

Urinary Polymerase Chain Reaction for Diagnosis of Urogenital Tuberculosis

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Introduction: The aim of this study was to evaluate diagnostic value of urinary polymerase chain reaction (PCR) in urogenital tuberculosis (UTB).

Materials and Methods: In 33 patients with confirmed diagnosis of UTB by urine culture and/or acid-fast staining, clinical symptoms and laboratory and radiological findings were evaluated. For each patient, 3 consecutive urine samples were examined by PCR for *Mycobacterium tuberculosis* and the results were compared with the standard microbiological methods and radiological findings.

Results: The mean interval between the appearance of the symptoms and the diagnosis was 12.3 ± 12.2 months. Symptoms were irritative bladder symptoms such as dysuria and diurnal or nocturnal frequency (51.5%), flank pain (27.3%), microscopic hematuria (18.2%), gross hematuria (9.1%), and suprapubic pain (9.1%). The laboratory findings included hematuria (27.3%), pyuria (12.1%), and hematuria with pyuria (48.5%). Diagnosis of UTB was made in 19 patients by positive urine culture for MT in 19 patients (57.6%), positive acid-fast staining in 6 (18.2%), and both in 8 (24.2%). Intravenous urography showed abnormal findings in 16 patients (61.5%), including pyelocaliceal dilatation (26.9%), ureteral stricture and hydroureter (23.1%), multiple small caliceal deformities (15.4%), severe parenchymal destruction (11.5%), autonephrectomy (11.5%), and calcification (7.7%). Urinary PCR was positive in 16 patients (48.5%) and in 10 (62.5%) with abnormal findings on intravenous urography.

Conclusion: A high index of suspicion is necessary for diagnosis of UTB even in patients with nonspecific manifestations. Urinary PCR is recommended for instant diagnosis and screening before further examinations, but it cannot be the sole diagnostic modality for diagnosis of UTB.

Keywords: urogenital diseases, tuberculosis, *Mycobacterium tuberculosis*, polymerase chain reaction

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INTRODUCTION

Tuberculosis is still one of the major global health issues, especially in the developing countries. Currently, it is the second cause of death due to infectious diseases following the acquired immunodeficiency syndrome, worldwide.⁽¹⁾ Extrapulmonary tuberculosis constitutes up to 20% of the total

cases of the disease, and with the involving rate of 14%, the urogenital system is of the most common affected sites.^(1,2)

Clinical manifestations and paraclinical findings of urogenital tuberculosis (UTB) are nonspecific, resulting in delayed diagnosis and treatment which can cause kidney dysfunction, ureteral stricture, and

shrunken bladder.⁽¹⁾ Currently, diagnosis of UTB is based on the acid-fast staining or urine sample culture. Acid-fast staining is a rapid screening test, but it is not sensitive enough especially in the specimens obtained from extrapulmonary sites.^(2,3) The more sensitive urine cultures in both solid and fluid media require 6 to 8 weeks and 13 days, respectively, to give the results with yet low sensitivity rates.^(2,4-6) Polymerase chain reaction (PCR) is an instant assay that recognizes very few amounts of bacterium within 24 to 48 hours and has been reported to be very useful for detection of *Mycobacterium tuberculosis* (MT).^(1,5-12) The sensitivity of this method has been variably reported to be 60% to 100% for diagnosis of UTB.^(1,2,13-16) We investigated urine samples of the patients with UTB to evaluate the diagnostic potential of urinary PCR in patients with UTB.

MATERIALS AND METHODS

Patients

We enrolled patients with UTB whose diagnosis had been confirmed by positive acid-fast staining and/or positive urine culture results for MT, but had not undergone treatment yet. They had been referred to the clinical centers affiliated to Isfahan University of Medical Sciences to receive treatment. Their demographic data, clinical symptoms, and laboratory and radiological findings were recorded. The PCR assay was performed on the collected urine samples of all patients for detection of MT.

Sampling Method

Three consecutive early morning urine specimens were taken from each patient. Twenty microliter of screening-processed distiller soluble 10% and 10 μ L of proteinase K (20 μ L/mL) were added to 200 mL of the centrifuged urine sample and incubated at 60°C for 8 hours. A specimen for PCR was then prepared using the standard DNA extracting and phenol-chloroform method. The needed product was proliferated using the primers relevant to ISO6110 insertion element regions (Techgene International, Les Ulis, France) including:

TGTGGTGGCCGGCGTGTCCGCC

AAGCGCTGCCGCGCGATCCGC

The final product was assessed by electrophoresis for a 245-bp band correspondent to MT.

RESULTS

A total of 33 patients aged 47.3 ± 16.1 years (range, 20 to 75 years) with confirmed UTB entered the study. Of these, 13 (39.4%) were men and 20 (60.6%) were women. The mean interval between the appearance of the symptoms and the diagnosis was 12.3 ± 12.2 months (range, 1 to 48 months). Manifestations of the disease included irritative bladder symptoms such as dysuria and diurnal or nocturnal frequency in 17 patients (51.5%), flank pain in 9 (27.3%), microscopic hematuria in 6 (18.2%), gross hematuria in 3 (9.1%), and suprapubic pain in 3 patients (9.1%). Urethral discharge, scrotal sinus, and general weakness each were detected in 1 patient (3.0%). The laboratory findings included hematuria in 9 (27.3%), pyuria in 4 (12.1%), hematuria and pyuria in 16 (48.5%), and no significant pathologic findings in 4 (12.1%) of the patients.

Diagnosis of UTB was made in 19 patients by positive urine culture for MT in 19 patients (57.6%), positive acid-fast staining in 6 (18.2%), and both in 8 (24.2%). Intravenous urography (IVU) was performed in 26 patients, which showed abnormal findings in 16 patients (61.5%). The main findings on the IVU were pyelocaliceal dilatation in 7 (26.9%), ureteral stricture and hydroureter in 6 (23.1%), multiple small caliceal deformities in 4 (15.4%), severe parenchymal destruction in 3 (11.5%), autonephrectomy in 3 (11.5%), and calcification in 2 (7.7%). Polymerase chain reaction assay detected MT in 16 patients (48.5%) in the studied group. In the patients with abnormal finding on the IVU, 10 (62.5%) had a positive PCR for MT.

DISCUSSION

Diagnosis of UTB is usually difficult since it manifests with nonspecific signs and symptoms. Irritative voiding symptoms are the most common symptoms, as they were in our patients (51.5%); more than half of the patients had hematuria and pyuria, which is consistent

with the other studies.⁽¹⁾ Abnormal radiological findings were previously reported in 63% to 95% of the patients.^(1,17) The most common findings are pyelocaliceal dilatation (hydronephrosis, hydroureter, and hydrocalicosis) and calcification.⁽¹⁸⁾ In our study, 61.5% of the IVUs showed abnormal findings including pyelocaliceal dilatation, urethral stricture and hydroureter, multiple small caliceal deformities, severe parenchymal destruction and autonephrectomy, and calcification. These findings, however, cannot help us make a definite diagnosis, but UTB should be always considered in the differential diagnoses, especially in endemic areas.

Diagnosis of UTB is usually made very late.⁽¹⁹⁾ Therefore, using a more sensitive method for diagnosis of the disease is of special importance. For a prompt detection of MT, we can use PCR assay. The sensitivity of PCR on urine samples was previously reported to be between 60% and 100% for diagnosis of UTB.^(1,2,14-16) The results of PCR may be affected by metabolites, drugs, or other biologic materials in the fluids of the body.⁽⁴⁾ There are enzyme inhibitors which may interfere with the routine PCR test. Some methods have been proposed to overcome this problem including the use of a proteolytic enzyme or sonication methods.⁽²⁾ Nonhomogeneous distribution of bacteria is another reason for the false-negative results. The best method is to test several specimens from a patient and select qualified specimens with good concentrations before the analysis.⁽¹⁾ In our study, the test was positive in 16 of 33 patients with a sensitivity rate of 48.5%. The reason for such a low sensitivity, other than the aforementioned factors, might be the possibility of lack of bacilli in the samples due to periodic bacilli excretion in the urine samples. In other studies, PCR test and culture or smear were performed on the same urine sample simultaneously.⁽¹⁴⁻¹⁶⁾ Thus, we might have more positive tests if we had used urine samples which were positive for MT in culture or acid-fast staining. Considering the mechanism of PCR assay and its ability to recognize very low amounts of the bacteria, it seems reasonable to report PCR highly positive if there is any bacterium in the sample and if the smear is positive.

The important point in our study was the relationship between the abnormal findings on the IVU and positive PCR tests; the PCR sensitivity was higher in the patients with an abnormal IVU than in all of the patients. We can speculate that in more severe infections with changes detectable on imaging, greater excretion of the bacterium occurs in urine; consequently, PCR is more likely to be positive for MT.

CONCLUSION

To diagnose UTB, a strong clinical suspicion is needed. In clinically suspicious cases, IVU findings are suggestive but nonspecific. Although PCR cannot be recommended as the only method in identification of UTB, it can be considered as one of the instant diagnostic tools before performing the other tests which are time consuming.

CONFLICT OF INTEREST

None declared.

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