Role of Urinary PCR in Diagnosis of Genitourinary Tuberculosis

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WF300,WC302,WF360

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***Abstract*-Context: Genito urinary tuberulosis accounts for approximately 15 to 20% of the cases of extrapulmonary tuberculosis. Objectives: To evaluate the role of urinary polymerase chain reaction (PCR) in the detection of Mycobacterium tuberculosis (MTb) in patients with a clinical suspicion of genitourinary tuberculosis (GUTB) and to compare its sensitivity with intravenous urography (IVU), bladder biopsy, and urine smear for acid fast bacilli (AFB). Methods and Material: The study was carried out between January 2001 to 2003 in patients with a clinical suspicion of GUTB. The diagnostic yield of urinary PCR for MTb and its sensitivity in comparision with routine urine smear for AFB, bladder biopsy, and IVU were assessed. Results: Urine smear for AFB was positive in 14 cases (36.6%); bladder biopsy was positive in 7 (23.3%); and urinary PCR for MTb was positive in 36 cases (94.4%). Of 38 cases of proven GUTB, IVU was suggestive of the diagnosis in 35 (90%). Conclusions: In clinically suspected cases, IVU may be suggestive of GUTB, but it is not specific. In the present study, IVU was suggestive in 90% of patients. MTb was isolated in the urine smear for AFB in only 36.6% of patients, and bladder biopsy was positive in 23.3%. Urinary PCR for MTb was the most sensitive indicator and was positive in 94.4% of patients.**

Keywords-Genitourinary tuberculosis, Polymerase chain reaction, extra pulmonary tuberculosis.

I. Introduction

Genitourinary tuberculosis (GUTB) accounts for approximately 15 to 20% of the cases of extra pulmonary tuberculosis.. Early diagnosis plays a vital role in control of TB.[1]Diagnosis of mycobacterial infections, however, remains an enigma. Although acid fast bacilli (AFB) microscopy, and conventional Lowenstein Jensen (L-J) culture remain the cornerstone of the diagnosis of TB, these traditional bacteriological methods are either slow or their sensitivity is quite low, especially with clinical samples that contain small number of organisms [2]. Polymerase chain reaction (PCR) is an instant assay that recognizes very few amounts of bacterium within 24-48 hours .In view of this, we intend to evaluate the role of urinary PCR in the detection of Mycobacterium tuberculosis (MTb) in patients with a clinical suspicion of GUTB and to compare its sensitivity with intravenous urography (IVU), bladder biopsy, and urine smear for acid fast bacilli (AFB).

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II. Methods

The study was carried out between January 2001 to January 2003 in 38 patients with a clinical suspicion of GUTB. The college ethical committee approval was obtained before commencing the study. Their clinical features, organ involvement, and investigation results were studied. The PCR assay was performed on the collected urine samples of all patients for detection of MTb. Three consecutive early morning urine specimens were taken from each patient. In our study SRL's MYCO tect (developed by RANBAXY) was used. Here IS 6110 gene sequence present in mycobacterium was used. MYCO tect also detects the 16s RNA gene sequence in mycobacterium and hence rules out the chances of false negative results. The needed product was proliferated using the primers relevant to IS 6110 insertion element regions (custom synthesized by Bangalore Genei, India) including upstream primer 5' CCTGCGAGCGTAGGCGTCGG 3' and down stream primer 5' CTCGTCCAGCGCCGCTTCGG 3'.The final product was assessed by electrophoresis for a 123-bp band . Urine is sent for routine and microscopic examination for red blood cells and leukocytes & AFB. Overnight urine sample for three consecutive days is sent for AFB staining. Urine is sent for culture and sensitivity. The diagnostic yield of urinary PCR for MTb and its sensitivity in comparision with routine urine smear for AFB, bladder biopsy, and IVU were assessed.It is an established practice to start medical treatment only after microbiological confirmation of the disease for institution of chemotherapy in cases of tuberculosis of any system. In cases where urine smear was negative for AFB,We started on antitubercular therapy wherever there was gross urographic and or endoscopic evidence of tuberculosis or in cases where bladder biopsy confirmed tubercular cystitis. In borderline or doubtful cases patients were followed up for development of GUTB features before the treatment was started.

III. Results

An analysis of 38 cases of genitourinary tuberculosis studied between January 2000 to January 2003 is presented here. Out of 38 cases studied, age of patients varied from 12 years to 78 years with a mean age of 40 years. There was a preponderance of male patients (male patients 26 and females 12) male and female ratio was 2:1. Patients suspected of having GUTB most often presented with irritative voiding symptoms. [Table1] Extra renal manifestations were present in 8 cases i.e. in 26.66% out of which pulmonary involvement was seen in 6 cases i.e. 20% three patients of pulmonary tuberculosis had been treated previously (within 2-3 years). In three patients it was

detected only after coming with symptoms of urogenital tuberculosis. Unilateral cervical lymph node tuberculosis was founded in two of our patients 6.66%.Renal calcification on X –ray KUB was noticed in 15 cases (43 %). Aciduria was seen in as many as 70 % of cases where as sterile pyuria was seen in 63 % which is a classic urinary finding on routine urinalysis and culture in genito urinary tuberculosis. [6] Urine smear for AFB was positive in 14 (36.6 %) cases which were high compared to 18.2% in a study done in Iran [4].The abnormal pyelographic changes were seen in as high as 53.3% of cases. [Table2] The incidence of ureteric strictures in our series was also high. In majority, the strictures were at the vesicoureteric junction. But four cases had additional strictures pyeloureteric and vesicoureteric junction.PCR was done in 36 of 38 patients .the other two patients were not able to afford the PCR test. Out of the 36 patients PCR was positive in 34 patients (94.4%) PCR was positive 100% in all patients in whom urine smear was AFB positive (14 Cases . i.e. 37 %) in the other 20 patents in whom urine smear for AFB was negative PCR positive in only 18 cases (90 %).[Table 3]

In patients of low clinical suspicion of GUT i.e. of 30 cases PCR was positive in 28 cases (93 %) in patients of high clinical suspicion of GUTB i.e. of 8 cases PCR was positive in all the cases (100 %) So even in patients in whom there was low clinical suspicion we were able to start ATT early so that complications of GUTB were low. But in patients of high clinical suspicion i.e. extra genital TB or patients with pulmonary , lymph nodal involvement PCR was 100 % positive in whom long term ATT was given subsequently .

Bladder biopsy was taken in as many as 7 cases (23.3 %) all of them being positive for granulomatous disease i.e. tuberculosis. Kidney biopsy was done in 8 cases (26 %), two of them following Nephroureterctomy and two after partial nephrectomy and in four patients in whom renal calculi had to be removed. One patient with epididymo orchitis due to tuberculosis underwent Epididymectomy and proved to be tuberculosis. One more patient in whom we did testicular biopsy also proved to be tuberculosis.

IV. Discussion

Diagnosis of UTB is usually difficult since it manifests with nonspecific signs and symptoms. 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. Irritative voiding symptoms are the most common symptoms, as they were in our patients (46%); 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.[3] The most common findings are pyelocaliceal dilatation (hydronephrosis, hydroureter, and hydrocalicosis) and calcification. In our study, 52% of the IVUs showed abnormal findings including pyelocaliceal dilatation, urethral stricture and hydroureter, multiple small caliceal deformities, severe parenchymal destruction, autonephrectomy, and calcification. These findings, however, cannot help us to 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. (4) Therefore, using a more sensitive method for diagnosis of the disease is of special importance. Identification of the tuberculosis bacillus in the urine is achieved through Ziehl-Neelsen's acid–fast staining technique or through urine culture in Lowenstein-Jensen medium [5,6]The former is quick, with 96.7% specificity but only 42.1% to 52.1% sensitivity.[5,6] Culture is the diagnostic gold standard for urogenital tuberculosis. Because bacilluria is sporadic and faint, 3 to 6 early morning midstream samples are required. Sensitivity varies widely, from 10.7% to 90%, and the results can take 6 to 8 weeks to obtain.[2] Studies have described direct detection of *M tuberculosis* DNA by the urinary PCR technique as a rapid, non-invasive, and highly specific method for the diagnosis of GU TB.[1] The sensitivity of PCR on urine samples was previously reported to be between 60% and 100% for diagnosis of UTB.[Table 4][4] Studies have described direct detection of *M tuberculosis* DNA by the urinary PCR technique as a rapid, non-invasive, and highly specific method for the diagnosis of GU TB.[2,3]There are enzyme inhibitors which may interfere with the routine PCR test. 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.[6] In our study, the test was positive in 34 of 36 patients with a sensitivity rate of 94.4%. Two patients could not afford the test and was not done, In a recent study from Iran, using 33 patients with confirmed diagnosis of urinary tuberculosis, a detection rate of 48.5% for PCR, 57.6% for culture and 18.2% for acid fast staining were reported[7]. The important point in our study was that 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. There are reports of higher incidence rates of GUTB in AIDS [8]. According to the information on patients in the present study, we had two patients who were HIV positive.

V. Conclusion

A high index of suspicion is necessary for a diagnosis of GUTB. In clinically suspected cases, IVU may be suggestive of GUTB, but it is not specific. In the present study, IVU was suggestive in 90% of patients. MTb was isolated in the urine smear for AFB in only 36.6% of patients, and bladder biopsy was positive in 23.3%. Urinary PCR for MTb was the most sensitive indicator and was positive in 94.4% of patients. It is evident from this series that PCR provides a much faster diagnosis of urinary MTb. It is a rapid, sensitive, specific diagnostic method and avoids a delay in starting treatment.

VI. References

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Table 1 Showing the various symptoms with which the patients presented

Symptoms	No of cases[N=38]	Percentage%
Dysuria	18	46.6
Frequency of micturition	15	40.6
Renal pain	9	30
Pyuria	9	30
Fever	6	20
Haematuria	5	16.6
Respiratory symptoms	3	10
Weight loss	7	23.3
Nocturia	2	6.6
Retention of urine	2	6.6
Decreased semen/ haemospermia	3	10
Testicular swelling	5	16.6
Sinus formation	2	6.6

Table 2 showing the pyelographic changes in comparision with other studies

	Present study %	Prasad et al [9] %	Ardalan MR et al[10] %
Normal pyelogram	10	55	10
Abnormal pyelogram	90	45	90
Calyceal abnormalities	20	33	20
Hydronephrosis/Hydroureter	23	06	20
Non visualization of kidney	06	06	48
Ureteric stricture	23	NA	52
Thimble baldder	06	NA	20
Vesico ureteric reflux	03	NA	NA

Table 3 Comparing PCR with urine AFB smear

PCR	Positive	Negative
AFB Positive	14	Nil
AFB Negative	18	02

Table 4: Results of a comparison study comparing PCR, IVU, biopsy and urine AFB in various studies of proven cases of GU TB

Investigation Method	Hemal et al[3] (N35)	Khosravi et al[1] (N11)	Moussa et al[6] (N1000)	Yazdani et al[7] (N33)	Present study (N38)
IVU suggestive of diagnosis	32 (91.42)	NA	NA	NA	35 (90)
Mtb isolated in urineAFB smear	13 (37.14)	4 (41.6)	520 (52.0)	(18.7)	14 (36.6)
Mtb isolated in urineAFB culture	13 (37.14)	11 (100)	NA	(57.6)	NA
Biopsy positive	11 (45.83)	NA	NA	NA	17 (45)
PCR positive	33 (94.2)	10 (100) *	956 (95.6)	(48.5)	34 (94.4)

*Ignoring one positive culture which the organism was identified as nontuberculous mycobacterium by further tests. This specimen showednegative PCR result, since specific primers for MTB were used by the authors.