Evaluation of Polymerase Chain Reaction in the Diagnosis of Tuberculosis


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Abstract

The diagnosis of tuberculosis may depend on examination of the sputum, examination of the pleural fluid for Mycobacterium tuberculosis, and histopathological examination of the parietal pleura. Still, in many cases, there is no conclusive proof for the diagnosis. The present study aimed at evaluating the role of the polymerase chain reaction (PCR) in the diagnosis of different forms of tuberculosis from different tissue samples and buffy coat of the peripheral blood. This study included 37 patients, divided into three groups. Group I included 20 patients with active tuberculosis sputum smear and/or culture positive for acid fast bacilli. Group II included 13 patients with unilateral tuberculous pleural effusion with highly positive tuberculin test and exudative lymphocytic pleural effusion. The diagnosis was confirmed by culture of the pleural fluid on LJ media, also by histopathological examination for caseating tuberculous granuloma of the parietal pleural biopsy. Group III included 4 patients with right side transudative pleural effusion secondary to congestive heart failure. Sputum PCR was positive in 18 out of 20 patients in group I but negative in all cases of group II and III (sensitivity 90.9% and specificity 100%). Pleural fluid PCR was positive in 10 patients out of 13 cases in group II but negative in group III (sensitivity 81.2% and specificity 100%). Peripheral blood PCR was negative in all patients of the three groups (sensitivity zero% and specificity 100%). From this study it can be concluded that PCR is a useful, sensitive, specific and rapid technique for the diagnosis of tuberculosis from the tissue samples of the affected organ and not from the peripheral blood.

Introduction

Tuberculosis is a preventable and generally curable infectious disease, it remains an important public health problem all over the world. Conventional methods of diagnosis of tuberculosis depend upon the demonstration of tubercle bacilli in the sputum, pleural fluid, pleural biopsy, urine and cerebrospinal fluid specimens. The definitive bacteriological diagnosis of tuberculosis depends on direct microscopy of the sample stained with Ziehl-Neelsen (Z.N.) staining for acid fast bacilli and then cultural confirmation. The conventional culture procedure using Lowenstein-Jensen (LJ) medium is sensitive but require long time (3-6 weeks) due to slow growth of most pathogenic Mycobacteria. The smear examination though simple, rapid and economic, its estimated detection limit is 10^4 bacilli/ml of sputum, while that of the culture is 10^2/ml, so some specimens with positive culture results have negative smear results. Smear sensitivity may be also influenced by the efficiency of decontamination, concentration, the thickness of the smear and the training of examining eyes.

Polymerase chain reaction (PCR) offers a possible alternative technique to the conventional diagnostic techniques. Biological specimens such as sputum, blood,
bronchoalveolar lavage (BAL) fluid, pleural fluid, cerebrospinal fluid and urine are all amenable to PCR analysis(4). The PCR allows the specific detection of single Mycobacterial cell in clinical samples in a matter of hours(5).

Evidence of immune activation in peripheral blood of patients with tuberculosis whether pulmonary or extrapulmonary suggests that organisms commonly enter the blood stream where they sensitize the effector cells(6).

The clinical course of chemotherapy-treated tuberculosis is lengthy so the development of diagnostic procedures capable of rapidly identifying Mycobacterium tuberculosis in clinical samples remains a worth while goal(6).

**Aim of the Work**

The aim of this work was a trial to evaluate the PCR technique in the diagnosis of tuberculosis in different clinical samples and its correlation with some other conventional methods.

**Patients and Methods**

Thirty-three patients with tuberculosis and four patients with non tuberculous pleural effusion were included in this study from those patients admitted to Chest Department of Benha University Hospitals during the period from March 1996 to October 1996.

The patients were classified into the following groups:

- **Group I** : Include 20 patients with active newly diagnosed pulmonary tuberculosis.
- **Group II** : included 13 patients with active conformity diagnosed tuberculous pleural effusion.
- **Group III** : Included 4 patients with transudative pleural effusion secondary to congestive heart failure.

All patients were subjected to the following:

1. Thorough history taking and clinical examination.
2. Chest x-rays, P-A and lateral views.
3. Tuberculin skin testing (mantoux technique) using 2 tuberculin unit of purified protein derivative(7).
4. Erythrocyte sedimentation rate.
5. Total and differential white blood cell count.
6. Sputum examination : direct by Ziehl-Neelsen (Z.N.) staining to detect acid fast bacilli (AFB) and cultivation on Lowenstein-Jensen (L.J.) media.
7. Thoracentesis to obtain specimens of pleural fluid for the study in groups II and III only.
8. Needle biopsy of the parietal pleura by Abram’s needle only in group II and III. The tissues were stained and examined for caseating granuloma in Histopathology Department, Benha Faculty of Medicine.
9. Peripheral blood samples were collected from all patients for separation of buffy coat layer, which includes the mononuclear cells, and stored as a pellet at -70°C for DNA extraction and amplification by PCR technique.
10. PCR examination of the sputum, pleural fluid and buffy coat of the peripheral blood.

The sensitivity and specificity was calculated according to the formula:

Sensitivity = TP / TP + FN
Specificity = TN / TN + FP

Where TP=true positive, TN=true negative, FP=false positive, and FN=false negative.

**Separation of mononuclear cells from peripheral blood**:

Separation of buffy coat from the peripheral blood was done according to the method described by Boyum, (1968)(9) in the following steps:
Two ml of blood were withdrawn by venepuncture under complete aseptic conditions, and delivered immediately to tube containing heparin preservative free (Flow Laboratories) 10 IU/ml blood. For each blood sample, 3 ml ficoll-Isopaque (Flow Laboratories) were put in a siliconized centrifuge tube and kept at room temperature before use. Blood was diluted with 3 parts of phosphate buffer saline (PBS). The diluted blood was layered very gently on top of each 3 ml ficoll-Isopaque, then the tubes were centrifuged at 2000 rpm for 20 min. at room temperature. After centrifugation, the supernatant was aspirated and removed to 5 mm just above the turbid interface. The interface was aspirated and transferred to a new centrifuge tube. The interface layer was diluted with at least 4 volumes of PBS, mixed well and centrifuged at 2000 rpm for 5 min., then the sediment of peripheral blood mononuclear cells (PBMC) was washed two times with PBS. To sterile epindorff tube, the sediment was transferred, washed with 3-4 volumes saline and centrifuged for 5 min. at 14000 rpm, the supernatant was discarded and the pellet was kept in the deep freeze at -70°C for subsequent DNA extraction.

Sputum and pleural fluid samples:

Sputum : Decontamination, liquefaction and concentration of the sputum samples were done using NaOH-N acetyl-cystein (NALC) mixture solution. Equal volumes (5-10ml) of NALC-NaOH mixture and provided samples of sputum were mixed and incubated for 20 minutes at room temperature with frequent shaking. Phosphate buffer saline was added up to 5 ml with proper mixing, then centrifugated at 3000 rpm for 15-20 minutes in a cold centrifuge. The supernatant was decanted and the sediment was used for Z.N. staining and inoculation on L.J. (Oxoid) slopes medium. The slopes were incubated at 37°C and checked weekly for 8 weeks for bacterial growth. Bacterial colonies were tested by niacin strips (Dififico, USA) to identify M. tuberculosis. The remaining of the sediment was resuspended in 1-2 ml phosphate buffer and kept in the deep freeze at -70°C for subsequent DNA extraction.

Pleural fluids : Samples of pleural fluid were centrifuged and the sediment was used for Z.N. smear, culture on L.J. media and DNA extraction for PCR.

PCR assay:

PCR was assayed by using AB analytical kit (produced by AB ANALILICAsrl, Via a formis, 8-35020 PADova). It is a complete kit for DNA extraction, amplification and visualization on agarose gel for the detection of M.tuberculosis. The method was performed as described by manufacturer.

[I] DNA extraction:

DNA was extracted from the pleural fluid, blood and sputum as the following:

The pellets were frosted-defrosted 3 times, 500 ul of lysing buffer, 15 ul sodium dodecyl sulphate (SDS) and 50 ul proteinase K were added to each pellet and incubated overnight at 55°C. Suspension of Mycobacterium tuberculosis culture (positive control) and sterile distilled water (negative control) were included in each run. 700 uL from each sample including the control tubes were added to 700 uL of buffered phenol chloroform in a sterile epindorff tubes. The tubes were shacked well and centrifuged at 12000 rpm for 5 minutes, the supernatants were transferred to a new microtube by a micropipette with a sterile tip. 700 uL. chloroform was added to each tube, shaken well and centrifuged for 5 minutes at 12000 rpm, the supernatant was transferred to a new sterile microtube. 70 uL. sodium acetate were added to each tube and one ml cold absolute ethanol (stored at -20°C) was added
and mixed gently by turning the tube up-down. The tubes were freeze-dried at -20°C for 30 min., after that the tubes were centrifuged at 14000 rpm for 20 min. in a refrigerated centrifuge. Ethanol was discarded leaving the pellets dry, they were left turned up-side down in a sterile vertical flow cabinet. The pellet was suspended with a suitable volume (20 ml) of sterile Tris [TE] = 10 mM Tris HCl, 1 mM EDTA, pH8] and the tubes were mixed well using vortex for one minute.

II) DNA amplification:

The DNA target for amplification used was a pair of primers of insertion sequence (IS) 6110 from *Mycobacterium tuberculosis* genome. For a single amplification reaction a mixture of 2.5 ul buffer, 2.5 ul dNTP, 1 ul primer F, 1 ul primer R, 10 ul DNA solution, 0.5 ul AB taq, 25 ul bidistilled sterile water to final volume and mineral oil 25 ul was used. All components except DNA solution and mineral oil were mixed before starting the amplification and the right amount of the mixture were put into the microtubes. The microtubes was put in the thermal cycler (Biometra personal cycler Nr, 9505332, Biotron, Germany), programmed with the following sequence: 94°C for 5 minutes (first step), 94°C for 2 minutes (denaturation), 68°C for 2 minutes (annealing) and at 72°C for 2 minutes (extension). This was repeated for 30 cycles. Following amplification, the DNA was concentrated by alcohol precipitation and dissolved in 30 ul of sterile distilled water. The amplified products were analyzed by agarose gel electrophoresis. In an epindorff microtube the following were mixed 2.5 ul bromophenol blue 6x, 10 ul of amplified DNA and 2.5 ul distilled water. For staining, ethidium bromide was added to agarose gel and running buffer in concentration of 0.05 μl/ml. This mixture was put in the gel well and connected with power supply 64 volt/min. After 30 minutes the DNA was visualized by florescence of ethidium bromide when the gel was transferred to the ultraviolet transilluminator. The DNA bands at 123bp were observed and comparing with that of the positive control and the molecular size marker. A molecular size marker Hae III digested OX174 replicative form DNA (New England Biolabs) was used as a molecular size marker.

Results

This study was carried out on 37 patients, 20 males (54%) and 17 females (46%), their ages were ranged from 13-56 years with the mean age of 31.9 ± 17.4 years. They were classified into three groups, group I included 20 patients with active newly diagnosed pulmonary tuberculosis, group II included 13 patients with tuberculous pleural effusion and group III included 4 patients with transudative pleural effusion secondary to congestive heart failure.

The results of this study were statistically analyzed in the following tables:

Table 1. Statistical analysis of erythrocyte sedimentation rate and tuberculin tests in different groups of the study

<table>
<thead>
<tr>
<th>Group</th>
<th>ESR1 in mm</th>
<th>P value</th>
<th>Tuberculin test in mm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>X + SD</td>
<td>&lt; 0.05</td>
<td>Range</td>
</tr>
<tr>
<td>Group I (No. 20)</td>
<td>45-84</td>
<td>68.8±9.1</td>
<td>&lt; 0.05*</td>
<td>13-18</td>
</tr>
<tr>
<td>Group II (No. 13)</td>
<td>51-92</td>
<td>71.4±13.4</td>
<td>&lt; 0.05**</td>
<td>15-21</td>
</tr>
<tr>
<td>Group III (No. 4)</td>
<td>13-24</td>
<td>15.6±4.9</td>
<td>&lt; 0.05*</td>
<td>5-7</td>
</tr>
</tbody>
</table>

ESR1: Erythrocyte sedimentation rate in the first hour.
* : Group I versus group III.
** : Group II versus group III.
Table 2. Statistical distribution of the results of the sputum smear (Z.N. stain) and culture on L.J. media.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sputum smear Z.N. stain</th>
<th>Sputum culture on L.J. media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Group I (No. 20)</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Group II (No. 13)</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Group III (No. 4)</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Sensitivity of the sputum smear stained with Z.N. stain = 86.9%
Specificity of the sputum Z.N. stained smear = 100%
Sensitivity of the sputum culture on L.J. media = 100%
Specificity of sputum culture on L.J. media = 100%

Table 3. Distribution of the results of different methods for diagnosis of *M. tuberculosis* in pleural fluid in the different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Z.N.</th>
<th>Culture on L.J. media</th>
<th>Abram's Pl. Biopsy</th>
<th>Pleural biopsy tuberculous granuloma</th>
<th>Protein content in gm/dl</th>
<th>% of lymph content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Group I (No. 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II (No. 13)</td>
<td>0</td>
<td>13</td>
<td>4</td>
<td>9</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Group III (No. 4)</td>
<td>0</td>
<td>4</td>
<td>00</td>
<td>4</td>
<td>00</td>
<td>4</td>
</tr>
</tbody>
</table>

Percentage of the pleural fluid +ve stained smear = zero%
Percentage of the pleural fluid +ve culture on L.J. media = 30.8%
Percentage of pleural biopsy tuberculous granuloma = 38.5%
Table 4. Statistical analysis of the results of PCR in all tissue samples

<table>
<thead>
<tr>
<th>Group</th>
<th>Sputum PCR</th>
<th>Pleural fluid PCR</th>
<th>Blood PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Group I (No. 20)</td>
<td>18</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>Group II (No. 13)</td>
<td>00</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Group III (No. 4)</td>
<td>00</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Sensitivity of the sputum PCR = 90.9%
Specificity of the sputum PCR = 100%
Sensitivity of the pleural fluid PCR = 81.2%
Specificity of the pleural fluid PCR = 100%
Sensitivity of the blood PCR = zero%
Specificity of blood PCR = 100%

Table 5. Statistical analysis of PCR of the sputum and peripheral blood in comparison with Ziehl-Neelsen stain and Lowenstein-Jensen media in group I (active pulmonary tuberculosis)

<table>
<thead>
<tr>
<th>References test</th>
<th>Screening test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sputum PCR</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Sputum ZN</td>
<td>16</td>
</tr>
<tr>
<td>Stain +ve</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Sputum culture</td>
<td>18</td>
</tr>
<tr>
<td>On L.J. media</td>
<td>18</td>
</tr>
</tbody>
</table>

The percentage of positive sputum for T.B. by Z.N. Smear, culture and PCR were 85%, 100% and 90% respectively.
Table 6. Statistical analysis of PCR of the pleural fluid and peripheral blood in comparison with Z.N. stain, L.J. culture and pleural biopsy tuberculous granuloma in group II (tuberculous pleural effusion)

<table>
<thead>
<tr>
<th>References test</th>
<th>Pleural fluid</th>
<th></th>
<th>Blood PCR</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td>Positive</td>
</tr>
<tr>
<td>Pleural fluid ZN</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>+ve</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>-ve</td>
<td>10</td>
<td>76.9</td>
<td>3</td>
<td>23.1</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>76.9</td>
<td>3</td>
<td>23.1</td>
</tr>
<tr>
<td>Pleural fluid culture on LJ media</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>+ve</td>
<td>3</td>
<td>23.1</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>-ve</td>
<td>7</td>
<td>53.8</td>
<td>2</td>
<td>15.4</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>76.9</td>
<td>3</td>
<td>23.1</td>
</tr>
<tr>
<td>Pleural biopsy tuberculous granuloma</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>+ve</td>
<td>4</td>
<td>30.8</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>-ve</td>
<td>6</td>
<td>46.1</td>
<td>2</td>
<td>15.4</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>76.9</td>
<td>3</td>
<td>23.1</td>
</tr>
</tbody>
</table>

The percentage of positive pleural effusion for T.B. by Z.N. Smear, culture, biopsy and PCR were 0%, 30.8%, 38.5% and 76.9% respectively.

Discussion

Pulmonary tuberculosis is still one of the major health problems in the developing countries including Egypt(11). Diagnosis of tuberculosis depends upon the demonstration of tubercle bacilli in the sputum, pleural fluid, pleural biopsy specimen or granuloma in the pleura(12). The PCR will have to show that it provides advantages over the conventional methods such as Z.N. staining and culture. It allows specific detection of single bacterial cells in clinical material in a matter of hours(13).

This study included 37 patients, they were classified into three groups, group I included 20 patients clinically diagnosed as suspected pulmonary tuberculosis and the diagnosis was confirmed by sputum smear with Z.N. staining followed by culture on L.J. medium. Group II included 13 patients with tuberculous pleural effusion, as all had exudative lymphocytic pleural effusion and PPD-highly positive tuberculin reactive persons (mean diameters of tuberculin test was 17.6±4.2 mm) (Table I), also mean of ESR in the first hour in this group was 71.4 ± 13.4 mm, which was higher than that of the control group III (15.6 ± 4.9 mm) and the difference was statistically significant (Table 1). These results are in agreement with that of Barnes et al., (1991)(14). Group III (control group) include 4 patients with transudative pleural effusion secondary to
congestive heart failure as the mean protein content of the pleural fluid was 1.6 ± 0.4 gm% (Table 3).

Sputum smear examination after staining with Z.N. stain was positive in 17 out of 20 patients with pulmonary tuberculosis, group I, with a sensitivity of 86.9% and specificity of 100% as it was negative in tuberculous pleural effusion, group II, and congestive heart failure with pleural effusion, group III (Table 2). These results are in agreement with that of Chia et al., (1990) and Tenover et al., (1993). Sputum culture on L.J. media was positive in all cases of group I (sensitivity 100%) because all patients of this group were selected as culture positive and specificity 100% as it was negative in group II and III (Table 2). These results are in agreement with that of Michael, (1994).

In this study pleural fluid smears stained with Z.N. stain were negative in all studied patients (Table 3). Routine smears of Mycobacterium tuberculosis in the pleural fluid are not indicated because they are almost always negative, unless the patient has a tuberculous empyema. Culture results of the pleural fluid for Mycobacterium tuberculosis was positive in 4 cases of group II out of 13 patients (30.8%), but negative in all patients of group III (Table 3). These results agree with that of Berger and Mejia, (1973). Pleural biopsy has its greatest utility in establishing the diagnosis of tuberculous pleurisy. The demonstration of caseating granuloma in the parietal pleura is highly suggestive of tuberculous pleurisy. In the present study, 5 patients out of 13 patients in group II was positive (38.5%) and all cases of group III was negative, these results are in agreement with that of Sharer, (1968). The remaining 61.5% of group II showed chronic non specific inflammation (Table 3). Group II showed high percentage of lymphocytic effusion (91.6 ± 4.8%), high protein content (6.7 ± 1.8 gm%) and highly positive tuberculin test (17.6 ± 4.2 mm). All exudative lymphocytic pleural effusion in PPD-tuberculin reactive persons was considered to be tuberculous by some investigators, and should be treated for tuberculosis.

As regarding sputum PCR technique IS, 6110 DNA segment was used as the amplification target because it is reported to increase the sensitivity of the assay. This protocol detected 18 patients out of 20 in group I (sensitivity of 90.9%) and specificity of 100% as the sputum PCR in group II and III were negative (Table 4). These results are in agreement with that of Huh et al. (1995). Pleural fluid PCR was positive in 10 out of 13 patients with tuberculous pleural effusion, group II (sensitivity of 81.2% and specificity of 100%) as the pleural fluid PCR in group III was negative (Table 4), these results are in agreement with that of De-Wit et al. (1992), but higher than that of Hamdy and Elnawawi, (1997). This difference can be explained by different pathogenesis of the pleural fluid in pleural tuberculosis. It might result from rupture of a subpleural caseous focus in the lung into the pleural space. Delayed hypersensitivity also plays an important role in the development of tuberculous pleural effusion. It may increase the permeability of the pleural capillaries to protein and the increased protein levels in the pleural fluid results in a much higher level of pleural fluid.
formation. Also, the intense inflammatory reaction in the parietal pleura impedes the lymphatic drainage from the pleural space and leads to accumulation of the fluid.\(^{[17]}\).

As regarding PCR in the buffy coat of the peripheral blood it was negative in all the three groups with a sensitivity of zero% and specificity of 100% (Table 4), these results were similar to the study of Kalk et al. (1992)\(^{[21]}\), who attributed this negative result to the localized nature of the infection. But these results disagree with that of Schlugier et al. (1994)\(^{[6]}\), who examined buffy coat of 8 patients with active pulmonary tuberculosis, 6 of them were infected with HIV. All their patients had positive PCR results for Mycobacterial DNA in the buffy coat. This can be explained by the fact that these patients were immunocompromised and the liability to blood dissemination was high.

Comparing sensitivity of PCR by the other conventional bacteriological procedures, it was noticed that:

1. Percentage of the sputum PCR was 90% while that of the sputum Z.N. stained smear was 90% and that of the sputum culture on L.J. media was 100% (Table 5).
2. Percentage of the pleural fluid PCR was 76.9% while that of the pleural fluid Z.N. stained smear was zero%, that of the pleural fluid culture on L.J. media was 30.8% and that of the pleural biopsy tuberculous granuloma was 38.5% (Table 6).

So, it was noticed that the sensitivity of the sputum PCR was near to the sputum Z.N. stained smear and sputum culture on L.J. media, but the percentage of pleural fluid PCR was higher than pleural fluid culture on L.J. media and pleural biopsy.

**Conclusion**

From this study it can be concluded that:

1. Detection of *Mycobacterium tuberculosis* DNA in peripheral blood in patients with pulmonary tuberculosis by PCR technique is doubtful and sputum has been starting point for most of molecular amplification techniques.
2. PCR cannot be used for the diagnosis of extrapulmonary tuberculosis from the peripheral blood.
3. Uses of PCR in the diagnosis of tuberculous pleural effusion could be limited only to the following situations:
   - Negative results of conventional methods inspite of strong clinical suspicions or when rapid diagnosis is essential.
4. PCR is an efficient, rapid, sensitive and specific procedure for the recovery of tubercle bacilli and isolation of DNA from the clinical tissue samples of the affected organ and not from the buffy coat of the peripheral blood.

**References**

4. Eisenstein BL (1990) : The polymerase chain reaction, a new diagnostic method of


