Comparative Efficacy of Different Laboratory Techniques Used in Diagnosis of Tuberculosis in Human Population

Tanzeela Talat, Bashir Mahmood Bhatti and M. Yaqoob

In order to test the efficacy of different laboratory techniques used in diagnosis of tuberculosis, 60 Tb cases along with 50 control cases in Faisalabad area were studied, using different laboratory tests. Mantoux's test showed no correlation with hematological parameters. Acid fast test (AFB-Test) was routinely used in labs, but there was still chances of contamination of sputum sample with a typical mycobacteria, so increasing the chances of error in the results. Chest radiograph was also a specific technique to some extent but in case of small children and other lungs diseases, it became unreliable and confusing test. When a comparative evaluation was made between different diagnostic tests, polymerase chain reaction (PCR) proved to be a very sensitive, highly specific, qualitative and quantitative technique for the confirmation of pulmonary tuberculosis. However, there was no correlation of PCR results with other hematological findings. Hematological studies showed significantly high level of erythrocyte sedimentation rate (ESR) in Tb cases (64.3 ± 38.2) (P<0.00) than in control cases (16.57 ± 5.26) were significantly increased (72.5 ± 39.9). Hemoglobin level (Hb) of Tb patients was significantly decreased (8.84 ± 2.33) than in control cases (11.8 ± 1.65). Total Leucocyte count (TLC) in Tb patients was significantly (P<0.00) higher (8882 ± 1650) as compared to control cases (82134 ± 1161.68). Differential leucocyte count (DLC) analysis of Tb cases in comparison with control cases showed significantly high (P<0.00), neutrophil count in Tb cases (69.4 ± 4.69) as compared to control cases (60.04 ± 5.15). Lymphocyte count in Tb cases was significantly decreased (P<0.00) (24.55 ± 3.64) than in control cases (32.39 ± 4.63) and eosinophil count in Tb cases was (2.16 ± 1.34) and control cases (3.2 ± 1.55) at probability level of (0.0004). Monocytes and basophil count was non-significantly different (P<0.1127; P<1.00) in Tb cases (3.7 ± 1.72, 0.08 ± 0.27) from control cases (4.38 ± 2.55, 0.08 ± 0.27). It may be concluded that PCR test is reliable, which may be employed in routine testing of Tb cases to safeguard against misdiagnosis.

Key words: Tb, Bacilli tuberculi, epidemiology, Mantoux/Tuberculine test, X-Ray analysis, microscopic smears, acid fast test, PCR technique, hemoglobin, hematology

1Physiology and Biochemistry Division, Poultry Research Institute, Punjab, Rawalpandi, Pakistan
2Department of Chemistry, University of Agriculture, Faisalabad, Pakistan
Introduction
Diagnosis of tuberculosis requires knowledge of the strengths and shortcomings of various diagnostic methods and experience in their use. No diagnostic method, by itself, can be relied on to confirm or rule out tuberculosis. Well tested diagnostic methods of chest radiograph, tuberculin skin testing, smear and culture have recently been supplemented by rapid diagnostic test based on amplification of bacterial DNA. Failure of compliance can be a significant problem in patients who are homeless or drug abusers, or for various reasons cannot complete a course of therapy.

Directly observed therapy is strongly recommended for those patients (Mc Dermott et al., 1997). About one third of the world population is infected with Tuberculosis bacilli, causing eight million new cases of tuberculosis and three millions deaths each year. A more complete epidemiology of Tb, will lead to a better identification of index cases and to a more effective treatment of the disease. Recently, new molecular tools have become available for the identification of Mycobacterium Tuberculosis (MTB) strains, allowing a better recognition of transmission routes of defined strains. Both a standardized restriction fragment length polymorphism (RFLP) based methodology for epidemiological studies on a large scale and DNA amplification based methods that allows rapid detection of outbreaks with Mycobacterium Drug Resistant Strains (MDR), often characterized by high mortality rates, have been developed (Suffys et al., 1997).

Tb of man is caused by a group of very closely related species forming the Mycobacterium tuberculosis complex. These include M. tuberculosis (cause of most infections) M. bovis, (endemic in cattle, spread to man by milk) and a typical mycobacteria (rare compared with human type), cause cervical lymph node infection in children. MTB is mostly transmitted in cough sprays in patients having overt pulmonary Tb and gain access to the body by inhalation of injective droplets. Initial lesion is usually in the lung from which organism reaches other organs through lymphatic and blood stream (Collier et al., 1986). The initial primary Tb occurs in the lungs but occasionally in the tonsil or alimentary tract. The primary infection differs from the subsequent infection in that the primary focus in lung, tonsil or bawl is almost invariably accompanied by caseous lesions in the regional lymph nodes. Post primary pulmonary Tb is the term used to describe lung disease, characteristic feature of which is tuberculous cavity which is formed when the caseded and liquefied center of a tuberculous pulmonary lesion is discharged into bronchi. Extension of infection to pleura causes Tb pleurisy, when accompanied by effusion, develop tuberculous empyema (Edwards and Boucher, 1991).

DNA fingerprinting studies have established that exogenous re-infection occurs more often than previously believed. Drug resistant tuberculosis is an other form of Tb, which is usually defined as a resistant strain of MTb to isoniazid and rifampicin with or without additional drug resistance. Mycobacterium Drug Resistant Tuberculosis (MDR TB) is an increase in many parts of the world. Secondary resistance is the result of preferential replication of mutants in patients receiving inadequate therapy. Primary resistance occurs when an untreated person is infected by a strain that is already resistant (Collier et al., 1998).

Tuberculosis may be partially explained by the higher rate of infection in the immunocompromised states associated with old age, renal failure, cirrhosis, malnutrition hematologic malignancies and AIDS. Different forms of extra pulmonary Tb are endobronchial tuberculosis, lymphatic, genital, kidney, arthritis, abdominal, intestinal, cutaneous, meningitis, disseminated and mixed tuberculosis (Wyngarden and Smith, 1988). Common clinical features of all the types of tuberculosis includes anorexia, weight loss, sleep sweats, evening pyrexia, cough, spuath and hemoptysis. Case findings depends upon the common symptoms of disease, chest radiograph and sputum smear examination.

These are the important and common methods of case finding in developing countries. This study was undertaken to identify the status of tuberculosis in population of Faisalabad city and using different diagnostic methods to evaluate their relative efficacy.

Materials and Methods
This project was designed to determine the true situation of Mycobacterium tuberculosis infection in Faisalabad area, seek out the communities at a great risk and comparative evaluation of polymerase chain reaction (PCR) test with the existing remaining conventional diagnostic techniques.

Epidemiological data: In order to know the disease status of Tb in Faisalabad, retrospective descriptive epidemiological data were obtained from District Tb Hospital, Circular Road, Faisalabad. Previous data available at the hospital for the last decade (1989-98) were collected on a questionnaire having the following information:
(a) Date of admission of the case, (b) Name sex of patient, (c) Age of patient, (d) Residential address, (e) Clinico-analytical epidemiological data.

The detailed information of 100 Tb cases admitted at the hospital was obtained on a predesigned questionnaire having following information.

Youngsters (under 23 years and unmarried) + ve/ve or relapse case: (a) Name, (b) Sex, (c) Age, (d) Father's Profession, (e) Source of income of family, (f) Person's status in the family, (g) Education, (h) Date of diagnosis, (i) Domestic animals, (j) Residential environment, (k) Family background related to Tb, (l) Dietary habits.

Male (married) + ve/ve or relapse case: (a) Name, (b) Age, (c) Address, (d) Profession, (e) Total income, (f) Working hours, (g) Date of marriage, (h) Number of children, (i) Dietary habits, (j) Date of diagnosis, (k) Type of domestic animal if any, (l) Residential environment, (m) Family background related to Tb.

Female (married) + ve/ve or relapse Case: (a) Name, (b) Age, (c) Address, (d) Type of official work/home assignments, (e) Husband's profession, (f) Total income, (g) Date of marriage, (h) Number of children, (i) Dietary habits, (j) Date of diagnosis, (k) Type of domestic animal if any, (l) Residential environment, (m) Family background related to Tb.

Diagnostic studies
Selection of patients: Fifty (50) clinical Tb cases from District Tb Hospital, Circular Road, Faisalabad were selected for diagnostic tests along with 50 control cases.

Mantoux/tuberculin test: The tuberculin skin test was used based on principle given by Collier et al. (1998).

In the Mantoux/tuberculin test, an exact amount (usually 0.1 ml) of tuberculin (2TU 0.1 ml⁻¹) was injected intracutaneously in the forearm, after 72 hours, injection site was palpated and measured induration as transverse diameter. Results were noted as an induration of 10mm diameter measured transversely indicated a positive reaction (Collier et al., 1998).

X-Ray analysis: In disease, around the area of infection, activated macrophages aggregate so, that a compact palisade, which is many cell thick, is formed to produce the characteristic lesion of tuberculosis and many other chronic infections, namely the granuloma. The apparatus used included, X-ray machine, illuminating screen and the method employed included observation of anteroposterior chest radiographs on illuminator. Results were recorded according to the presence or absence of radiopaque characteristic lesions of Tb on Apex, center and base regions of both of the lungs in case of pulmonary tuberculosis patients versus control cases (Collier et al., 1998).

Microscopic smear examination: Acid fast bacilli (AFB) Staining/Ziehl-Neelsen Staining. The staining technique is based on
the resistance of Mycobacteria to decolorization by acid or a mixture of acid and alcohol after staining by amyathane dyes i.e., acid fastness’ (Colliers et al., 1998).

Mycobacterium were stained bright red. Results were noted on
the basis of presence or absence of acid fast bacilli. The sputum
smears containing acid fast bacilli were called + ve slides while
others as -ve (Merchant and Packer, 1984).

Polymerase chain reaction (PCR) technique: PCR is based on
the principle of amplification of specific DNA segment in vitro, after
separating DNA stands by heating, then annealing to an excess of
short synthetic DNA primers that flank the region to be amplified
in the presence of taq-polymerase and dNTPs (Lehnig et al.,
1993). Digestion and decontamination of specimens were done by
(Maniatis et al., 1982).

PCR amplification: PCR amplification was done using apparatus
such as Multi tube vortex (Mickle apparatus); PCR Machine
(thermocycler) PTC-100 programmable thermal controller.
Microdispensers with tips, PCR tubes and the reagents (50µL total
cocktail) used were: 10µL of PCR product, 5 µL reaction buffer
containing, 10mM Tris-HCl (pH 8.3), 50mM KCl (potassium
chloride), 1.5mM MgCl2, (magnesium chloride), 0.01% gelatin,
0.2mM (O.2µL) dNTPs (deoxy nucleotide triphosphates) dATP,
dGTP, dTTP, dCTP, dNTPs – ABI Master piece, 500mg dissolved
in autoclaved distilled H2O, 0.2 µLprimers (each) available in
commercial packing of company GENOSYS: 2µL (1µL) of Taq
Polymerase. Taq polymerase was extracted from a thermostable
bacteria called Thermus aquaticus and donated by the Restriction
lab of CEMB, Lahore, 10 µL Genomic DNA of clinical sample of
MTB Template, 10µL of Genomic DNA of Mycobacterium species
other than MTB (NTM or MOTT), Distilled H2O, to make final volume
50µL.

Mycobacterium tuberculosis insertion sequences IS 986 were
used for amplification which was of 641 bp. The 33 6UL of distilled
H2O was added to all PCR tubes, then added 5µL of reaction buffer,
containing 10mM Tris-HCl (pH 8.3), 50mM KCl, 1.5mM MgCl2,
and 0.01% gelatin was added to all PCR tubes individually: 0.2µM
(0.2µL) of dNTPs (dATP, dGTP, dTTP, dCTP) was poured in each
above PCR tube using another tip (0.2µL) contained 20 p mole);
2µL of Taq polymerase was dispensed off in all PCR tubes,
individually: 0.2µL of each primer named as Pr-B and Pr-A
which correspond to pp 105-124 (S-GCGCGATGTTGCCAGAGAGAT-3) and
Pb 626-645 (6-CTGATGCCTCAGGTT CA-3) respectively of the
TS986 sequence were dispensed in all PCR tubes, using
different tips again; 10µL of isolated DNA from each sample was
added in all tubes at last for every sample tip of microdispenser
was used to avoid contamination. In -ve control PCR tubes
one was devoid of DNA containing all other PCR cocktail and other
contained 10µL of genomic DNA of NTM (non-tuberculosis
microbacterium) + ve control PCR tube contained 10µL of DNA
of clinical sample of MTB (Mycobacterium tuberculosis): After
vortexing the samples were shifted to thermocycler (PCR machine)
and programmed as 2 minute initial denaturation at 95 °C 1 minute
annealing at 65 °C and 30 seconds of primer extension at 72 °C.
After 40 cycles final extension was done for 2 min at 72 °C (Idrees
et al., 1998).

Detection of PCR products: The gel containing 641 bp fragments,
was visualized by ( UV transillumination after making comparison
with markers (Maniatis et al., 1982).

Biochemical studies: Complete blood picture (CBP) of 50 (Fifty)
clinical TB patients and 50 control cases was done by using
following tests.

Erythrocyte sedimentation rate (ESR) test: Westergen method
was used to perform ESR test. ESR, also known as sed rat, is a
non-specific lab. Test does not give the physician information
about a specific disease but they indicate presence of acute or
chronic inflammation. Level of red blood cells was read as ESR
(Kolmer et al., 1961; Baker and Silverton, 1982).

Hemoglobin level (Hb level) detection: Hemoglobin (Hb) in
the sample is oxidized in the presence of ferricyanide to hemoglobin
(Hi, also called methemoglobin), which then reacts with cyanide at
pH 7.2, producing hemoglobin cyanide (Hi CN or cyanmethemoglobin).
Reaction of all haemochromogens is completely developed in 3 minutes, at room temperature. Readings are performed at 620 nm.

Homogenized the samples thoroughly before use. After setting
the instrument at zero with reagent, 20 µL of each homogenized
blood sample in a clean dry micro-pipette was mixed with 5 ml of
Hb reagent, previously kept in cuvette, rinsing three times with
reagent, mixed by inversion and kept for 3-5 minutes in stand.
Then read the absorbance at A=620nm after keeping the cuvettes
one by one in the slot of colorimeter. Absorbance was carefully
noted and recorded. Hb level measured by multiplication of
sample’s absorbance with factor.

Hb level of unknown sample =
Absorbance of unknown sample x factor
(Wedding and Toenjes, 1989).

Total leucocyte count (TLC): For TLC counts, 3% acetic acid
(CH3COOH) is used which hemolyses red blood cells and preserves
white cells. The dilution of the blood to diluting fluid is 1:20.
(Wedding and Toenjes, 1989)

Differential leucocyte count (DLC): For this purpose polychromatic
stains were used each having different staining character. Some
cellular structures except one type of dye, while other cellular
structures are stained with another. Basic part of cell (cytoplasm)
is stained by acidic portion of stain, giving pink appearance and
acid part of cell (nucleic acid) is stained by basic portion of stain
giving it purple to blue appearance, according to the type of stain
used. On the basis of shapes of nuclei, size of cells and granulation,
assign them different names like neutrophil, eosinophil, basophil monocyte or lymphocytes. The differential count gives only the relative number (percentage) of the different
type of white blood cells (Wedding and Toenjes, 1982; Kolmer
et al., 1951; Baker and Silverton, 1982).

The experimental data relating to all laboratory methods used
to diagnose tuberculosis in human beings was subjected to statistical
analysis (Steel and Torrie, 1980)

Results and Discussion
Epidemiological studies spread over ten years were conducted in
order to know the ways of transmission of disease in the
community and impact of control measures. Seasonal trends of
reported TB cases were also noted, which showed a consistent
remarkable increase from the month March to September, with
highest trends in July and August. From 1989-96 there was endemic situation of TB but remarkably increased in 1997 and 1998 (Table I).

Relative incidence (RI) of TB in males and females, reported in each
year was also calculated, showing high mean relative incidence of
TB in males (63.14%) as compared to females (36.86%) (Table I).
These findings were substantiated by the findings of Coller et al.
(1998), who reported that the TB rate was significantly higher in
males than in females as the males were more exposed to the
outer environment, like male subjects working mainly as miners,
were exposed to silica dust.

Age based epidemiology increased TB incidence in age group from
(15-39 years), with a mean age for TB was 33 years. An average
age for tuberculosis was 35 years, as reported by Pollador et al.
(1991). The highest incidence of disease was reported in
males above 30 years. Caminero et al. (1996) carried out the
distribution of tuberculosis, the TB incidence peaked in age group 40-48 and
30-39 years.
Mantoux’s test: Of the total observed clinical Tb cases, 54% cases showed a positive Mantoux’s test. Thirtyfour percent (34%) were male Tb cases and 18% were female cases, so females showed less incidence of positive test. These findings were exactly in accordance with the findings of Collier et al. (1998), who stated that the positive Mantoux’s test was shown mostly by males than the females patient. He defined the positive Mantoux’s test > or = 10mm in duration. The major reasons for not showing the positive Mantoux’s test might be the immunosuppression due to the previous or current antimicrobial treatment. A positive response to skin testing with tuberculin occurred only between 3 and 8 weeks after primary infection.

Furthermore, weak or negative reaction did not exclude a diagnosis of tuberculosis. Some persons were intrinsically poor reactors and in others reactivity was depressed due to advanced disease, old age malnutrition and immunosuppression, including HIV infection. Janis et al. (1996) declared a positive PPD (purified protein derivative) response of > or = 10mm induration. Independent risk factors for + ve, PPD test included age more than 55 years. Male sex with hypertension, HIV infection, current steroid use, history of cancer were associated with – ve PPD response. All Mantoux’s test + ve cases (54%) showed + ve chest radiograph (54%); 6% AFB + ve and 12% PCR + ve results. Moreover the Mantoux – ve cases were all + ve (46%) by X-ray, 12% were AFB + ve and 20% PCR + ve showing no significant relationship between these tests. On the analysis of hematologic tests performed, the ESR values of Mantoux’s + ve cases were slightly higher (65.51 ± 41.216) than – ve case (62.26 ± 34.77) but the increase was statistically non-significant with a probability of 0.0069 (Table 2). Chan et al. (1990) elevated ESR and Mantoux test were valuable indicators for MTb infection.

Hb level of Mantoux’s Test + ve cases was low (9.52 ± 2.766) than – ve cases (8.065 ± 1.44). The difference of Hb level between both + ve and – ve cases were statistically significant (P<0.0003) (Table 2). The total leukocyte count (TLC) + ve cases were 8144.81 ± 1917.74, neutrophil count (88.791 ± 6.758), lymphocyte count (24.77 ± 3.516), eosinophil count (2.111 ± 1.87), monocyte count (3.444 ± 1.423) and basophil (0.109 ± 0.315). While of – ve cases, TLC were 8690.86 ± 1252.28, neutrophil count (68.21 ± 6.38), lymphocyte count (24.43 ± 3.80), eosinophil count (2.30 ± 1.49), monocyte count (3.96 ± 1.87) and basophil count (0.043 ± 0.21) (Table 2). The probability values were 0.2594 for TLC, 0.95 for neutrophil, 0.7421 for lymphocyte, 0.608 for eosinophil, 0.2778 for monocyte and 0.3744 for basophil, showing a non-significant difference between these parameters.

Chest radiograph (X-Ray): All studied cases were showing abnormal radiological shadows on X-Ray with 18% AFB + ve, 34% PCR + ve and 54% Mantoux’s test + ve. Many of these cases had different extent of cavities with different localities on lung area. Some had healed cavities with fibrosis and calcification. When the evaluation was made on the basis of hematological parameters, an increase of ESR values were seen in 100% cases (63.3 ± 38.2) in X-Ray + ve cases, while (18.57 ± 5.26) in – ve cases with a probability P < 0.0000 showing a significant difference between both ESR values. Hemoglobin level (Hb level) in X-Ray + ve cases was decreased (8.84 ± 2.33) than X-Ray – ve cases (11.81 ± 1.66) while probability calculated was 0.0000 showing highly significant difference. According to the study of Mahotra et al. (1986), Pulmonary Tb cases were 80.6% + ve on Chest X-ray with 27.5% + ve by Mantoux test and 30.6% sputum + ve. ESR of all patients was raised. According to another report, mean age for Tb was 33 years, raised ESR, mild anaemia and histological examination gave + ve diagnosis in 100% cases with 66% AFB + ve cases (Wells et al., 1986). Data analysis (Table 2) revealed a remarkable significant increase of total leukocyte count in + ve cases (6882 ± 1550) while in – ve cases (6212.4 ± 1161.68) with probability 0.000. A significant result with probability 0.000 was obtained when neutrophil count was compared between X-ray + ve (69.4 ± 4.69) and – ve case (60.04 ± 5.15).

Lymphocyte count in + ve cases was slightly decreased (24.55 ± 3.64) than in – ve cases (32.29 ± 4.63), P < 0.000 and result was significant statistically. Eosinophil count in + ve cases was (2.16 ± 1.34), while in – ve cases (3.2 ± 1.65), so significant difference was found. Monocyte count also showed no significant difference in + ve (3.7 ± 1.77) and – ve cases (4.38 ± 2.65) while probability was 0.1127. Basophil count of + ve and – ve cases was same (0.08 ± 0.27) with probability 1.00, showing no statistical difference of both values. These findings were further corroborated as drawn by Jain et al. (1991) who observed clinical and radiological differences and compared + ve and – ve case of pulmonary Tb. He noticed anaemia, leucocytosis, raised ESR and abnormal radiological shadows in + ve pulmonary Tb cases. Ounoubali and Scott (1988) declared no correlation between cellular immunity, radiological extent of disease, TLC and ESR.

Acid fast bacilli staining test (AFB test): 18% of all clinical Tb cases were found + ve with high incidence in males (12%) than females (6%); 6% of AFB + ve cases, were Mantoux’s Test + ve also, while 50% were Mantoux’s test + ve but AFB – ve showing no correlation of immunologic response to number of bacilli present. All cases were X-Ray + ve irrespective of AFB test + ve or – ve; 12% cases were PCR + ve which were also – ve by AFB test but 20% PCR test + ve was shown by the AFB – ve cases. 12% cases among 18% were + ve by both PCR and AFB test, 6% AFB + ve were PCR test – ve. So AFB test + ve but PCR test – ve showed, high chances of lab-contamination with other mycobacteria. The + ve AFB and + ve PCR test showed a low number of mycobacteria (< 103/μl) which was not detectable by simple AFB staining technique (Collier et al., 1999). When a comparison of hematological tests was made between AFB + ve and – ve case, the ESR values of + ve cases were decreased (61.22 ± 36.85) than – ve cases (67.14 ± 38.39) with probability level of 0.262 showing non-significant difference. Hb level of AFB-test + ve cases was slightly increased (9.16 ± 2.506) than – ve cases (8.751 ± 2.359), showing non-significant difference (P<0.5984). Total leukocyte count (TLC) was (8211.11 ± 1076.6) in + ve cases while (8419.76 ± 1756.67) in – ve cases, with non significant difference (P<0.7361) (Table 2). Neutrophil count in + ve case was (68.66 ± 5.3) while in – ve case (69.34 ± 4.6) so non-significant difference was shown (P<0.8531). Lymphocyte count in + ve cases was (23.88 ± 4.16) and in – ve cases (24.7 ± 3.5) showing non-significant difference (P<0.6427). Eosinophil count in AFB + ve cases was (2.22 ± 0.971) and in – ve cases (2.82 ± 3.24) so, non significant difference was present (P<0.567). Monocyte count in + ve case was (4.11 ± 2.26) and in – ve cases (3.7 ± 1.63) so P<0.4315) non-significant difference existed. Basophil count in + ve cases was (0.1111 ± 0.333) while in – ve cases (0.073 ± 0.263) with p<0.711, so no significant difference was present. In all Tb cases whether AFB test + ve or – ve no difference in hematological parameters were shown. So a Tb case can not be declared AFB test + ve or – ve on the basis of results of hematological parameters (Table 2).

Polymerase chain reaction (PCR) test: 34% of all observed clinical Tb cases showed + ve PCR-test result, in which 24% were males and 10% females. So mostly males showed a high ratio of PCR test positively than females. All PCR test + ve or – ve were X-ray + ve showing no relationship with extent of cavities at lung areas (Table 2). 16% of PCR test were + ve and Mantoux’s test + ve, while 40% of PCR test were – ve but Mantoux test + ve also, no correlation of immunological responses to PCR test was detected. 12% cases were AFB + ve and PCR test + ve, 6% cases were AFB – ve but PCR test – ve because, AFB test could not distinguish between typical (MTb) and atypical (MOTT or MTMM) Mycobacteria (Collier et al., 1998). 22% PCR test + ve cases (34 - 22%) were AFB
Table 1: Relative incidence of Tb in males and females reported at district Tb Hospital, Circular Road, Faisalabad (1989-98)

<table>
<thead>
<tr>
<th>Years</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of cases</td>
<td>Relative incidence %</td>
</tr>
<tr>
<td>1989</td>
<td>250</td>
<td>60.38</td>
</tr>
<tr>
<td>1990</td>
<td>250</td>
<td>64.96</td>
</tr>
<tr>
<td>1991</td>
<td>336</td>
<td>65.36</td>
</tr>
<tr>
<td>1992</td>
<td>291</td>
<td>67.36</td>
</tr>
<tr>
<td>1993</td>
<td>258</td>
<td>66.28</td>
</tr>
<tr>
<td>1994</td>
<td>269</td>
<td>60.33</td>
</tr>
<tr>
<td>1995</td>
<td>251</td>
<td>59.31</td>
</tr>
<tr>
<td>1996</td>
<td>262</td>
<td>62.11</td>
</tr>
<tr>
<td>1997</td>
<td>363</td>
<td>63.62</td>
</tr>
<tr>
<td>1998</td>
<td>399</td>
<td>66.06</td>
</tr>
<tr>
<td>Total</td>
<td>3029</td>
<td>63.14</td>
</tr>
</tbody>
</table>

Table 2: Statistical analysis of different laboratory methods and hematological parameters used to diagnose tuberculosis in human population

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No of cases</th>
<th>Mantoux test %</th>
<th>X-Ray test %</th>
<th>AFB test</th>
<th>PCR test</th>
<th>ESR mm/h</th>
<th>HB level g/dl</th>
<th>TLC mm3</th>
<th>Neutrophil %</th>
<th>Lymphocytes %</th>
<th>Eosinophils %</th>
<th>Monocytes %</th>
<th>Basophils %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>Males (+ve)</td>
<td>33 8 0 0 0 18.30 ± 12.39 6082.42</td>
<td>60.84 ± 32.07 3.06 ± 3.39</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females (+ve)</td>
<td>17 8 0 0 0 18.02 ± 12.62 6082.42</td>
<td>60.84 ± 32.07 3.06 ± 3.39</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>60 ± 4.47 ± 1.29</td>
<td>6219.4 ± 60.04 ± 22.99 2.2 ± 0.78</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB cases</td>
<td>Males (+ve)</td>
<td>32 40 64 12 24 66.96 ± 9.49 8470.31</td>
<td>70.27 ± 24.07 1.97 ± 3.44</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females (+ve)</td>
<td>60 30 30 6 10 72.16 ± 7.74 8225.56</td>
<td>67.63 ± 26.38 2.5 ± 4.11</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>60 ± 5.99 ± 1.50</td>
<td>6669 ± 69.42 ± 26.46 2.16 ± 3.7</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR test</td>
<td>Males (+ve)</td>
<td>17 16 34 12 34 73.17 ± 5.04 5858.86</td>
<td>71.23 ± 23.41 1.76 ± 3.47</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females (+ve)</td>
<td>10 34 54.03 1385.65 ± 5.37 ± 0.04</td>
<td>1.65 ± 13.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>60 ± 9.72 ± 2.65</td>
<td>1678.32 ± 11.9 ± 9.40</td>
<td>1.97 ± 2.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFB test</td>
<td>Males (+ve)</td>
<td>9 6 18 18 12 61.22 ± 9.18 8211.11</td>
<td>69.61 ± 29.88 2.22 ± 4.11</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females (+ve)</td>
<td>5 6 56 179.67 ± 5.31 ± 0.15 0.971 ± 2.25</td>
<td>0.333</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>60 ± 66.41 ± 2.99</td>
<td>7496 ± 68.64 ± 26.21 2.36 ± 3.28</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mantoux test</td>
<td>Males (+ve)</td>
<td>27 64 64 8 12 66.66 ± 5.62 6144.51</td>
<td>66.791 ± 24.77 2.111 ± 3.444</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females (+ve)</td>
<td>18 64 51.74 7658.06 ± 5.76 ± 3016</td>
<td>1.68 ± 3.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>60 ± 41 ± 2.62 ± 10.74 30.8</td>
<td>4.40 ± 6.00</td>
<td>2.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Statistical analysis of different biochemical-hematological parameters of control cases (Age based)

<table>
<thead>
<tr>
<th>Age grouping</th>
<th>ESR (mm hr^-1)</th>
<th>Hb level (g dl^-1)</th>
<th>TLC (l mm^-3)</th>
<th>Neutrophil (%)</th>
<th>Lymphocytes (%)</th>
<th>Eosinophils (%)</th>
<th>Monocytes (%)</th>
<th>Basophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(12-22) n = 14</td>
<td>14.13</td>
<td>12.11</td>
<td>6080.71</td>
<td>57.0</td>
<td>34.14</td>
<td>3.07</td>
<td>4.78</td>
<td>0.14</td>
</tr>
<tr>
<td>S.D.</td>
<td>± 4.853 ± 1.86</td>
<td>± 126</td>
<td>± 71</td>
<td>± 80</td>
<td>± 80</td>
<td>± 80</td>
<td>± 80</td>
<td>± 80</td>
</tr>
<tr>
<td>(23-32) n = 12</td>
<td>18.52</td>
<td>11.83</td>
<td>6680.33</td>
<td>60.68</td>
<td>32.33</td>
<td>6.66</td>
<td>4.5</td>
<td>0.083</td>
</tr>
<tr>
<td>S.D.</td>
<td>± 4.316 ± 1.68</td>
<td>± 999.58</td>
<td>± 4.56</td>
<td>± 3.22</td>
<td>± 1.15</td>
<td>± 2.33</td>
<td>± 0.29</td>
<td></td>
</tr>
<tr>
<td>(33-42) n = 7</td>
<td>17.16</td>
<td>12.9</td>
<td>6300.60</td>
<td>61.00</td>
<td>33.16</td>
<td>2.66</td>
<td>3.16</td>
<td>0.00</td>
</tr>
<tr>
<td>S.D.</td>
<td>± 4.49 ± 1.96</td>
<td>± 1143.67</td>
<td>± 8.6</td>
<td>± 7.9</td>
<td>± 2.16</td>
<td>± 2.71</td>
<td>± 0.00</td>
<td></td>
</tr>
<tr>
<td>(43-52) n = 8</td>
<td>16.25</td>
<td>11.31</td>
<td>8482.60</td>
<td>68.27</td>
<td>31.62</td>
<td>3.63</td>
<td>3.76</td>
<td>0.00</td>
</tr>
<tr>
<td>S.D.</td>
<td>± 5.04 ± 1.43</td>
<td>± 1506.82</td>
<td>± 2.74</td>
<td>± 2.87</td>
<td>± 0.74</td>
<td>± 3.11</td>
<td>± 0.00</td>
<td></td>
</tr>
<tr>
<td>(53-62) n = 6</td>
<td>16.0</td>
<td>11.8</td>
<td>5820.00</td>
<td>60.2</td>
<td>30.8</td>
<td>4.40</td>
<td>6.00</td>
<td>2.000</td>
</tr>
<tr>
<td>S.D.</td>
<td>± 3.39 ± 0.77</td>
<td>± 739.694</td>
<td>± 6.18</td>
<td>± 1.64</td>
<td>± 1.14</td>
<td>± 2.44</td>
<td>± 0.47</td>
<td></td>
</tr>
<tr>
<td>(63-72) n = 6</td>
<td>14.4</td>
<td>10.4</td>
<td>5550.00</td>
<td>62.8</td>
<td>29.6</td>
<td>3.4</td>
<td>3.8</td>
<td>0.00</td>
</tr>
<tr>
<td>S.D.</td>
<td>± 6.56 ± 0.41</td>
<td>± 360.55</td>
<td>± 1.82</td>
<td>± 1.67</td>
<td>± 1.3</td>
<td>± 1.0</td>
<td>± 0.00</td>
<td></td>
</tr>
</tbody>
</table>
test –ve because PCR being highly sensitive and specific could detect MTB in number 10^{-3} ml of given sample which could not be detected by simple AFB staining test (Coller et al., 1998). Fursegawa et al. (1995) evaluated the clinical diagnostic value of PCR assay for detection of MTB. PCR test lead to rapid diagnosis of TB. PCR test was important to examine samples especially when these were smear –ve. Noordhock et al. (1995) declared the specificity and sensitivity of PCR 99.9 and 82.1 %, respectively. Rapid and simplified PCR was slightly more sensitive than culture method (Zambardi et al., 1996).

By the comparative evaluation of hematological parameters of PCR test – ve and – ve cases, it got clear that there was a significant difference (P<0.049) of erythrocyte sedimentation rate (ESR) between PCR – ve (71.37 ± 34.03) and – ve cases (56.41 ± 37.92). Hemoglobin level was not significantly (P>0.0239) decreased in PCR – ve (8.80 ± 2.56) than PCR test – ve cases (8.96 ± 1.99). Neutrophil count of PCR – ve case (71.23 ± 3.78) was non-significantly (P>0.096) increased than PCR – ve case (88.45 ± 11.81). Lymphocyte count was also non-significantly (P>0.096) decreased in PCR – ve (23.41 ± 3.84) than PCR – ve cases (25.21 ± 3.4). When eosinophil count was compared, there was a non-significant (P>0.175) difference between PCR – ve (1.76 ± 1.2) and PCR – ve cases (2.36 ± 1.39). Monocyte count in PCR – ve (23.23 ± 2.32) and PCR – ve cases (23.12 ± 1.36) were not significantly different (P>0.5063). Basophil count difference was also statistically non-significant (P>0.2718) between PCR – ve (0.117 ± 0.332) and PCR – ve cases (0.280 ± 0.242) (Table 2).

From these analysis it was clear that no assessment about PCR test could be made on the basis of hematological parameters and PCR was independent, sensitive and specific technique. AFB-Test could detect 5,000-10,000 microbacteria/ml of sample, while culture could detect 10-100 organism/ml but it was lengthy and time consuming method, DNA amplification by PCR and related methods were more sensitive and could detect 100 organisms/ml of clinical samples within short time (Coller et al., 1998).

### Table 4: Statistical analysis of different biochemical/hematological parameters of TB cases (Age based)

<table>
<thead>
<tr>
<th>Age grouping (year)</th>
<th>ESR (mm hr^{-1})</th>
<th>Hb level (g dl^{-1})</th>
<th>TLC/ (%)</th>
<th>Neutrophil (%)</th>
<th>Lymphocytes (%)</th>
<th>Eosinophils (%)</th>
<th>Monocyte (%)</th>
<th>Basophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(12-22) n = 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.D.</td>
<td>57.52</td>
<td>9.014</td>
<td>9182.72</td>
<td>8.54</td>
<td>26.45</td>
<td>2.09</td>
<td>2.06</td>
<td>0.00</td>
</tr>
<tr>
<td>(23-32) n = 12</td>
<td>77.92</td>
<td>8.150</td>
<td>8008.33</td>
<td>69.83</td>
<td>24.08</td>
<td>2.08</td>
<td>3.67</td>
<td>0.25</td>
</tr>
<tr>
<td>S.D.</td>
<td>±36.17</td>
<td>±1.69</td>
<td>±1788.38</td>
<td>±4.76</td>
<td>±2.94</td>
<td>±1.22</td>
<td>±1.45</td>
<td>0.00</td>
</tr>
<tr>
<td>(33-42) n = 7</td>
<td>47.14</td>
<td>9.74</td>
<td>8086.71</td>
<td>67.87</td>
<td>26.64</td>
<td>2.86</td>
<td>3.67</td>
<td>0.14</td>
</tr>
<tr>
<td>S.D.</td>
<td>±32.50</td>
<td>±1.71</td>
<td>±1512.65</td>
<td>±5.06</td>
<td>±3.05</td>
<td>±3.19</td>
<td>±2.23</td>
<td>±0.37</td>
</tr>
<tr>
<td>(43-62) n = 6</td>
<td>61.37</td>
<td>9.45</td>
<td>9020.06</td>
<td>71.26</td>
<td>23.12</td>
<td>1.76</td>
<td>3.75</td>
<td>0.00</td>
</tr>
<tr>
<td>S.D.</td>
<td>±40.43</td>
<td>±2.11</td>
<td>±898.94</td>
<td>±5.06</td>
<td>±4.42</td>
<td>±3.18</td>
<td>±3.16</td>
<td>0.75</td>
</tr>
<tr>
<td>(63-72) n = 6</td>
<td>80.98</td>
<td>9.04</td>
<td>7640.00</td>
<td>69.6</td>
<td>25.2</td>
<td>1.80</td>
<td>3.40</td>
<td>0.00</td>
</tr>
<tr>
<td>S.D.</td>
<td>±18.90</td>
<td>±1.9</td>
<td>±1062.01</td>
<td>±3.36</td>
<td>±3.27</td>
<td>±1.1</td>
<td>±0.39</td>
<td>0.00</td>
</tr>
</tbody>
</table>

P<0.05

Omvubali and Scott (1988) stated many abnormalities during tuberculosis, one of them was raised ESR with hyperglobulinemia. According to Hannisdal and Engan (1981) ESR is a prognostic factor. In tuberculosis patients, ESR values were significantly raised (Jurik et al., 1982; Laudet et al., 1980; Sharp and Goldman, 1987; Syrjala, 1986). When data were analyzed on the basis of age, age group (12-23) of control cases showed ESR (14.13 ± 4.88) and TB cases (87.63 ± 38.59), so there was a significant increase of ESR in TB cases (P<0.0002). Group II (23-32) of control subjects showed ESR (18.62 ± 4.3) while, TB cases showed ESR (77.92 ± 39.17) (P<0.001) so, difference was statistically high (Table 3 and 4).

Group III (33-42) of control subject had ESR (17.16 ± 4.49), while TB cases had (47.14 ± 31.6). P (0.0346) so significant difference was present in group IV (43-52), had ESR (18.25 ± 0.04), while TB cases had ESR (61.37 ± 40.43). P (0.0109) showing significant difference.

Group V (63-62) of control subjects showed ESR (18.6 ± 3.39), while TB cases showed ESR (88.0 ± 19.8). P (0.0000) highly significant difference was shown. Group VI (63-72) of control subjects showed ESR (14.4 ± 6.88) while TB cases (42.33 ± 29.5). P (0.0663), the difference of values was non-significant. So, most significant difference of ESR values was shown by age group (23-32) and (63-62) (Table 3 and 4). When the data was analyzed on the basis of sex, there was a statistically non-significant (P<0.113) increase in ESR values of control males (18.9 ± 5.02) than in control females (15.92 ± 4.7). According to the standard values, the ESR values were relatively higher in normal females than in normal males (Kolmar et al., 1991).

In case of TB patients, the ESR values of females were significantly (P<0.0000) increased (72.60 ± 39.90) than males (56.65 ± 37.06).

### Hemoglobin (Hb) level:
After analysis of data (Table 3 and 4) the Hb level of control cases was 12.11 ± 1.86, while of TB cases was significantly decreased (P<0.0000) (9.014 ± 3.007). An iron deficiency in TB patients was reported by (Luntz and Bogie, 1956), Wells et al. (1986), Baynes et al. (1986) and Jain et al. (1991). According to Coller et al. (1998), iron was essential for the growth of mycobacteria both in vivo and in vitro, Mycobacteria had evolved a sophisticated system for scavenging iron from the environment and their eukaryotic hosts. Iron was necessary for the bacterial growth and for the lipodissaccharide. A part of intracellular iron was bound to special proteins, particularly Ferritin, some iron was complexed to sulphur and haem proteins. The supply of intracellular iron was, therefore, controlled by density of surface expressed transferrin receptor. Bacteria mobilize iron by releasing it from host iron binding proteins or by means of specialized iron binding proteins, the siderophores or by combination of both. Age based analysis showed age group (112-22 years) of control subject had Hb level (12.11 ± 1.88) while, TB patients had Hb level (9.04 ± 3.007), (P<0.0004) so, showing significant difference. Age group II (23-32 years) of control subjects showed Hb level (11.83 ± 1.68) while TB patients showed Hb level (8.15 ± 1.59) expressing (P<0.00) most significant difference. Age group III (33-42 years) of control subjects showed Hb level (12.6 ± 1.98), while TB patient had Hb level (9.74 ± 3.67), (P<0.076) so, statistically, difference was non-significant.
Age group IV (43-62 years) of control subjects showed HB level (11.31 ± 1.43), while TB cases showed (9.47 ± 2.11) (P < 0.0019) as non-significant difference was shown by this age group. Age group V (53-62) of control subjects showed HB level (11.38 ± 1.19), while TB cases showed HB level (9.42 ± 2.01) (P < 0.003), so significant difference was shown. Group VI (63-72) of control subjects showed HB level (10.4 ± 0.41) while TB cases showed HB level (7.97 ± 1.06), so P < 0.001, showing significant difference between the groups (Table 1). So, statistically significant difference of HB level was expressed by three age groups, like 23-32, 53-62 and 63-72 years. It means that among these above three age groups there was a significant drop of HB level in TB patients as compared to normal ones. When the data were analyzed on the basis of the control group showed significant difference of HB level was greater in case of males than females. HB level in different age group was also different (Kolmer et al., 1951). In case of TB patients males had HB level (9.48 ± 2.52) while females (7.74 ± 1.51), with probability level of 0.1129 (Table 2), so showing non-significant difference. Fishman (1980) reported that a mild anaemia was often present. It resembled the anaemia of other chronic infections but was usually less severe.

Total leucocyte count (TLC): Total leucocyte count of normal subjects was 6213.4 ± 1161.68 (Table 2), while of TB cases was 8882 ± 1650 (Table 2) with probability value 0.000, expressing highly significant difference of TLC between TB patients and control subjects. Fishman (1980) reported that TLC in peripheral blood was often normal and rarely over 15000/mm. Over 20000/mm suggested some infectious process. High ESR values and high leucocyte count in case of pulmonary TB was narrated by Miller et al. (1989). Wisniewski and Blikava (1989) also stated that data were analyzed on the bases of different age groups (Table 3 and 4). TLC of age group 11-22 (control) was 6090.71 ± 1348.00 (Table 3) while, in TB patients (9182.72 ± 1686.86) so, highly significant difference was present (Table 4). In age group II (23-32) of control subjects, TLC was (6888.33 ± 999.58) while in TB cases (8008.33 ± 1778.38), (P < 0.00318) showing significant difference. In age Group III (33-42) of control subject has TLC (5300.00 ± 1143.67) while, TB cases had (8055.71 ± 2162.65), P < 0.0016 expressing non significant difference. In age Group IV (43-52) of control subjects the TLC was (6462 ± 1506.82), while of TB case was (9025 ± 999.94) with (P < 0.00139) showing significant difference of values. While in TB cases was (7640 ± 1052.6) with probability level (P < 0.001) expressing significant difference. In age group V (53-72 years) the TLC values in normal subjects was (5560.0 ± 360.56) while in TB Cases was (8066.6 ± 1692.06) with (P < 0.00456) so expressing highly significant difference of values in both TB cases and control cases. From the data, it was concluded that in age group (23-42 years) non significant difference of TCL in TB patients as compared to control subjects (Table 3 and 4). When sex based analysis of TLC was made, there was a non-significant increase in male TB patients (6352.3 ± 1108.29) than female control subjects (5692.94 ± 1238.0), same case was present in TB male (8470.31 ± 1684.2) and females (5225.58 ± 1508.9), with probability level (P < 0.11) so, non-significant difference was present (Table 2).

Differential leucocyte count (DLC): The neutrophil count of control subjects was (80.04 ± 6.15), lymphocyte count (32.39 ± 6.65) eosinophil count (3.2 ± 1.55). Monocyte count (4.38 ± 2.56) and basophil count (0.68 ± 0.27) while the TB level in neutrophil count had neutrophil count (59.94 ± 4.62), lymphocyte count (24.55 ± 3.6), eosinophil count (2.16 ± 1.34), monocyte count (3.7 ± 1.72) and basophil count (0.08 ± 0.27) (Table 2).

Significance of DLC values of normal and TB cases yielded such as neutrophil showed significant difference with P < 0.000 eosinophil showed significant difference with P < 0.0004, while monocyte showed non-significant difference (P < 0.1127) and basophil non-significant difference (P < 1.00) (Table 2).

Fishman (1980) demonstrated, that differential white blood cell count was usually normal except when the tuberculous disease was advanced and active. Although, changes did occur in relative numbers of lymphocyte, monocyte and polymorphonuclear leucocyte, these had not proved useful either as clinical or prognostic indexes. According to the findings of Oonubail and Socion (1988), during TB there was a series of metabolic and immunologic abnormalities, including evidence of under nutrition, anaemia, neutrophil leucocytosis, monocytosis, lymphopenia, hyperglobulinaemia and raised ESR. A comparative study of Robles and Reyes (1994) revealed leucocytosis, neutrophilia, high ESR and high frequency in case of tuberculosis. Age based data revealed the different DLC values in age group (12-22) of control persons was neutrophil (67.5 ± 7.01), lymphocyte (34.14 ± 8.69), eosinophil (0.37 ± 1.89), monocyte (4.78 ± 2.09) and basophil (0.14 ± 0.26) (Table 3). While in TB cases, neutrophil (68.54 ± 4.16), lymphocyte (25.45 ± 2.94), eosinophil (2.05 ± 1.22), monocyte (2.91 ± 1.45) (Table 4).

Data showed non significant difference of DLC on the basis of sex in normal cases (Table 2). From the analysis of data (sex based) it was clear that there was unclear differentiation of DLC values in male and female TB patients (Table 2).

Analytical epidemiology: Analytical epidemiology study was conducted on three groups, divided on the basis of age as younger (unmarried) including males and females of age below 24 years, males (married) and females (married). On the basis of detailed analytical epidemiological data, it came to know that many of village dwellers were using raw milk. Collier et al. (1998) discovered that animal reservoirs of M. bovis posed a serious threat to human health. TB in cattle principally involved the lung and it spread from animal to animal by cough spray. Humans in direct contact with cattle might like wise be infected and developed primary pulmonary lesions. Milk was the principal vector of transmission of M. bovis to town dwellers. Such transmission was facilitated by the pooling of milk from many cows and herds. The analysis of data also showed that many patients were having chains of TB patients at home and one person mostly developed TB from previously existing TB source. These findings, also supported their lack of knowledge about prevention of the disease for the children existing in the household in contact with subjects with marked residual post-tuberculosis alteration (Grishko and Vasilev, 1996). Koralev et al. (1999) stated that contact with TB patients was responsible for new cases, discharging drug resistant MTB. Family members and neighbours of patients in active TB of the population were at great risk, to develop TB than other population (Samusensov, 1996). Forty seven percent (47%) of total TB patients were misdiagnosed and received incomplete treatment. According to the studies elaborated by Koralev and Gvozdikin (1984) the tuberculosis had a risen by chance as a result of misdiagnosis and poor control over persons to be treated. Mass media should play an important role in bringing awareness among the people about curing the disease sooner its symptoms occurred. Previous admission to hospital, failure to isolate positive patients in a single room and the absence of positive pressure ventilation were associated with spread of TB. Control measures could halt the transmission of MDR strains (Collier et al., 1999). 82% of admitted TB patients were smokers among males. Collier et al. (1998) demonstrated the higher incidence of TB in men, who smoked and drank exclusively. Hospital records were reviewed by Pratt et al. (1980) to ascertain smoking history and occupation. Data showed significant effects of smoking on the disease. Bronchitis and Broncholithis were associated with textile industry. A significant association was found between cotton dust exposure and both mucous and chronic bronchitis. The comparative efficacy of different tests used in the study may be inferred from the findings that Mantoux test had no correlation with hematological values and the AFB test was limited by the size of the sample. In AFB test two positives cases are detectable if the bacterial count in the sample is about 10^4/ml. The detection of TB through X-ray is not reliable in small children. The hematological
values in all tests were found to be affected by the age and sex of the population. These values were found to have no consistent correlation with any method used in this study. PCR technique was found to be more sensitive, specific and a tool to confirm TB qualitatively and quantitatively and thus being the only reliable technique is worthy of use in reliable diagnosis of TB in human beings.

Acknowledgments
The authors are grateful to Mr. K. Rahman, Department of Chemistry and M. Irsad, S.T.A.K. Sindh and M. Siddique, Department of Microbiology, University of Agriculture, Faisalabad and M. Idrees, Center of Excellence in Molecular Biology (CEMB) University of Punjab for technical assistance. Mr. Tariq Aitz is appreciated for skillful computerized typing of manuscript.

References
Syriale, H., 1986. Peripheral blood leukocyte counts, erythrocyte sedimentation rate and C-reactive protein in tuberculosis caused by the Type B-strain of Francisella tularensis. Infection, 14: 51-54.