Polymerase chain reaction is a good diagnostic tool for *Mycobacterium tuberculosis* in urine samples

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Abstract

Tuberculosis is a major public health problem in developing countries. Tuberculosis of the urinary tract is not uncommon in all over world, and it continues to be an important clinical problem, mainly because of its nonspecific clinical presentation and variable radiographic appearance, which often mimic other pathologic lesions. Detection of suspects bacterial population; direct smears can be used but they are often negative and do not differentiate tuberculosis from nontuberculous mycobacterium. Culture, which is more sensitive, takes 6 to 8 weeks because of the slow growth rate of mycobacterium. Polymerase chain reaction (PCR) is a technique used to amplify extremely small amounts of a specific genomic sequence rapidly. The presence of an extremely small number of bacteria can thus be detected within 24 to 48 hours. Therefore PCR is a promising tool for rapid detection of urinary tract tuberculosis in urine samples.

*Key words:* PCR, *Mycobacterium tuberculosis*, diagnosis

**Introduction**

Tuberculosis is a major public health problem in developing countries. Despite the decline in incidence seen in the 1980s, resurgence has occurred and 8% to 15% of patients with pulmonary tuberculosis go on to develop urinary tract tuberculosis (UTB). The estimated involvement is 400 per 100,000 populations.
Tuberculosis of the urinary tract is not uncommon in all over world, and it continues to be an important clinical problem, mainly because of its nonspecific clinical presentation and variable radiographic appearance, which often mimic other pathologic lesions (Figure 1).

The earliest urographic change occurs in the minor calices, and caliceal dilation is the first sign. However, it is often so minimal that the diagnosis can be extremely difficult (Ney and Friendenberg, 1981). A slight loss of sharpness of the calix, which represents mucosal edema, is another subtle initial sign (Elkin, 1990). As the disease progresses, the calix appears moth-eaten or feathery (Taylor, 1939). An instance of calcification in renal tuberculosis was found in 7% of cases by Crenshaw (1930) and in 14.4% of cases by Gow (1965). Urethral strictures have been reported in about 10% to 56% of patients, and bladder involvement is found in one third of cases of UTB (Royalance et al., 1970).

Early diagnosis of the disease allows administration of antitubercular treatment at a stage at which it may be curative. However, more often than not, the diagnosis is delayed because of a delay in presentation and in making a definitive diagnosis, with the consequence that a number of patients present with nonfunctioning kidneys, ureteral stricture, and shrunken bladders. These changes can be avoided if the diagnosis is made early and treated effectively.

The diagnostic criterion for UTB is the isolation of Mycobacterium tuberculosis from urine. This is not easy to achieve, as the discharge of organisms into the urine is sporadic and, more importantly, involves few organisms (Gow et al., 1984; Gow, 1992). Direct smears are often negative and do not differentiate tuberculosis from nontuberculous mycobacterium. Culture, which is more sensitive, takes 6 to 8 weeks because of the slow growth rate of mycobacterium (Manjunanth et al., 1991). This study conducted by the Şişli Etfal Research and Training Hospital 1st Urology Clinics. These urine specimens used for both routine culture and PCR techniques.

DNA extraction

100 ml urine samples were used in 2 hours after taken and concentrated to 2 ml. Bacterial DNA extraction and PCR for amplification of the Mycobacterium tuberculosis complex (Yamaguchi et al., 1989) were carried out according to a previously published protocol (Seth et al., 1996). DNA was extracted with QIAamp DNA Mini Kit (Qiagen).

PCR analysis

The urine specimens were prepared for PCR amplification according to the following protocol.
Sediments were washed three times with an equal volume of Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA; pH: 8.0) at 5000-x g for 5 min. The resulting pellet was resuspended in 0.25 mL of Tris-EDTA buffer and then boiled for 20 min. After centrifuged at 5000-x g for 5 min, 5 µl of the supernatant was analyzed by PCR in a 50 µl reaction mixture. The PCR reaction mixture contained 50 mM KCl, 10 mM Tris-HCl, pH: 8.3, 1.5 mM MgCl₂, 200 µM deoxynucleoside triphosphates (dNTP), 2.5 U Taq polymerase (Boehringer Mannheim, Germany) and 0.5 µM (each) of the primers. The primer sets used to amplify the 123-bp IS 6110 gene fragment consisted of TBC1 (CCT GCG AGC GTA GGC GTC GG) and TBC2 (CTC GTC CAG GGC CGC TTC GG) (Eisenach et al., 1991; Bennedsen et al., 1996 and Desjardin et al., 1998-Qiagen). The reaction mixture was subjected to 30 cycles of amplification (95°C, 30 sec; 68°C, 30 sec; 72°C, 30 sec) followed by a 5 min extension at 72°C (Thermocycler; Techne). 15 µl of the amplification products were analyzed by electrophoresis in an ethidium bromide stained 2% agarose gel (Sambrock et al., 1989).

Results

Total of 29 urine specimens were collected from 15 women and 14 men. Their average ages for women 35 and for men were 42.9 patient were positive for tuberculosis by both test, while remain of the study group were negative. 4 urine samples could not evaluated by PCR due to the presence of inhibitory substances of nature. The bladder biopsy was diagnostic of tuberculosis in 5 (20%) of the 24 patients biopsied. Fine needle aspiration cytological examination was done in 1 patient with epididymal swelling and was suggestive of tuberculosis. The urinary PCR was falsely positive in 1 (4%) patients (Table 1).

Mycobacterium tuberculosis organisms were found to be excreted intermittently in the urine of infected patients. 7 men patients were showing testicular swelling, perianal sinus or genital ulcer. 2 women and 3 men patients had secondary coliform infection. 1 woman and 5 men had gross hematuria. Microscopic hematuria was present 9 men and 11 women. All patients had frequent urination, initially during the day; later, at night. Dysuria, frank pain, suprapubic pain, blood or pus in the urine presented in all patients. Cystourethroscopy was performed in 29 patients. The bladder revealed evidence of chronic cystitis in 24 patients. After detailed consultation other fungal infection for 2 patient, pyonephrosis for 1 patient, calyceal diverticula for 2 patients and bladder cancer for 1 patient were detected. Complete blood cell count, sedimentation rate, serum chemistry were investigated for all suspect patients. The erythrocyte sedimentation rate was elevated in 16 patients. Chest and spine radiographs showed negative results for all patients. Two patients had abnormal renal parameters. Kidney, urethra and bladder radiographs reveal calcifications in the kidney for 4 patients. Calcifications were intraluminal. Bladder calcification was not obtained from radiographic results. There was no HIV positive patient. All positive patients were consulted by infection consultants and treated with INH, Rifampin, pyrazinamide and ethambutol for first 2 months and rifampin and INH treatment were continued for resistance to either agent exists.

Discussion

Tuberculosis is still rampant in underprivileged societies and continues to take a heavy toll. It can affect any organ system in the body and produce protean manifestations. UTB occurs in 8% to 20% of patients with pulmonary tuberculosis, with a prevalence of 400 per 100,000 population (Styblo, 1980; Peterson, 1994). The most common presenting symptoms in patients with UTB are irritative voiding symptoms and hematuria, with the reported incidence in world literature at around 60% and 50%, respectively (Narayan, 1982). The speed and simplicity of amplification techniques can greatly assist TB diagnosis, therapy and epidemiology. This improvement will continue with the rapid advances in the tools available in molecular biology. No long series has been reported on the sensitivity and specificity of urinary PCR in UTB. Although, PCR test has been extensively studied and proved highly sensitive, specific and rapid. In various studies, data show sensitivity ranging from 87-100% (usually >90%) and specificity from 92.2-99.8 % (usually >95%).
compares to cultures (37%), bladder biopsies (47%) and intravenous pyelogram (IVP) examinations. Along an accurate clinical assessment, PCR is the best available tool to avoid delay in starting treatment because it takes only 6 hours (Davies et al., 1999). False-negative findings may result from the presence of inhibitors; non-homogeneous distribution of bacteria in the specimen so that the fraction tested does not contain mycobacterium; or low numbers of mycobacterium in the specimen, which decreases the probability of the presence of organisms in the fraction analyzed by PCR (Missirliu et al., 1996; Kolk et al., 1992). False-positive results with PCR may be caused by contamination due to the presence of amplicons or Mycobacterium tuberculosis complex bacilli or DNA (van Vollenhoven et al., 1996).

Most PCR assays employed for the diagnosis of tuberculosis do not distinguish between infections caused by Mycobacterium bovis and Mycobacterium tuberculosis. The species-specific PCR assays developed for the differentiation of Mycobacterium tuberculosis and Mycobacterium bovis have been invalidated due to false negative results owing to absence of specific target sequences such as mtp40 in some Mycobacterium tuberculosis strains (Gillespie and McHugh, 1997 and Cousins, 1992).

In conclusion, in this study PCR results were same with routine culture results. Therefore, PCR is a rapid tool to detect Mycobacterium tuberculosis in urine samples for UTB patients.

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References


