ORIGINAL PROF-2218

TUBERCULOUS PATIENTS;

CROSS SECTIONAL STUDY OF BCG VACCINATION AND ANTIBODY TITRE IN HOUSE HOLD CONTACTS OF TUBERCULOUS PATIENTS

Dr. Nasir Aziz, Dr. Hamid Mahmood, Dr. Hashim Riaz, Dr. Zahid Mahmood, Dr. Akhtar Murad Qadir, Dr. Muhammad Munir, Dr. Muhammad Tayyab

ABSTRACT... Objective: This study was designed to analyze the BCG vaccination status and compare the immune globulins levels in the household contacts of tuberculous patients with non contacts of unexposed healthy peoples. **Place:** Gulab Devi Hospital, Post Graduate Medical Institute and Defense Housing Authority Lahore. **Study design:** Cross sectional study. **Materials and Methods:** Investigations like ESR were done and the sera of 200 persons included in the study were tested for anti tuberculous antibodies by ELISA. BCG vaccination present or absent and scars were positive or negative in both groups were included. **Results:** The combined serological positivity of the household contacts was 65.8% and for non-contacts was 34.1%. BCG scars were mostly absent in the household contacts as well as in non-contacts; But statistically IgG and IgA were present significantly by higher number in the household contacts as compared to non-contacts, where as no significant difference in the IgM levels. These immunoglobulins status were compared with BCG scars in both study and control groups. The results were analyzed by SPSS version 14. **Conclusions:** Household contacts of tuberculosis patients are more susceptible to tuberculosis as compared to the non-contacts, as shown by positive and negative status of antituberculous antibodies in the house hold contacts. More over BCG vaccination has no significant role in humoral evaluation.

Key words: Tuberculosis, BCG, Immunoglobulins

Article Citation
Aziz N, Mahmood H, Riaz H, Mahmood Z, Qadir AM, Munir M, Tayyab M. Cross sectional study of BCG vaccination and antibody titre in house hold contacts of tuberculous patients. Professional Med J 2013;20(5): 678-683.

INTRODUCTION

Tuberculosis is a chronic infectious disease of worldwide importance and 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 three million people dying of TB each year[1]. 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[2]. In many populations there is an excess of tuberculosis in young women and older men. Possible explanations for these patterns, includes of human immunodeficiency virus, pregnancy, smoking, smoke exposure during cooking, contact with tuberculosis cases within the household or outside areas, and gender differences in health service usage and diagnostic delay[3].

Tuberculosis has been neglected as public health issue for many years and 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[4]. 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 maxim of tuberculosis control has been inadequate therapy, which is worse than no therapy at all[5].

Tuberculosis may develop any where in the body, but usually presents as pulmonary infection, ranging from mild infiltration to chronic, cavity formation, and severely destructive disease. The different manifestations of infection with Mycobacterium tuberculosis reflect the balance between the bacillus and host defense mechanisms, in which the quality of host defense determines the outcome[6]. The disease produced by tuberculous bacilli is characterized by granulomas that typically undergo central necrosis[7]. 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[8].

Humoral immunity plays a secondary role in the host defense against mycobacteria[9]. Elimination of Mycobacterium tuberculosis infection mainly depends on the success of the interaction between infected macrophages and T-lymphocytes. The immunological response of the human body to Mycobacterium tuberculosis is mostly and essentially cell mediated[10]. The role of humoral immune response in protection against Mycobacterium tuberculosis is controversial. Passive immunization provided little or no protection against Mycobacterium tuberculosis[11]. The recent contributions towards tuberculosis, immunology should provide new opportunities for developing vaccines that may be more effective than BCG[12].

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. There are many laboratory methods including detection of Mycobacterium tuberculosis after staining by Ziehl-Neelsen method, fluorochrome dye, and culture of the micro-organism have been widely used for the diagnosis of tuberculosis. Although culture of the bacteria is the reference standard of diagnosis and follow-up of disease[13].

Tuberculosis continues to be a public health problem in Pakistan. There is little epidemiological date available in Pakistan about tuberculosis. In Pakistan from 110,000 to 130,000 new tuberculosis cases are found each year. During 1990-91 there were 105000 admissions with medical problems in thirty major hospitals in Pakistan out of which 65000 had pulmonary tuberculosis[14]. Tuberculosis (TB) affects predominately young people in developing countries where it is still an endemic infection. Therefore the same trend may be seen in women at childbearing age[15].

Tuberculosis is included in the top list of health problems of Pakistan. Its diagnosis and surveillance is even bigger problem in this country due to inappropriate health facilities, poverty, illiteracy and ignorance. Therefore, studies was designed to evaluate the effects of disease exposure in its initial stage by evaluating IgM; IgG and IgA antibodies against Mycobacterium tuberculosis in these subjects and were compare through ELISA with the levels of these immunoglobulin in the unexposed people with or without BCG vaccination. Enzyme linked immuno sorbent assay is a serological test to detect exposure to Tuberculosis.

MATERIALS AND METHODS

The study included 120 persons selected among the family members suffering from active pulmonary tuberculosis and 80 normal healthy persons who did not have any history of exposure with the patients of pulmonary tuberculosis. Different categories of subjects irrespective of age and sex were living in the same house of patient 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 this study BCG positive and negative subjects among the household contacts and non contacts were also included on the basis of history and presence of scar in all the 200 subjects. The other infections and vaccinations of recent past to effect immunoglobulin levels were excluded in the study.

Two ml of blood was collected and transferred to the vial containing anticoagulant for routine hematological investigation like ESR, three ml of the blood delivered into a sterilized centrifuge tube and allowed to clot. The clotted sample was allowed to stand at room temperature for one hour more then they were centrifuged at 3000 rpm for 15 minutes to extract serum, they were stored in three vials in almost equal quantity. The vials were properly labeled and put into a freezer at -20˚C having uninterrupted power supply.

The serum was kept frozen until test carried out for 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 , was used. The data of antibodies levels were compared with the BCG vaccination scars presence. The data was analyzed by the SPSS and statistically compared.

RESULTS AND OBSERVATIONS

Our study included 200 subjects, out of these 120 subjects were the peoples 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. Levels of immunoglobulins in BCG vaccinated and natural exposure based subjects show same findings. No difference of immunoglobulin levels (IgM,IgG,IgA) between groups BCG positive and natural exposed is found, so equally found sero positive.

The results of the present study are shown below in the tabulated form

Subjects	BCG Scar Present	%	BCG Scar Absent	%
House hold contacts (n=120)	43	35.8	77	64.2
Non- contacts (n=80)	38	47.5	42	52.5
Total	81	40.5	119	59.5

Table-I. Presence of BCG scar in house hold contacts and no contacts
P-value : 0.01 (Not significant)

Immunoglobulin	Scar Present	Scar Present
IgM +ve	1 (2.0%)	2 (5.7%)
IgM –ve	50 (98.8%)	33 (94.2%)
IgG +ve	30 (58.8%)	10 (28.6%)
IgG –ve	21 (41.2%)	25 (71.4%)
IgA +ve	24 (47.1%)	1 (2.9%)
IgA –ve	27 (52.9%)	34 (97.1%)

Table-II. Comparison of antibiotics status among BCG scare present between house hold contacts and non-contacts

Immunoglobulin	Scar Present	Scar Absent
IgM +ve	6 (8.7%)	2 (4.4%)
IgM –ve	63 (91.3%)	43 (95.6%)
IgG +ve	59 (85.5%)	17 (37.88%)
IgG –ve	10 (14.57%)	28 (62.2%)
IgA +ve	42 (60.9%)	5 (11.1%)
IgA –ve	27 (39.17%)	40 (88.9%)

Table-III. Comparison of antibodies status among BCG scar Absent between house hold contacts and non-contacts

DISCUSSION

120 household contacts and 80 controls were subjected to BCG vaccination and their seras examined for immunoglobulin titre.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 (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 (1995), 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 (1995).Tuberculosis is more endemic and exposure is more due to poverty and over crowding, in our situation[16].

Bothamely et al (1992) 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[17].

Fada et al (1992) 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 (1992); 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 (1992) are totally different from those of the present study[18].

Gevaudan et al (1992) 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[19]. 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 ($P<0.05$).18 and El-Barrawy et al (1991) have shown that antibodies against Mycobacterium tuberculosis existed in 6.4% - 25.7% of the healthy persons[20]. These figures are quite comparable with those of our study[20].

CONCLUSIONS

The level of awareness about BCG vaccination was equal among the household contacts and non-contacts as was revealed by the history of BCG vaccination ($P>0.05$) and the presence of BCG scar ($P>0.05$). It is viewed that household contacts of patients suffering from active pulmonary tuberculosis have more affection of being infected with Mycobacterium tuberculosis as the non-contact, by the presence higher levels of antituberculous antibodies.

So that BCG vaccination still debatable provision in health care facilities in the developing countries to decrease tuberculoses meningitis than other parts of the body tuberculosis.

REFERENCES

1. Al-Attiyah R, Shaban FA, Wiker HG, Oftung F, Mustafa AS. **Synthetic peptides identify promiscuous human Th1 cell epitopes of the secreted Mycobacterial antigen MPB70.** Infect Immun 2003; 71: 1953-60.

2. Moosa FA, Sultan N, Shah S. **Incidence of abdominal tuberculosis presenting with intestinal obstruction.** Med Chan 2002; 8: 56-58.

3. Crampin AC, Glynn JR, Floyd S, Malema SS, Mwinuka

VK, et al. **Tuberculosis and Gender: exploring the patterns in case control study in Malawi.** INT J TUBER LUNG DIS 2004; 8: 194-203.

4. Cantwell MF, Snider DE jr, Cauthen GM, Onorato IM. **Epidemiology of tuberculosis in the United States, 1985 through 1992.** JAMA 1994; 272: 535-9.

5. Iqbal R, Shabbir I, Mirza MN, Hasan M. **TB drug resistance an alarming challenge – answer dots.** Pak J Med Res 2003; 42: 134-8.

6. Crevel RV, Ottenhoff THM, Vandermeer WM. **Innate immunity to Mycobacterium tuberculosis.** Clin Micrbiol Rev 2002; 15: 294-209.

7. Daniel MT and Debanne MS. **The serodiagnosis of Tuberculosis and other Mycobacterial Diseases by Enyme Linked immunosorbent Assay.** Am rev Resp Diseases 1987; 135: 1137-1151.

8. Lichtenberg FV. Infectious diseases. In: Cotran RS, Kumar V, Robbins SL. **Robbins pathological basis of diseases.** 4th ed ,Phildealphia. WB Saunders 1989; 307-90.

9. Cocito CG: **Properties of the mycobacterial antigen complex A-60 and its applications to the diagnosis and prognosis of tuberculosis.** Chest 1991;100:1687-93.

10. Crevel RV, Ottenhoff THM, Vandermeer WM. **Innate immunity to Mycobacterium tuberculosis.** Clin Micrbiol Rev 2002; 15: 294-209.

11. Casadevall A. **Antibody-mediated immunity against intracellular pathogens: Two-dimensional thinking comes full circle.** Infect Immunity 2003; 71: 4225-28.

12. Ali J. **Immunotherapy and mycobacterial disease with focus on interferon-gamma: Is it a viables supplemental therapeuticapproach a mirage?** Infect Dis J Pak2003; 17-20.

13. Raqib R, Rahman J, Kamaluddin AKM, Kamal SMM, Banu FA, **Rapid diagnosis of active tuberculosis by detecting antibodies from lymphocyte secretions.** JID 2003;188:364-70.

14. Aurangzeb M, Masroor M, Ahmad I, Qamar R, Sattar A, Imran K, Khan MH. **Revelance of pulmonary tuberculosis in close contact of diagnosed cases of Tuberculosis.** Pak J Chest Med 2004; 10: 11-22.

15. Zekioglu O, Ozol D, Cavusoglu C, Saydam CC, Ozhan MH, et al. **Miliary tuberculosis with endometerial spread in a pregnant woman: a case report.** Ann Saudi Med 2003; 23: 296-97.

16. Malati T, Kumari GR, Dinakar I. **Evaluation of A60 antibodies in pulmonary and neurotuberculosis.** Indian J Clin Biochem 1995; 10: 72-76.

17. Bothamley GH, Beck JS, Potts RC, Grange JM, Jiro T, Ivanyi J. **Specificity of antibodies and tuberculin response after occupational exposure to tuberculosis.** J Infect Dis 1992; 166: 182-186.

18. Fadda G, Grillo R, Ginesu F, Sanotru L, Zanetti S, Dettori G. **Serodiagnosis and followup of patients with pulmonary tuberculosis by enzyme-linked immunosorbent assay.** Eur J Epidemiol 1992; 8:81-7.

19. Gevaudan MJ, Bollet C, Charpin D, Mallet MN, Micco De et al. **Serological response of tuberculosis patients to antigen 60 of BCG.** Eur J Epidemiol 1992; 8: 66-76.

20. El-Barawy MA, Hafez SA, Mokhtar SA, Abou Rayan MA. **Enzyme linked immunosorbent assay (ELISA) in the diagnosis of active pulmonary tuberculosis.** J Egypt Public Health Assoc 1991; 66: 279-89..

PREVIOUS RELATED STUDIES

Iltifat Sultan, Muhammad Sadiq, Jeved Akhtar, Rashid Jaleel. TUBERCULOUS PATIENTS (Original) Prof Med Jour 16(1) 67-69 Jan, Feb, Mar 2009.

AUTHOR(S):

1. **DR. NASIR AZIZ**
   Professor of Pathology,
   AIMC Abbottabad
2. **DR. HAMID MAHMOOD**
   Associate Professor of Bio Chemistry,
   FMC, Abbottabad
3. **DR. HASHIM RIAZ**
   Assistant Professor of Community Dentistry
   FMC, Abbottabad
4. ***Dr. Zahid Mahmood***
5. ***Dr. Akhtar Murad Qadir***
6. ***Dr. Muhammad Munir***
7. ***Dr. Muhammad Tayyab***

Correspondence Address:
Dr. Hamid Mahmood
Professor of Bio Chemistry,
Islam Medical and Dental College, Sialkot.
drhamidmahmood373@gmail.com

Article received on: 26/02/2013
Accepted for Publication: 14/06/2013
Received after proof reading: 16/09/2013

CORRECTION

The amendment of the Professional Vol: 19, No.05 (Prof-2042) on page 630 (in title) is as under;

INCORRECT

LOCAL FLAPS AND SPLIT;
Sensory deficit in term of two point discrimination (TPD) thickness skin grafts (STSG).

CORRECT

SENSORY DEFICIT IN TERM OF TWO POINT DISCRIMINATION (TPD);
Local flaps do better than split thickness skin grafts (STSG).

CORRECTION

The amendment of the Professional Vol: 20, No.03 (Prof-2163) on page 390 and 398 is as under;

INCORRECT
Page No: 390

Dr. Irfan Ishaq, Prof. Ghulam Qadir Fayyaz

Article Citation:
Ishaq I, Fayyaz GQ.

CORRECT
Page No: 390

Dr. Irfan Ishaq, Dr. Ayesha Choudhary, Prof. Ghulam Qadir Fayyaz

Article Citation:
Ishaq I, Choudhary A, Fayyaz GQ.

INCORRECT
Page No: 398

AUTHOR(S):

1. **DR. IRFAN ISHAQ**
   Registrar
   Department of Plastic Surgery
   Services Institute of Medical Sciences, Lahore.
2. **PROF. GHULAM QADIR FAYYAZ**
   MBBS, M.S, D.S.S.
   Head Department of Plastic Surgery
   Services Institute of Medical Sciences, Lahore

CORRECT
Page No: 398

AUTHOR(S):

1. **DR. IRFAN ISHAQ**
   Registrar
   Department of Plastic Surgery
   Services Institute of Medical Sciences, Lahore.
2. **DR. AYESHA CHOUDHARY, FCPS**
   Assistant Professor General Surgery
   Sir Ganga Ram / FJMC Hospital, Lahore
3. **PROF. GHULAM QADIR FAYYAZ**
   MBBS, M.S, D.S.S.
   Head Department of Plastic Surgery
   Services Institute of Medical Sciences, Lahore