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 year⁴. 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 housing⁵. 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 delay⁶.

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 system⁷. 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 all⁸.

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 outcome⁹. The disease produced by tuberculous bacilli is characterized by granulomas that typically undergo central necrosis⁰. 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 body⁰.
Humoral immunity plays a secondary role in the host defense against mycobacteria. 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. The role of humoral immune response in protection against Mycobacterium tuberculosis is controversial. Passive immunization provided little or no protection against Mycobacterium tuberculosis. The recent contributions towards tuberculosis, immunology should provide new opportunities for developing vaccines that may be more effective than BCG.

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.

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. 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.

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:

<table>
<thead>
<tr>
<th>Subjects</th>
<th>BCG Scar Present</th>
<th>%</th>
<th>BCG Scar Absent</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>House hold contacts</td>
<td>43</td>
<td>35.8</td>
<td>77</td>
<td>64.2</td>
</tr>
<tr>
<td>(n = 120)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-contacts</td>
<td>38</td>
<td>47.5</td>
<td>42</td>
<td>52.5</td>
</tr>
<tr>
<td>(n = 80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>40.5</td>
<td>119</td>
<td>59.5</td>
</tr>
</tbody>
</table>

Table-I. Presence of BCG scar in house hold contacts and non contacts

P-value : 0.01 (Not significant)

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>Scar Present</th>
<th>Scar Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM +ve</td>
<td>1 (2.0%)</td>
<td>2 (5.7%)</td>
</tr>
<tr>
<td>IgM –ve</td>
<td>50 (98.8%)</td>
<td>33 (94.2%)</td>
</tr>
<tr>
<td>IgG +ve</td>
<td>30 (58.8%)</td>
<td>10 (28.6%)</td>
</tr>
<tr>
<td>IgG –ve</td>
<td>21 (41.2%)</td>
<td>25 (71.4%)</td>
</tr>
<tr>
<td>IgA +ve</td>
<td>24 (47.1%)</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>IgA –ve</td>
<td>27 (52.9%)</td>
<td>34 (97.1%)</td>
</tr>
</tbody>
</table>

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

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

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 sera 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.

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.

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.

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. 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. These figures are quite comparable with those of our study.

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

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


PREVIOUS RELATED STUDIES

TUBERCULOUS PATIENTS

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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:
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INCORRECT
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CORRECT
Page No: 390
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