Combined use of Polymerase Chain Reaction, Interferon-γ and soluble IL-2 Receptor for Differential Diagnosis of Tuberculous Pleural Effusion

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ABSTRACT

Background and Objectives: Rapid and early differential diagnosis between tuberculous and non-tuberculous pleural effusion (TPE & NTPE) is a critically important clinical problem. The paucibacillary nature of TPE and inefficiency of conventional biochemical and microbiological investigations challenge the application of more comprehensive markers. In this study we evaluated the relevance of applying polymerase chain reaction (PCR), for detection of Mycobacteria Tuberculosis- DNA (TB-DNA), in association with interferon gamma (IFN-γ) and soluble IL-2 receptor (sIL-2R) levels in pleural fluid for differential diagnosis of TPE. Patients and Methods: Study population included 60 patients with pleural effusion (PE); 40 patients with TPE (7 patients with confirmed tuberculosis (TB) and 33 patients with probable TB), and 20 patients with non-tuberculous, non-infectious pleural effusion (NTPE) (10 cases due to malignancy and 10 cases due to heart failure). PE samples were assessed for: biochemical markers (total protein and glucose), lymphocytic count, presence of acid fast bacilli in Ziehl-Neelsen (Z.N.) stained direct smears and by culture on Löwenstein Jensen (L.J.) medium, TB-DNA using conventional PCR, as well as levels of IFN-γ and sIL-2R using commercial ELISA kits. Results: Biochemical markers, in particular total protein level, confirmed the exudative nature of TPE and malignant PE. The percentage of lymphocytes in PE was significantly higher in patients with confirmed TB (>80%) than all patients in other studied groups. All patients with confirmed TB were positive for TB-DNA PCR and had IFN-γ and sIL-2R levels more than calculated cut off points. However, probable TB group showed a wide range of variability. None of patients with malignant PE but three of heart failure patients were positive for TB-DNA PCR. All patients with NTPE had IFN-γ level less than cut off point. On the other hand, all patients with heart failure but 50% of patients with malignancy had sIL-2R level less than cut off point. Conclusion: Clinical data together with simultaneous detection of TB-DNA by PCR and measurement of IFN-γ and sIL-2R levels as well as lymphocytosis (>80 %) in PE could provide the basis for rapid and efficient diagnosis of pleural TB in different clinical settings.

INTRODUCTION

Tuberculous pleurisy is the second most frequent form of extra-pulmonary tuberculosis (TB) after TB lymphadenitis and the most common cause of pleural effusion (PE) in many countries. Tuberculous pleural effusion (TPE) can follow early post-primary, chronic pulmonary, or miliary TB. Pleural TB is often an acute illness with cough, pleuritic chest pain, fever, or dyspnea. Although TPE may resolve over a period of several months without treatment, failure to diagnose and treat pleural TB can result in progressive disease and the involvement of other organs in as many as 65% of patients. However, treatment based on clinical suspicion rather than on microbiological diagnosis results in over treatment, delay in accurate diagnosis, and potentially greater morbidity. So, reliable clinical markers providing physicians with rapid and accurate diagnosis of TPE is greatly needed.

Conventional methods for the diagnosis of TPE have proven inefficient. Direct examination of pleural fluid and Ziehl-Neelsen (Z.N.) staining of acid-fast bacilli is rapid and inexpensive but requires bacillar concentration of 10,000/mL and has a low sensitivity of approximately 0–1%. Although culture is more sensitive (11 to 50%), it requires 2 to 6 weeks to grow Mycobacterium tuberculosis (M. tuberculosis) and a minimum of 10 to 100 viable bacilli. The paucity of bacilli and the nonspecific cytochemical characteristics of PE in pleural TB mandate more invasive procedures such as pleural biopsy for differential diagnosis. Pleural biopsy has high sensitivity (70–80%), but the procedure requires expertise, may increase the morbidity of the patient and can cause complications.

Early diagnosis of TPE has been greatly facilitated by the detection of specific sequences of the M. tuberculosis genome in PE using polymerase chain reaction (PCR) with sensitivities ranging from 20 to 80% and specificities of 78 to 100%, depending on the area of the genome that is amplified and the technique used for DNA extraction. However, it is indicated that PCR alone has limited value in diagnosis of TPE with negative smear and its role in day-to-day clinical practice needs to be defined. A negative PCR result never eliminates the possibility of TB, and a positive result is not always confirmatory. The high biological sensitivity and specificity of PCR for M. tuberculosis suggest that this method, when used in combination with easily applicable methods, could be highly beneficial for differential diagnosis of TPE.
Estimation of pleural effusion levels of different cytokines by enzyme-linked immunosorbent assay (ELISA) have been implicated to aid in the diagnosis of TPE such as interferon-\(\gamma\) (IFN-\(\gamma\))\(^{10,11}\) and soluble interleukin-2 receptor (sIL-2R).\(^{12,13}\) IFN-\(\gamma\) is a key cytokine in the immunopathogenesis of TB.\(^{14}\) It has been proved that patients with TPE have significantly higher levels of IFN-\(\gamma\) in the pleural fluid as compared to peripheral blood thus exhibiting localisation of predominantly Th1-type immunity in the pleural fluid.\(^{15}\) In addition, released sIL-2R, a surrogate marker of T-cell activation and proliferation, could serve as a marker of disease activity in patients with TB.\(^{16}\) However, the application of these simple diagnostic aids have not being widely utilized for routine laboratory diagnosis of pleural TB, particularly for the risk of false-positive results in populations with a low prevalence of TB. In this study, we have evaluated the relevance of combined use of PCR, for detection of \(M.\) tuberculosis-DNA, (TB-DNA PCR) and determination of IFN-\(\gamma\) and sIL-2R levels in pleural fluid for differential diagnosis of TPE.

**PATIENTS AND METHODS**

**Study groups of patients:**

The current study was conducted on 60 patients presenting with pleural effusion and attending EL Maamora Chest Hospital or chest department of Alexandria University Hospital. Patients under study included 40 patients with TPE and 20 patients with NTPE. Patients with TPE were first classified to characteristic clinical picture, suggestive X-ray findings, positive tuberculin test as well as lymphocytosis and total protein in pleural effusion. Confirmed pleural TB based on the extracted DNA and 13 \(\mu\)l sterile distilled water. The cycling conditions were as follows; an initial denaturation cycle at 94°C for 10 minutes, 35 cycles of (denaturation at 94°C for 1 second, annealing at 56°C for 1 second and extension at 72°C for 1 minute), and one cycle of final extension at 72°C for 10 minutes. The amplified DNA products were run on 2% agarose containing ethidium bromide (0.5\(\mu\)g/ml) and visualized under UV light for the positive band (150 bp).

Estimation of IFN-\(\gamma\) and sIL-2R receptor levels:

IFN-\(\gamma\) and sIL-2R levels were measured in PE using corresponding commercially available ELISA kit (Bender MedSystems GmbH, Campus Vienna Biocenter 2, Vienna, Austria, Europe) following manufacturer recommendations. IFN-\(\gamma\) and sIL-2R sample concentrations were determined from corresponding standard curve of diluted IFN-\(\gamma\) and sIL-2R standards, respectively. The sensitivities of the applied ELISA kits for...
None of patients with malignant PE but Z.N. stained smears as well as cultures on L.J. media of PE for all cases with TPE were negative for M. tuberculosis acid fast bacilli.

TB-DNA-PCR:
None of patients with malignant PE but only three out of the 10 patients with heart failure (30%) were positive for TB-DNA by PCR. On the other hand, all patients of the confirmed TB group and only 19 out of the 33 patients (57.6%) of the probable TB group were positive for TB-DNA PCR with no significant difference between both tuberculous groups. (Fig 1)

Microbiological markers:
Z.N. stained smears as well as cultures on L.J. media of PE for all cases with TPE were negative for M. tuberculosis acid fast bacilli.

Biochemical markers, lymphocytic count:
The mean total protein levels in PE of the malignant, confirmed TB and probable TB groups (4.39 ± 0.15, 4.39 ± 0.19 & 4.33 ± 0.12 g/dl, respectively) were significantly higher than corresponding level in the heart failure group (2.30 ± 0.16 g/dl), p<0.001. The mean glucose level was significantly lower in PE obtained from patients with malignant PE (5.07% ± 2.31 and 103.36 ± 4.5E mg/dl, respectively), p<0.001. In addition, the mean glucose level was significantly lower in the confirmed TB group than in the probable TB group, p=0.004. The percentage of lymphocytes in PE of patients with confirmed TB (91.57 ± 2.68 %) was significantly higher than corresponding percentage in PE from patients with probable TB (55.18 ± 5.42 %, p<0.001), malignancy (21.0 ± 5.07 %, p<0.001), and heart failure (67.80± 9.43 %, p=0.035). (Table 1)

Statistical analysis:
Statistical analysis of the data was carried out using SPSS version 15. Chi-square test and student t-test were used for comparing qualitative and quantitative data of each two groups, respectively. Significant difference was considered at p value ≤ 0.05. Numerical values are presented as mean ± SE.

RESULTS

**Biochemical markers, lymphocytic count:**
The mean total protein levels in PE of the malignant, confirmed TB and probable TB groups (4.39 ± 0.15, 4.39 ± 0.19 & 4.33 ± 0.12 g/dl, respectively) were significantly higher than corresponding level in the heart failure group (2.30 ± 0.16 g/dl), p<0.001. The mean glucose level was significantly lower in PE obtained from both confirmed TB and probable TB groups of patients (47.93 ± 2.69 and 65.16 ± 4.82 mg/dl, respectively) than in the malignant and heart failure groups (85.91 ± 2.31 and 103.36 ± 4.5E mg/dl, respectively), p<0.001. In addition, the mean glucose level was significantly lower in the confirmed TB group than in the probable TB group, p=0.004. The percentage of lymphocytes in PE of patients with confirmed TB (91.57 ± 2.68 %) was significantly higher than corresponding percentage in PE from patients with probable TB (55.18 ± 5.42 %, p<0.001), malignancy (21.0 ± 5.07 %, p<0.001), and heart failure (67.80± 9.43 %, p=0.035). (Table 1)

**Microbiological markers:**
Z.N. stained smears as well as cultures on L.J. media of PE for all cases with TPE were negative for M. tuberculosis acid fast bacilli.

**TB-DNA-PCR:**
None of patients with malignant PE but only three out of the 10 patients with heart failure (30%) were positive for TB-DNA by PCR. On the other hand, all patients of the confirmed TB group but only 19 out of the 33 patients (57.6%) of the probable TB group were positive for TB-DNA PCR with no significant difference between both tuberculous groups. (Fig 1)

**Immunological markers:**
The sensitivities of the ELISA kits used for detection of IFN-γ and sIL-2R were 1.5pg/ml and 0.04 ng/ml, respectively. The mean levels of IFN-γ in NTPE of the malignant and heart failure groups were 1.54 and 1.73 pg/ml, respectively. The mean levels of sIL-2R in NTPE of the malignant and heart failure groups were 19.61 and 1.73 ng/ml, respectively. Therefore, the cut off levels of IFN-γ and sIL-2R calculated according to these values were 3 pg/ml and 20 ng/ml, respectively. (20)

**IFN-γ:**
The mean levels of IFN-γ in the malignant and heart failure groups were 1.54 ± 0.1 and 1.78 ± 0.11 pg/ml, respectively. None of the patients belonging to these groups had IFN-γ level more than cut off point (3 pg/ml). All patients included in the confirmed TB group had an IFN-γ level > 3 pg/ml while in the probable TB group only 45.45 % of them (15/33) had IFN-γ level > 3 pg/ml. The mean levels of IFN-γ in the confirmed TB and probable TB groups (61.56 ± 13.58 and 38.45 ± 8.49 pg/ml, respectively) were significantly higher than corresponding levels in PE due to malignancy and heart failure (p <0.001 & = 0.008, respectively). Moreover, the mean level of IFN-γ in the pleural fluid of confirmed TB group was significantly higher than that in the probable TB group of patients, p = 0.011. (Table 2, Fig.2)

**sIL-2R:**
50% of patients with malignant PE (5/10) and none of the heart failure group had sIL-2R levels more than the cut off point (20ng/ml). All patients included in the confirmed TB group and only 51.51% of the probable TB group (17/33) had sIL-2R levels more than the cut off point (20 ng/ml). The mean level of sIL-2R in PE of the confirmed TB group was significantly higher than corresponding level in the malignant and heart failure groups (27.08 ± 1.0, 19.61 ± 1.86; p=0.044 and 12.51 ± 1.65 ng/ml; p=0.001, respectively). sIL-2R in PE of the probable TB group (19.37 ± 1.38 ng/ml ) was significantly higher than corresponding level in heart failure group, p=0.003, but still significantly lower than that in the confirmed TB group. (Table 2, Fig.3)

**Combined cytological, molecular and immunological data:**
None of patients with malignancy had lymphocyte percentages > 80%, positive TB-DNA PCR results or IFN-γ > cut off point. However, 50% of patients enrolled in this group had sIL-2R > cut off point. None of patients with heart failure had IFN-γ or sIL-2R > corresponding cut off point. However, positive TB-DNA PCR results and lymphocyte percentages > 80% were observed in 30% of patients belonging to this group. On the other hand, All patients with confirmed TB had lymphocyte percentages >80%, positive TB-DNA PCR results as well as IFN-γ and sIL-2R levels > corresponding cut off point. However, Probable TB group showed a wide range of variability. Therefore, this group of patients was subdivided...
into 4 subgroups (I-IV). Subgroup I (n=11) was similar to the confirmed TB group of patients. Subgroup II (n=9) had lymphocyte percentages < 80%, negative TB-DNA PCR and IFN-γ and sIL-2R below corresponding cut off point. In subgroup III (n=8), TB-DNA PCR was positive but IFN-γ and sIL-2R were below corresponding cut off point. Subgroup IV had negative TB-DNA PCR but IFN-γ and sIL-2R were >corresponding cut off point. Regarding associated lymphocytosis (lymphocyte percentages >80%), subgroups III and IV also showed wide variability. The specificities of TB-DNA PCR, IFN-γ and sIL-2R were 65.0%, 100% and 75.0%, respectively.

### Table 1: Cytological and biochemical characteristics of PE in different study groups.

<table>
<thead>
<tr>
<th></th>
<th>Malignant (n=10)</th>
<th>Heart failure (n=10)</th>
<th>Confirmed TB (n=7)</th>
<th>Probable TB (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total protein g/dl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3.6-4.9</td>
<td>1.9-3.1</td>
<td>3.6-5.0</td>
<td>3.0-5.7</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>4.39 ± 0.15</td>
<td>2.30 ± 0.16</td>
<td>4.39 ± 0.19</td>
<td>4.33 ± 0.12</td>
</tr>
<tr>
<td>P1</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>0.812</td>
<td></td>
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<td><strong>Glucose mg/dl</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Range</td>
<td>73.3-96.8</td>
<td>80.0-123.0</td>
<td>33.5-55.2</td>
<td>25.0-153.7</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>85.91 ± 2.31</td>
<td>103.36 ± 4.57</td>
<td>47.93 ± 2.69</td>
<td>65.16 ± 4.82</td>
</tr>
<tr>
<td>P1</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>P2</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>P3</td>
<td>0.852</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lymphocytes (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2-42</td>
<td>15-100</td>
<td>80-100</td>
<td>0-100</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>21.00 ± 5.07</td>
<td>67.80 ± 9.43</td>
<td>91.57 ± 2.68</td>
<td>55.18 ± 5.42</td>
</tr>
<tr>
<td>P1</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>P2</td>
<td>0.035*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P1: Significant level when compared to malignant group; P2: Significant level when compared to heart failure group; P3: Significant level when compared to confirmed TB group; (*): Significant difference at p ≤ 0.05.

### Table 2: Levels of IFN-γ (pg/ml) and sIL-2R (ng/ml) in PE of different study groups.

<table>
<thead>
<tr>
<th></th>
<th>Malignant</th>
<th>Heart failure</th>
<th>Confirmed TB</th>
<th>Probable TB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>33</td>
</tr>
<tr>
<td><strong>IFN-γ (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.19-2.25</td>
<td>1.30-2.40</td>
<td>15.95-125.08</td>
<td>1.47-163.41</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>1.54 ± 0.10</td>
<td>1.78 ± 0.11</td>
<td>61.56 ± 13.58</td>
<td>38.45 ± 8.49</td>
</tr>
<tr>
<td>P1</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.008*</td>
</tr>
<tr>
<td>P2</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.008*</td>
</tr>
<tr>
<td>P3</td>
<td>0.011*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Positive &gt; 3</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Negative ≤ 3</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td><strong>sIL-2R (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>12.1-29.2</td>
<td>4.0-20.0</td>
<td>22.0-29.9</td>
<td>4.7-28.4</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>19.61±1.86</td>
<td>12.51±1.65</td>
<td>27.08±1.00</td>
<td>19.37±1.38</td>
</tr>
<tr>
<td>P1</td>
<td>0.044*</td>
<td>&lt;0.001*</td>
<td>0.003*</td>
<td>0.03*</td>
</tr>
<tr>
<td>P2</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>P3</td>
<td>0.03*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive &gt; 20</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Negative ≤ 20</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>16</td>
</tr>
</tbody>
</table>

P1: Significant level when compared to malignant group; P2: Significant level when compared to heart failure group; P3: Significant level when compared to confirmed TB group; (*) : Significant difference at p ≤ 0.05.
Fig. 1: PCR results of ten PE samples. Lane 6: DNA marker, Lane 14: Negative control, Lane 1, 2, 3, 8, 10: Negative PCR results, Lane 4, 5, 7, 9, 11, 12: Positive PCR results (TB-DNA band at 150 bp).

Fig. 2: Distribution of pleural effusion IFN-γ among different study groups. #Cut off point: 3pg/ml.
DISCUSSION

Pulmonary TB is a common worldwide infection and a medical and social problem causing high mortality and morbidity, especially in developing countries. Despite numerous diagnostic tools, TPE remains difficult to diagnose. PCR is now a well-developed technique and has been extensively evaluated for routine diagnosis of TPE. However, it is assumed that establishing a set of biomarkers is greatly required and much effort has been devoted to the identification of immunologically important factors to improve the differential diagnosis of TPE. In this study we evaluated the relevance of applying TB-DNA PCR in combination with IFN-\(\gamma\) and sIL-2R levels in pleural fluid for differential diagnosis of TPE. Patients under study included 40 patients with confirmed TB, only 19 out of the 33 patients with probable TB (57.6%), none of patients with malignant PE and 3 out of the 10 patients with heart failure were positive for TB-DNA by PCR. Nagesh et al. found that PCR represents a rapid and sensitive method for the detection of mycobacterial DNA in tuberculous pleural effusion. It was also found that the overall accuracy of PCR of pleural biopsy was similar to the results of pleural biopsy culture; however, PCR of the pleural biopsy was much faster in reaching diagnosis. On the contrary, Liu et al. found that PCR alone has limited value in diagnosis of tuberculous pleurisy with negative smear, but when used in combination with pleural biopsy, it can be used to increase early detection of TB pleurisy in such patients. Basically, the low bacterial load in tuberculous pleural effusion seems to limit the clinical utility of PCR testing because the sensitivity of \(M.\ \text{tuberculosis}\) PCR testing largely depends on the bacillary load.

Our results revealed that IFN-\(\gamma\) was significantly higher in TPE than in PE due to malignancy or heart failure. Moreover, IFN-\(\gamma\) was significantly higher in confirmed TB than in probable TB group. All patients with confirmed TB and 45.45% of probable TB but none of patients with malignancy and heart failure groups had IFN-\(\gamma\) more than cut off value (3 pg/ml). Observed data were in agreement with nearly all previous reports that looked for the utility of IFN-\(\gamma\) levels in differential diagnosis of tuberculous pleural effusion. Yamada et al. have reported pleural fluid IFN-\(\gamma\) sensitivity and specificity of 91% and 100%, respectively for TB pleurisy. Villena et al. observed that pleural INF-\(\gamma\) levels (>3.7 IU/ml) were very valuable in diagnosing pleural TB. A recent meta-analysis was carried out and proved that the measurement of IFN-\(\gamma\) in pleural effusion is a useful tool for diagnosis of tuberculous pleural effusion. Several previous reports have documented the statistically significant higher levels of IFN-\(\gamma\) in tuberculous pleurisy compared to malignant.
effusions. \[24,31,32\] It was also observed that IFN-γ was significantly higher in TPE than pleural effusions due to various non tuberculous, non malignant etiologies (pneumonia, rheumatoid arthritis, heart failure and renal failure). \[20,23\] Sharma et al., also proved the localization of predominant Th1 response in pleural effusion of patients with pulmonary TB. They observed significantly higher IFN-γ levels as well as increased percentage of CD4+ lymphocytes expressing IFN-γ in their pleural effusion as compared to that of peripheral blood of the same patients. \[15\]

As regards sIL-2R all patients included in confirmed TB group, 51.51% of probable TB group, 50% of malignant group and none of heart failure group had sIL-2R > cut off point (20 ng/ml). In addition, sIL-2R in PE of confirmed TB group was significantly higher than corresponding level in malignant and heart failure groups. However, sIL-2R in PE of probable TB group was only significantly higher than corresponding level in heart failure group. Moreover, the level of sIL-2R in pleural effusion of confirmed TB group was significantly higher than corresponding level in probable TB group of patients. In agreement with our results Harita et al. and Porcel et al., \[13,34\] demonstrated that sIL-2R level was significantly higher in TPE than in carcinomatous and transudative PE.

Current results also supported previous reports looking for simultaneous assessment of IFN-γ and sIL-2R in patients with TPE. \[12,13,36\] Kim et al., found statistically significant higher levels of IFN-γ and sIL-2R in tuberculosis pleural effusion than pleural effusion due to other causes (malignancy, parapneumonia, empyema and acute eosinophilic pneumonia). \[35\] Tsao et al., found that patients with higher-grade pulmonary TB, revealed significantly higher epithelial lining fluid levels of IFN-γ and sIL-2R-α compared to those with lower grade pulmonary TB. \[36\] It was also observed that IFN-γ was the most sensitive and specific indicator of tuberculous pleuritis and the next most sensitive was sIL-2R when compared with IL-12p40, IL-18, adenosine deaminase and immunosuppressive acidic protein.\[12\]

Tuberculous and malignant PEs are characteristically exudative in nature with characteristic accumulation of CD4+ T lymphocytes. The accumulation of T cells in tuberculous pleural effusion results from a local cellular immune response to M. tuberculosis, producing local activated T cells and macrophages. In carcinomatous pleural effusion, the accumulation of T cells might equally represent an immune response to tumor antigens. It has been observed that the local immune reaction in tuberculous pleurisy is in favor of the Th1 pathway (enhanced cellular immunity) causing higher levels of IFN-γ and IL-2. On the other hand, in malignant pleural effusion local immune response was in favor of the Th2 pathway (depressed cellular immunity) causing high levels of IL-10 with low IFN-γ levels. \[37,38\]

According to our results the specificities of IFN-γ, sIL-2R and TB-DNA PCR were 100%, 75.0% and 65.0%; respectively. So negative results of IFN-γ but not those of sIL-2R and TB-DNA PCR could provide a sure exclusive diagnostic marker for negative cases. Therefore the results of sIL-2R and TB-DNA PCR needs to be confirmed by those of IFN-γ.

Theoretically, PCR for the M. tuberculosis genome should be highly effective and rapid in diagnosing tuberculous pleuritis. However the solitary application of TB-DNA PCR for the diagnosis of tuberculous pleural effusion has important limitations among them are false negative and false positive results. \[39-40\] Therefore, in this study we have attempted to construct clinical definition of TB based on the combination of several data including clinical history, cytological examination, biochemical analysis, TB-DNA PCR and the levels of cytokines (IFN-γ, sIL-2R).

In our study false negative results were suggested in 5 patients with probable TB (subgroup IV) who had coincident high levels of IFN-γ and sIL-2R above the cut off values. In addition, false positive results were also postulated in 3 patients with heart failure and in 9 patients with probable TB (subgroup III) because those patients had IFN-γ and sIL-2R levels less than the cut off values while TB-DNA PCR was positive. False positive PCR results are especially problematic when the rate of the culture is low and this is the case in our study due to paucibacillary nature of the disease. Many studies have reported problems with false positive PCR results at rates ranged from 0.8 % to 30.0 %. \[41,42\] As regards patients belonging to the heart failure group, they all had a transudative pleural effusion with relative low lymphocytic infiltration and no clinical manifestations nor X-ray findings suggestive of TB. Those observations supported the false positive results of TB-DNA PCR observed in this group. The variability of results of TB-DNA PCR reported in previous studies could be attributed to the use of different primers to detect M. tuberculosis in PE samples, enrolment of small study populations, the application of diverse criteria for diagnosis of pleural TB, and/or the high content of inhibitor (blood) in pleural effusion. \[40\] The carryover contamination by amplicons which can be transferred accidentally to a new sample or can contaminate PCR reagents was also reported which might explain false positive results. \[45\]
In conclusion, the results of this study suggested that clinical data together with simultaneous measurement of IFN-γ, sIL-2R and detection of TB-DNA by PCR as well as lymphocytosis in pleural effusion could provide the basis for rapid and efficient diagnosis of pleural TB in different clinical settings. Such association was clearly observed in all cases (100%) confirmed TB group as well as in 19 out of 33 cases (57.57%) of the probable TB group.

REFERENCES