) are quite low than the positivity for IgM (6%) & IgG (64%) in the present study household contacts and positivity for IgM (2.5%) and IgG (18.75%) in the present study non-contacts moreover IgA positivity 48 (40%) in household contacts and 7 (8.7%) in non-contacts ($p<0.05$).

Cocito and El-Barrawy *et al* have shown that antibodies against Mycobacterium tuberculosis existed in 6.4%–25.7% of the healthy persons (Raheja *et al*). These figures are quite comparable with those of our study.

CONCLUSION

It is concluded that household contacts of patients suffering from active pulmonary tuberculosis have more chances of being infected with Mycobacterium tuberculosis as compared to the healthy non-contact, as shown by the higher levels of antituberculous antibodies & positivity of Mantoux test.

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