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DIAGNOSIS OF RENAL TUBERCULOSIS BY REAL-TIME POLYMERASE CHAIN REACTION IN RENAL BIOPSY SAMPLE

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ABSTRACT

Objective: Detection of renal tuberculosis (RTB) by real-time polymerase chain reaction (PCR) in renal biopsy tissue. **Methods:** Selected for fifty renal tuberculosis patients and twenty five healthy controls were taken in the present study. The renal biopsy tissue of these patients were used for *Mycobacterium tuberculosis* DNA detection by real-time PCR, using 35 and 40 as cycle threshold (C_T) cut-off values. The detection of *Microbacteria tuberculosis* is used by Real Time PCR Kit (Shanghai ZJ Bio-Tech Co., Ltd.). Early morning urine samples collected for *M. tuberculosis* culture. **Results:** Fifteen patients in renal tuberculosis of, urine culture was *Microbacteria tuberculosis* positive. overall sensitivity of renal *tuberculosis* RT-PCR for detected of *Microbacteria tuberculosis* (C_T40) cut off value result 86% sensitivity, 76% specificity, 87.75.% positive prediction value (PPV), 73.03% negative prediction value (NPV) and (C_T35) cut of value result 78% sensitivity, 84.% specificity, 90.69.% positive prediction value (PPV), 65.62% negative prediction value (NPV). Urine PCR for IS6110 in detected of *Microbacteria tuberculosis* AFB result 30% sensitivity, 93.% specificity, 88.23.% positive prediction value (PPV), and 39.65% negative prediction value (NPV). **Conclusions:** Detection of *M. tuberculosis* DNA in renal biopsy tissue by real-time PCR is highly sensitive. *M. tuberculosis* culture is still the gold standard for rapid and superior diagnosis of renal tuberculosis by real-time PCR.

KEYWORDS: Real- Time Polymerase Chain Reaction; Renal Tuberculosis; Renal Biopsy.

INTRODUCTION

Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can affect other sites as well (extra pulmonary TB).Genitourinary tuberculosis (GUTB) is a common form of extra pulmonary tuberculosis. It is almost always secondary to a lesion elsewhere in the body, usually in the lungs. The bacilli reach the kidneys by haematogenous spread from the lungs.[1] Diffuse haematogenous dissemination occurs at the time of initial pulmonary infection in approximately 25% of pulmonary cases.[2] Bladder lesions are always secondary to the renal tuberculosis and is found in one third of cases of GUTB.[3] The earliest from the infection starts around one or the other ureteric orifices, which becomes red, inflamed and edematous. With progression, bullous granulations appear and may completely obscure the ureteric orifices.[4] Consequently patients diagnosed incorrectly and lost initial treatment and development in the end stage kidney disease, in a life-aggressive condition. The diagnosis of renal TB is therefore very significant in preventing progressive obliteration of kidney.[5] The diagnosis of active TB has conventionally been based on clinical and radiological findings, acid-fast bacilli (AFB) smear microscopy and *M. tuberculosis* culture. Since the last decade, nucleic acid amplification tests (NAATs) and PCR have also been widely used.[6,7,8]

Molecular laboratory techniques like direct microscopic examination on microbacteria culture, solid medium or liquid medium, are far from being sufficiently sensitive and specific for rapid *M. tuberculosis* detection.[9] Even if urine microbacteria tuberculosis culture is more sensitized than AFB smears, culture is slow; most of the samples do not confirm visible colonies of *M. tuberculosis* before 4 weeks thus delay in diagnosis.[10] Initially Hans in 1989 used polymerase chain reaction to identify tuberculosis DNA.[11] PCR is a High sensitive

and specific technique and can detect DNA range upto 1-100 ng. Rapid diagnosis of renal tuberculosis can be done by PCR with high specificity.[12] Biopsy tissues were used in the present study and *M. tuberculosis* was quantitatively detection by real-time PCR. Real-time PCR was simultaneously compared with urine *M. tuberculosis* culture to determine the significance of real-time PCR in diagnosis of renal tuberculosis.

MATERIALS AND METHODS

Patients

Fifty samples of human renal biopsy tuberculosis and twenty five samples of healthy control were taken in the present study. All patients were selected from Nephrology and Urology Department, Sir Sundar Lal Hospital, Banaras Hindu University, Varanasi during September 2012 to December 2016. Mean age was 39.5 ± 14.4 year and male and female ratio was 1.94:1 (33 males and 17 females). Detail clinical history was recorded for each sample. Sample size was determined according to feasibility and availability of the samples during the given period.

Renal biopsy tissue and urine samples were collected for clinical investigation. All biochemical parameter analysis was done by biochemical analyzer. (Kobas Integra 400 Plus) The study was approved by Institute of Medical Sciences, Human Research Ethics Committee, Banaras Hindu University, Varanasi, India. All participants were thoroughly informed about the objective of the study, as well as the risk and precaution. Written consent was taken from each patient.

Urine M. tuberculosis culture

Early morning urine samples were collected in 50 ml polypropylene tubes and centrifuged at 1500 × g for 10 min to clear particulate matter. The pH of the urine was checked and adjusted to 5.5–7.5 using 1 N NaOH and stored at –20°C. Urine samples for the analysis of albumin and creatinine were mixed with sodium azide (0.1% w/v) and stored at 4°C.

DNA isolation and Quantification

Renal biopsy tissue was taken from all subjects. Each biopsy tissue had a length of about 1 cm. DNA purification was done by QIAamp DNA mini kit according to the instruction manual and quantification of DNA was done by Qubit 2.0 Fluorometer. (Invitrogen by life technology made in Austria).

Real-time PCR

Mycobacterium tuberculosis (TB) real time PCR kit is used for the detection of DNA provided by Shanghai ZJ Biotech Co., Ltd. (PuJiang Hi-tech Park Shanghai China. The positive control (1×107copies/ml) contains high concentration of the target DNA. The concentration of primer was 0.2μM. PCR was done based on 96-well plates using Applied Biosciences prismStep one plus real-time PCR system.

Table 1: PCR condition.

37°C for 2min	1 cycle
94°C for 2min	1 cycle
93°C for 15sec, 60°C for 1min HEX/VIC/JOE IC (Fluorescence measured at 60°C) 40cycles	40 cycle

Selection of fluorescence channels	
FAM	Target Nucleic Acid
HEX/VIC/JOE	IC

Renal function

There was a detailed health examination in each subject, which included systolic blood pressure and diastolic blood pressure. Analysis involves hemoglobin, sodium, potassium, chloride, calcium, phosphate and blood urea nitrogen, serum creatinine and protein levels. We have collected blood and urine samples in biochemical analysis. We investigated the lifestyle of each subject (cigarette smoking and drinking), medical history (diabetes and hypertension) and basic demographic information (age, gender, working year) through the questionnaire.

RESULT

In fifteen patients of renal tuberculosis, 14 patients were urine culture *Microbacteria tuberculosis* positive. For real-time PCR 39 samples were tested positive using 35 as C_T cut-off value and 43 using 40 as the cut off value.

In the present study overall sensitivity, specificity and PPV, NPV of urine *Microbacteria tuberculosis* culture were respectively 30% , 92% and 88.23.%, 88.23.%. The sensitivity, specificity and PPV, NPV of RT-PCR was detected (C_T40) were respectively 86% , 76% and 87.75% and 73.03% . When using 35 as the C_T cut-off value, the sensitivity, specificity and PPV, NPV were respectively 78%, 84% and 90.69%, 65.65%. Compared with C_T40, the specificity was significantly increased Table 2.

Table 2: Comparison between the results Sensitivity, specificity, Positive prediction value, Negative prediction value for of renal tuberculosis.

Test	Sensitivity %	Specificity %	PPV %	PNV %
RT-PCR (CT40)	86	76	87.75	73.07
RT-PCR (CT35)	78	84	90.69	65.62
Urine culture	30	92	88.23	39.65

Showed the distribution of renal tuberculosis patients according to age. It was found that the age of renal tuberculosis patients ranged between (15-60+) year, with a mean age 39.5 ± 14.4 year, as shown in table 3. Moreover, regarding renal TB patients, the males (66 %)

are more than females (34 %) with the ratio of (1.94:1) as shown in table 4.

Table 3: Distribution of renal TB patients according to age.

	Suspected patients	
Age groups (years)	**Number**	**%**
11-20	5	10.0
21-30	14	28.0
31-40	8	16.0
41-50	11	22.0
51-60	10	20.0
61 +	2	4.0
Total	50	100.0
Mean age (years)	**39.5 ± 14.4**	

Table 4: Sex distribution of studied group.

	Renal TB patients	
Sex	**Number**	**%**
Male	33	66
Female	17	34
Total	50	100
M/F ratio	1.94:1	

Table 5: Demography and clinical parameters for controls and cases.

Parameters	Groups		P-value
	Controls	Case	
Age (Year)	38.88±15.16	38.44±16.90	0.923
Weigh(kg)	58.40±8.82	53.80±08.61	0.068
SBP(mmHg)	132.96±17.68	143.76±40.81	0.231
DBP(mmHg)	75.20±10.77	84.48±17.58	0.029
Hb (gm %)	12.77±2.68	7.95±298	0.061
Creatinine (mg %)	0.98±0.32	6.85±3.25	0.000
Urea (mg %)	28.0±22.38	140.88±66.54	0.000
Glucose (mg %)	09.16±19.23	92.56±21.96	0.683
Protein 24hr Mg/day	231±166.77	1464±2682	0.026
Sodium (meq/l)	138.68±4.79	129.80±6.17	0.003
Potassium (meq/l)	4.37±0.60	4.75±1.18	0.162
Chloride (meq/l)	100.00±4.76	97.80±8.21	0.128
Calcium (mg/dl)	100.00±4.76	7.80±0.94	0.250
Phosphate (mg/dl)	3.97±0.85	6.55±2.30	0.000

Clinical parameters of Controls and Cases

Values of clinical parameters like blood pressure (both systolic and diastolic), haemoglobin, creatinine, urea, glucose and 24 hours protein of cases and control were analysis by biochemical analyzer. Serum phosphate was significantly increased in cases than controls (6.55±2.30 and 3.97±0.85mg/dl respectively, p-value < 0.001), while increase in calcium was non-significant (7.80±0.94 and 3.97±0.85 mg/dl respectively, p-value = 0.250). Serum sodium was significantly decreased (p-value = 0.003), while decrease in potassium and chloride were non-significant in cases than controls (p-value= 0.162, and 0.128 respectively), table 5.

DISCUSSION

Genitourinary TB prevalence 15% of all extra-pulmonary cases, and may involve any fraction of the genitourinary region.10 Urinalysis can reveal microscopic haematuria, and proteinuria starts to acidic urine.

Due to new, suggestive treatment, some of the major clinical manifestations of renal tuberculosis patients prefer renal disease rather than cystitis.

Diabetic nephropathy, glomerulonephritis ,end stage of renal disease and other disease associated with TB have been described in the literature.[13,14,15,16,17] The purpose of this study was to used real-time PCR, high-quality diagnostic methods relevant directly to clinical sample, allow a rapid detection of M. tuberculosis in addition evaluation of the microbacteria burden.[18,19] Real-time PCR has been shown to be helpful greater than other techniques for identification of bacteria, mainly those that are complicated to culture or are considered growing.[20]

Our study showed that the sensitivity, and specificity of urine M. tuberculosis cultures were respectively 30% and 92%, and RT-PCR (C_T35) were respectively 78% sensitivity, 84%, Compared with urine M. tuberculosis culture, the sensitivity of real-time PCR was significantly increased. Although the 86% sensitivity, 76% specificity using C_T40 cut-off value was slow. Real-time PCR may be useful 35C_T cut of value as a rapid method for diagnosis of renal tuberculosis. Four patients negative were Real Time Polymerase Chain Reaction (C_T35) in the renal tuberculosis. The renal biopsies showed interstitial fibrosis, and granulomas inflammation. We speculated that the may be false negative due to the sensitivity of the technique.

CONCLUSIONS

In this study, we showed that high sensitive, specificity, PPV and NPV in renal biopsy tissue DNA detection of MTB by real time polymerase chain reaction. Moreover, it can increase the diagnostic accuracy and provide valuable information which would complete other clinical data for the early diagnosis of RTB. We consider that medical perform, it determination a powerful implement for the urine M. tuberculosis culture is still the gold standard for rapid and perfect analysis of renal tuberculosis by real-time PCR.

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