Detection of *Mycobacterium Tuberculosis* in Peritoneal Dialysis Patients

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**Abstract**

**Background:** Peritoneal TB is a variant of abdominal TB, it poses a public health problem in endemic regions of the world, this type of tuberculosis usually secondary to hematogenous spread from lung and it's difficult to diagnosis because nonspecific clinical presentation with insidious onset, it is considered one of the medical challenges' doctors’ face in the diagnostic process.

**Aims:** This study was conducted to determine the occurrence rate of *M. tuberculosis* in peritoneal fluids using IS6110-based polymerase chain reaction and conventional methods.

**Patients and Methods:** Seventy-five leftover peritoneal fluid were enrolled in this study samples separated in three tubes, the first one underwent chemical analysis, the second one was centrifuged for 10 minutes at 2000 -3000 rpm, the sediment was resuspended for direct diagnosis of *Mycobacterium tuberculosis* using Ziehl–Neelsen staining, loopful from the sediments were inoculated in Lowenstein Jensen media, while the third tube from peritoneal fluid was stored in –20°C till DNA extractions.

**Results:** Fifteen samples (20%) out of 75 peritoneal fluid samples were detected by Ziehl-Neelsen stain (ZN), three of them were culture negative on Lowenstein Jensen (LJ) media. Positive results of peritoneal fluid samples for *Mycobacterium Tuberculosis* on Lowenstein Jensen (LJ) media were 11 (14.6%) out of 75 after an incubation period of 14-28 days, while PCR results improved that 28 (37.3%) out of 75 samples were positive for peritoneal Tuberculosis.

**Conclusions:** This study concludes PCR targeting IS6110, may play a major role in the diagnosis of peritoneal tuberculosis this will ensure early treatment of patients and restricted further dissemination of disease.

**Keywords:** *Mycobacterium Tuberculosis*, peritoneal tuberculosis, peritoneal dialysis, insertion sequence 6110.

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Introduction:

Tuberculosis (TB) remains one of the majority group of diseases have devastating effect with highly morbidity and mortality (1). Tuberculosis primarily disease of the lung, but it can disseminate to involve any organ through the body and it refer as extrapulmonary TB (2). Peritoneal TB is a variant of abdominal TB; it poses a public health problem in endemic regions of the world. Peritoneal tuberculosis is an uncommon site of extrapulmonary tuberculosis, this type of Tuberculosis usually secondary to hematogenous spread from lung and it's difficult to diagnose because nonspecific clinical presentation with insidious onset, it is considered one of the medical challenges' doctors face in the diagnostic process (3). The incidence of Peritoneal TB from extra-pulmonary tuberculosis representing 4%–10% while its account 25%–60% of abdominal TB cases, as other types of Tuberculosis, peritoneal TB can occur at any age group (4). Many studies reported that at less 20%–40% of patients with peritoneal tuberculosis required surgical intervention, mainly with those group of patients underwent abdominal complications such as confined perforation with abscess or fistula, complete obstruction and free perforation, so early diagnosis of peritoneal TB can prevent surgical managements (5). Delay in diagnosis and treatment is directly related to poor patient outcome. As the number of *Mycobacteria* in peritoneal fluid is extremely low and direct detection is therefore difficult and not sufficiently sensitive, the current gold standard for the diagnosis of these organisms remains the isolation of it from peritoneal fluid by culture (6). However, there is an urgent need for more rapid diagnostic techniques as culture can take up to 8 weeks in case of Tuberculosis (7). This study was conducted to study the occurrence rate of *M. tuberculosis* complex in peritoneal fluids using IS6110-based polymerase chain reaction and conventional methods.

Subjects and Methods

Over six months from January to July 2018 leftover peritoneal fluid specimens (15 ml) from Baghdad City hospital, Al-Yarmouk Teaching Hospital, AL-kadhimiya Teaching Hospital and Al-Elwia Pediatrics Hospital were collected in sterile specimen container from patients underwent peritoneal dialysis as a result of chronic renal insufficiency. The total number of peritoneal dialysis patients during the study period was seventy-five patients. All participants were interviewed by a questionnaire focused on personal and clinical data. Participants were enrolled in conformity with informed consent, privacy and confidentiality of patients who were sampled, the ethical aspects of this study have been approved by the ethical council in Medical College, Al-Nahrain University.

Processing of Sample:

Peritoneal fluid processing occur according to standard operating procedures (SOPs), samples were separated in three tubes, the first one underwent chemical analysis, cell count and differentiations; the second one was centrifuged for 10 minutes at 2000–3000 rpm, the supernatant aspirated with a sterile pipette into a sterile tube, the sediment was resuspended for direct diagnosis of *Mycobacterium*
tuberculosis using Ziehl–Neelsen staining, loopful from the sediments were inoculated in Lowenstein Jensen media, while the third tube from peritoneal fluid was stored in 20°C till DNA extractions.

**Extraction and Purification of genomic DNA:**

Due to the complexity of cell wall structure of *Mycobacterium Tuberculosis*, freezing–thawing technique according to the Reischl *et al* (8) were used to cell lysis, briefly cell pellets were re-suspended in 600 µl nucleic lysis buffer. The cell suspension was subjected to liquid nitrogen for 3 min, followed by boiling for 1 minute and 15 seconds for five cycles, genomic bacterial DNA presto TM mini g manufacture by Geneaid Company were used to purify DNA. Two primers were used targeting the insertion sequence IS6110. These were; forward primer IS1: 5’-CCT GCG AGC GTA GGC GTC GG 3’ and reverse primer IS2 5’ CTC GTC CAG CGC CGC TTC GG -3’ were synthesized in Alpha DNA® (Canada), to amplify 123 bp(9). The reaction was performed in 25 µL of reaction mixture containing 2 µL of DNA template, 200 mM of each deoxynucleotide triphosphate (dNTP), what exact conc mM MgCl2, 1 µL of each primer, and 0.6-unit Taq DNA polymerase (Bioneer/ Korea). The volume was adjusted to 25 µL with deionized water. The PCR conditions included an initial denaturation 98°C for 3 min, followed by 28 cycles at 98°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 2 min. The reaction was amplified by a thermocycler PCR system (Hybaid/ UK). The PCR products were stained with ethidium bromide and undergone 2% gel electrophoresis. The results were detected through UV transluminator with a camera.

**Statistical Analysis**

Quantitative variables were expressed as a mean± standard deviation (SD) and analyzed to student *t*-test while binomial variables were expressed as frequency and percentage and analyzed by Chi-squared test whenever possible. The statistically significant was set at P value ≤0.05.

**Results:**

**Biochemical analysis and cell count**

Differential and cell count were done on all peritoneal fluid samples (n=75), the mean of white blood cells was (418.90) predominantly, neutrophilic pleocytosis with a mean of (82.05) versus lymphocytosis (30.63). The biochemical analysis of sugar and proteins were described in the table (1). Analysis of the subgroup of patients that were subsequently proved to be positive for *Tuberculosis* (n=28) displayed elevated white blood cell predominance of neutrophils as well as high level of protein with low level of sugar.
Table (1): Laboratory investigations performed on peritoneal fluid samples.

<table>
<thead>
<tr>
<th>Test</th>
<th>Patients</th>
<th>Normal value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>WBC total count</td>
<td>418.90</td>
<td>1498.00</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>82.05</td>
<td>99.00</td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td>30.63</td>
<td>97.00</td>
</tr>
<tr>
<td>Glucose mg/dl</td>
<td>48.58</td>
<td>168.00</td>
</tr>
<tr>
<td>Protein mg/dl</td>
<td>123.28</td>
<td>360.00</td>
</tr>
</tbody>
</table>

Ziehl-Neelsen stain and Culture:

Fifteen samples (20%) out of 75 peritoneal fluid samples were detected by Ziehl-Neelsen stain (ZN), three of them were negative on Lowenstein Jensen (LJ) media. Positive results of peritoneal fluid samples for *Mycobacterium Tuberculosis* on Lowenstein Jensen (LJ) media were 11 (14.6%) out of 75 which after an incubation period of 14-28 days.

Molecular Diagnostic method for detection of *tuberculosis*

Insertion sequences (IS6110) conventional PCR assay was used for the detections of *Mycobacterium Tuberculosis* complex, results indicated that 28 (37.3%) out of 75 samples were PCR positive with amplifications product size to amplify 123 bp figure (1). All culture positive samples (n= 11) were positive by PCR,

The PCR sensitivity for *Mycobacterium tuberculosis* complex was 100% and the specificity was 76.36%. The negative predictive value (NPV) was 100% with a positive predictive value (PPV) of 48% table (2)

Table (2): Sensitivity and specificity of PCR based IS6110 compared with culture.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Positive</th>
<th>Negative</th>
<th>PPV</th>
<th>NPV</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>11</td>
<td>17</td>
<td>48.00%</td>
<td>100%</td>
<td>100%</td>
<td>76.36%</td>
</tr>
</tbody>
</table>

Total 11 100% 64 100.00%
Discussions:

Peritoneal tuberculosis (TB) remains one of the most serious diseases faced by doctors, particularly inside the developing nations, the diagnosis of peritoneal tuberculosis is regularly tough and cannot be made or excluded according to medical findings, which might be quite protean and nonspecific. (10). Among Seventy-five patients were enrolled in this study, the mean age was (35) years, Female's patients were between the ages of (20-50) years and the males were (10-60) year's table (2). From the whole study, populations 26 were females (34.66%) and 49 were males (65.33%). Results of current study revealed that, the mean age was (35) years, there was a statistically significant difference between age and sex distribution, this result was similar to many other investigations working in this field who mentioned that Peritoneal TB can develop at any age, and it is typically seen between 25 and 55 years of age. The gender among patients with peritoneal TB differs from study to study and from country to another (11, 12).

Some studies reported that disease predominantly found in females, while other observed peritoneal TB predominantly in males (13, 14, 15). Such results variations may be attributed to socioeconomic factors such as overcrowding, poverty, illiteracy, and limited access to health-care facilities, furthermore the presence of male immigrants in the workforce, especially in developing countries make tuberculosis mostly found in male than females.

In this study, peritoneal fluid profile was significantly abnormal in definite diagnosis and in all cases that have been studied. Analysis revealed high level of protein and low level of sugar in all samples positive for Tuberculous peritonitis and these finding is agreed with those in most previous studies by (16, 17).

This study reported that white blood cell was high number with predominance in neutrophilic pleocytosis. Most studies have indicated that in the case of tuberculosis is accompanied by high number of white blood cells, which are mostly lymphocytic pleocytosis however, in patients undergoing peritoneal dialysis predominance of neutrophils, may be observed (18, 19).
This indicates that each of cell count and differentiation in associated with chemical picture of peritoneal fluid were offers a good diagnostic prediction in patients with peritoneal tuberculosis and this comes in agreement with previous studies (20,21).

Microscopic examination of peritoneal fluid through both smear and culture strategies stays a helpful intend to analyze peritoneal TB. However, to increase the detection rate of both procedures, it requires increased volume of peritoneal fluid,somereported assumed that 1 litter of ascitic fluid could provide up to 83% of positive results (22).

This technique is impractical, as this large amount of fluid needs special centrifuge machines. Interestingly, conventional Mycobacterial culture takes up to 4–6 weeks to achieve results, even with liquid culture methods the process requires at least 12 days. This delay may increase morbidity and therefore, other diagnostic tools for early diagnosis of TB are needed (23).

The use of polymerase chain reaction (PCR) in diagnosis of peritonealtuberculosis by amplifying specific regions of the Mycobacterium Tuberculosis (MTB) genome emerged as a tool for rapid and accurate diagnosis of this infection. The performance of this test has been studied, with results disclosing high specificity and variable sensitivity. Its clinical usefulness appears to be in patients with underestimated by clinicians and the uses of less sensitive conventional methods (24).

Insertion element IS6110 sequence. Which is a repeat sequence present in a large number of Mycobacterium tuberculosis complex.repetitive nature of IS6110 insertion sequence in M. tuberculosis genome makes it an attractive target for PCR amplification, can be used as genetic markersin molecular assays which allow sensitive and specific detection of MTBC, The as it could contribute to a higher degree of sensitivity of the assay (24).

Results in this study, improved that 28(37.3%) out of 75 samples were PCR Positive Fifteen samples (20%) out of 75 peritoneal fluid samples were detected by Ziehl-Neelsen stain (ZN), on Lowenstein Jensen (LJ) media were 11 (14.6%).

The sensitivity of PCR for Mycobacterium tuberculosis complex based on IS6110 primer was 100% and the specificity was 76.36%. Concerning negative predictive value (NPV) was 100 % with a positive predictive value (PPV) of 48 %. The sensitivity and specificity of this procedure differ greatly among the different laboratories ranging from 50–90% and 60–100% respectively (25).

Results obtained in this study show agreement with some previous studies by Lin, et al (232) Haldar, et al (233) and it is in disagreement with various others such as those of Bonington, et al (10); Chaidir, et al(234). The difference in such studies may be due to the sample size as well as to the method used to extract the DNA from samples in addition to the volume of peritoneal fluid.

Conclusions: This study concludes that, increased occurrence rate of peritonealtuberculosis,in patients who suffering from peritoneal dialysis. However, PCR targeting IS6110, may play a major role in the diagnosis of peritoneal tuberculosis this will ensure early treatment to patients and restricted further dissemination of disease.

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Conflict of interest
The authors declare that they have no competing interests.
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