ORIGINAL ARTICLE

Accuracy of Urinary PCR as Compared with Urine Culture for Early Diagnosis of Genitourinary Tuberculosis

FAZAL-UR-REHMAN KHAN, FARHAN ARSHAD CHEEMA, MOHAMMAD USMAN KHAN

ABSTRACT

Objective: To determine the sensitivity and specificity of PCR in early diagnosis of genitourinary tuberculosis when compared with urine culture for detection of genitourinary tuberculosis.

Patient and methods: This observational cross-section study was conducted from December 2011 to May 2012 at Shaikh Zayed Hospital, Lahore in the in the Department of Urology It is a prospective study of 50 patients to evaluate various modes of presentation of genitourinary tuberculosis and efficacy of various diagnostic tests currently available.

Results: The patients age range from 20-67 (38.23±13.27 years). Male to female ratio was 1:1.16. Nineteen patients (100%) had growth of mycobacterium tuberculosis on routine culture on Lowenstein Jensen (LJ) medium (Gold standard) and were considered positive for tuberculosis. Thirteen patients showed acid fast bacilli in their urine on ZN staining (sensitivity 51.5%, specificity 94.6%). Eighteen (18) patients showed acid fast bacilli in their urine on PCR, out of which 16 patients showed growth on LJ medium with a sensitivity of 88.6% and specificity of 96.5% (positive predictive value 95.3% and negative predictive value 92.4%).

Conclusion: Genitourinary tuberculosis remains the most common form of extrapulmonary tuberculosis. Polymerase chain reaction (PCR) is a very sensitive, specific and rapid diagnostic test and the only disadvantage is that it can not differentiate between the live and dead bacteria.

Keywords: Genitourinary tuberculosis, Urinary polymerase chain reaction, Early diagnosis, Lower urinary tract symptoms, Urine culture.

INTRODUCTION

The World Health Organization (WHO) estimates that one third of the world's population is infected with mycobacterium tuberculosis and there are 8 to 10 million new active cases of tuberculosis (TB) diagnosed each year.[1] Tuberculosis of kidney and urinary tract is like other forms of the disease, caused by member of mycobacterium tuberculosis complex. By far the most common causative organism is the human Tubercle Bacillus, Bovine Tubercle Baccilus, Mycobacterium Bovis Bacillus can occasionally be responsible as well[2]. The infecting organism reaches the genitourinary organs by the hematogenous route from the lungs[3]. Urinary tuberculosis is a disease of young adults (60% of patients are between the ages of 20 and 40) and is more common in males than in females[4].

Genitourinary tuberculosis (GUTB) is commonest form of extrapulmonary TB and has been reported in 8-10% of all cases of tuberculosis in developed countries and about 20% cases in under developed countries[5]. The primary site of involvement of TB is lungs, the leading secondary site of involvement is the as genitourinary tract. Presentation of genitourinary tuberculosis is usually with vague symptoms. Urinary frequency is the most common symptoms in both genders[5]. Sterile pyuria, gross or microscopic hematuria, a non-tender, enlarged epididymis with a beaded or thickened vas, a chronic draining scrotal sinus, or induration or nodulation of the prostate and thickening of one or both seminal vesicles are few of the presenting features[6]. Genitourinary tuberculosis presents as a diagnostic dilemma especially in the early disease and many cases remain undiagnosed or misdiagnosed. Diagnosis of GUTB depends upon the demonstration of mycobacterium tuberculosis through smear Zheil Nelson (ZN) staining of AFB, culture on solid or liquid media of early morning urine specimens[7].

Mycobacterial components including DNA can be detected by polymerase chain reaction (PCR)[8]. The polymerase chain reaction is a technique that can be used to amplify a specific DNA genomic sequence, whereby the presence of an extremely small number of bacteria can be detected[9]. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase (after which the method is named) are

Department of Urology, Shaikh Zayed Hospital Lahore.
Corresponding to Dr. Fazal-ur-Rehman Khan, email: drfazaln@gmail.com

key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified.

METHOGOLOGY

A total of 50 patients with suspected genitourinary tuberculosis were evaluated for various modes of presentation. Each patient was tested for presence of mycobacterium tuberculosis in urine by ZN staining, culture on LJ medium and PCR in three consecutive morning urine samples. The results of the AFB smear and PCR were compared with results of culture of LJ medium. Patient with features suggestive of genitourinary tuberculosis. such as unexplained lower urinary tract symptoms, sterile pyuria, chronic draining scrotal sinus, gross or microscopic hematuria were included the study. Patients with recently diagnosed pulmonary tuberculosis and taking antituberculous treatment were excluded from the study.

RESULTS

The age range of patients was 20-76 years (38.23±13.27). Male to female ratio was 1:1.6. 10(20%) patients presented with fever, 6(12%) with loin pain, 4 (8%) patients had LUTS. 26 (52%) had haematuria and 4 (8%) patients had sterile pyuria. After specific diagnostic tests, 19(38%), out of 50 patients proved to have genitourinary tuberculosis. Nineteen patients (100%) had growth of mycobacterium tuberculosis on routine culture on LJ medium (Gold standard) and were considered positive for tuberculosis. Thirteen patients showed acid fast bacilli in their urine on ZN staining, out of which 8 patients showed growth on LJ medium with a sensitivity of 51.5% and specificity of 94.6% (positive predictive value 91.8% and negative predictive value 77.5%). Eighteen patients showed growth of acid fast bacilli in their urine on PCR, out of which 16 patients showed growth on LJ medium with a sensitivity of 83.4% and specificity of 95.4% (positive predictive value 95.3% and negative predictive value 92.4%) [Figs.1-2,Tables 1-3].

Table 1: Presenting complaints of patients.

Presenting Complaints	=n	%age
Fever	10	20.0
Loin pain	6	12.0
LUTS	4	8.0
Haematuria (Macro/Microscopic)	26	52.0
Pyuria	4	8.0

Table 2: Results of AFB Smear relative to the results of AFB Culture

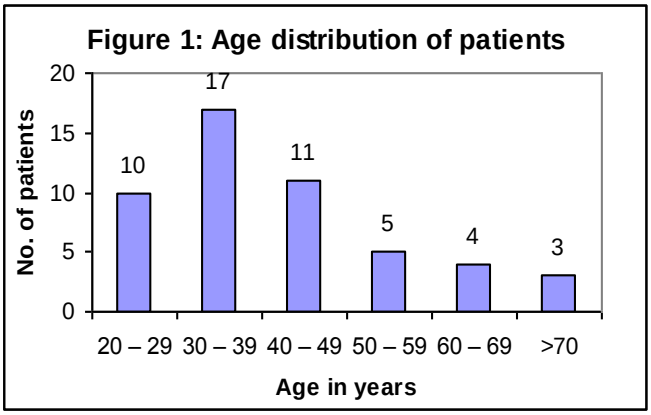
Results	Positive	Negative	Total
Positive	11	2	13
Negative	8	29	37

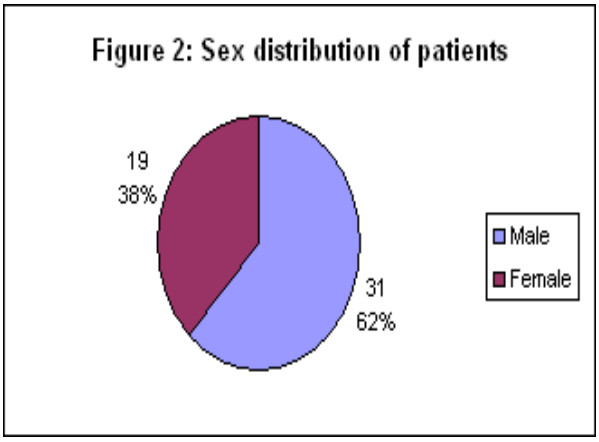
Sensitivity 51.5 Specificity 94.6 PPV 91.8 NPV 77.5

Table 3: Results of PCR relative to the results of AFB Culture

Results	Positive	Negative	Total
Positive	16	2	18
Negative	3	29	32

Sensitivity 88.6 Specificity 96.5
PPV 95.3 NPV 92.4





DISCUSSION

Tuberculosis remains the largest cause of death in the world from a single infectious disease. Genitourinary tuberculosis has been reported in 8-10% of all cases in the developed world and in 20% in third world countries[10]. The majority of the infected persons live in the developing countries. The factors responsible for failure to control tuberculosis in Pakistan are low socioeconomic conditions, lack of health education, non compliance and drug

resistance[11]. Genitourinary tuberculosis may present with nonspecific symptoms and a high index of suspicion may be kept to diagnose the disease at an early stage. The diagnosis of genitourinary tuberculosis requires demonstration of acid fast bacilli in urine which by traditional culture techniques are labour intensive and requires upto 8 weeks of incubation to achieve maximum sensitivity. The importance of rapid sensitive diagnostic tool is obvious in such circumstances.

The median age of patients with genitourinary tuberculosis is 38.23±13.27 years and patients were predominantly males (62%). In a study carried out by Khader et al which is comparable with our study.[12] In his study the mean age was 40 years and 62.8% were male patients. Out of 50 patients, bladder related symptoms were the most common mode of presentation. In our study, 4 patients (8%) had pyuria and 26 patients (52%) had haematuria. 12 patients (63.2%) had frequency of micturation. In a study done by Garcia et al, pyuria plus haematuria with sterile cultures was the most common mode of presentation.[13] According to Chattopadhya et al[14] gross haematuria was presenting feature in 44% patients. Similar to our study haematuria may be a leading feature of disease as discussed by Bernaschina et al.[15] In a study by Buchholz et al from Aga Khan University Hospital, Karachi the most common prevailing symptoms were lower urinary tract symptoms and haematuria.[16] Mycobacterium tuberculosis is termed acid fast, that it is resistant to decolorization with acid and alcohol. However, ZN staining can not usually detect the presence of organisms when they constitute less than 100,000 per ml of the specimen and therefore false negative results are common[17].

Eleven patients showed acid fast bacilli in their urine on ZN staining. Out of which 8 patients showed growth on LJ medium with a sensitivity of 51.5% and specificity of 94.6% (positive predictive value 91.8% and negative predictive value 77.5%). These results favourably compared with those reported by Moussa et al[18]. Their data indicated that utilization of acid fast stained smear was highly specific (96.7%), but the sensitivity was limited (52.1%). Further more this procedure can be legitimately criticized since it is observer dependent[18]. In favourable comparison of our study, Abdul Razic et al reported 50% positive rate of smear in their study[19]. However our results for ZN staining are in contrast with to that reported by Pfyffer et al with a positive smear rate of 20.8%[20].

PCR is a highly sensitive and a rapid diagnostic test and may prove to be a very helpful diagnostic tool in the diagnosis of genitourinary tuberculosis[21]. It is an in vitro enzymatic synthesis of specific DNA sequences using two oligonucleotide primers that hybridize to opposite strands of target DNA and flank the region of interest. The use of PCR to detect the presence of mycobacterium in clinical samples has been widely reported.[2] Most of these studies were limited to the materials obtained from respiratory tract and experience with the genitourinary origin is till limited. In our study we have analyzed its use in genitourinary tuberculosis. The PCR detected the mycobacterium tuberculosis in 92% of cases. Moussa et al in their study of 1000 cases reported 323 patients showed acid fast bacilli in their urine on PCR. Out of which 316 patients showed growth of mycobacterium on LJ medium with a sensitivity of 87.1% and specificity of 98.9% (positive predictive value 97.8% and negative predictive value 93.1%).[18] In our study, 18 patients showed acid fast bacilli in their urine on PCR. Out of which 16 patients showed growth on LJ medium with a sensitivity of 88.6% and specificity of 96.5% (positive predictive value 95.3% and negative predictive value 92.4%). In a study carried out by Gengnij, PCR for detection of M. tuberculosis was compared with acid fast staining and culture in 153 clinical specimens. It revealed 88.6% sensitivity and 89.2% specificity in smear positive specimens and 93.2% sensitivity and 85% specificity in culture positive specimens[25].

This test only detects the presence of mycobacterium DNA and does not differentiate between dead and alive bacteria. Under ideal condition this test can detect the presence of as little as 10-100 mycobacteria per ml of sputum compared with the ZN staining which can only pick up mycobacteria if their concentration equals or exceed 100,000 bacteria per ml. By using PCR, patients who are negative for AFB staining and culture positive for M. tuberculosis can now be identified within a day, allowing institution of therapy and reducing isolation time and medical costs[23].

CONCLUSIONS

It is concluded that genitourinary tuberculosis remains the most common form of extrapulmonary tuberculosis. It should be suspected in all the patients having long term chronic urinary tract symptoms with no obvious cause. A high degree of clinical suspicion will prevent the late diagnosis of disease and associated sequalae. ZN staining, though highly specific carries relatively low sensitivity for detection of mycobacterium tuberculosis. PCR is a very sensitive, specific and rapid diagnostic test and the only disadvantage is that it can not differentiate between the live and dead bacteria. However, when the clinical suspicion is high this test carries a very high yield and possess a great promise in the diagnosis of a disease whose detection by traditional

culture techniques remains laborious, tiresome and requires upto 8 weeks to achieve maximum sensitivity.

REFERENCES

1. Urology I, Campbell, Meredith F. (Meredith Fairfax) II. Wein, Alan J. III, Kavoussi, Louis R. IV. Campbell's Urology. V. Title: Urology [DNLM: 1. Urogenital Diseases. 2. Urology -methods. WJ 100 C192 2007].
2. Okadom A, Takeuchi F, Ishii T, Hiratsuka Y. Tuberculous epididmitis following intravesical BCG therapy. Nippon Hinyokika Gakkai Zasshi 2002;93: 580-2.
3. Lenk S, Schroeder J. Genitourinary tuberculosis. Curr Opin Urol 2001;11:93.
4. Cek M. EAU guidelines for the management of genitourinary tuberculosis. Eur Urol 2005;48:353.
5. Buchholz NP, Salahuddin S, Haque R. Genitourinary TB: A case profile of 55 patients. JPMA J Pak Med Assoc 2000;50:265-9.
6. Skoutelis A. Serious complications of tuberculous epididmyitis. Infection 2000;28:193.
7. Bennani S, Hafiani M, Debbagh A, elMrini M, Benjelloun S. Urogenital tuberculosis. Diagnostic aspects. J Urol 1995;101:187-90.
8. Hemal AK, Gupta NP, Rajeev TP. Polymerase in clinically suspected genitourinary tuberculosis: comparison with IVP, baldder biopsy and urine AFB culture. Urology 2000;56:570-4.
9. Moussa OM, Eraky I, El-Far MA, Osman HG, Ghoenim MA. Rapid diagnosis of genitourinary tuberculosis by polymerase chain reaction and non-radioactive DNA hybridisation. J Urol 2000;164:584-88.
10. Fischetti G, Lombardo G, Barrese F, Morello P, Cuzari S, Cristini C. TB-test in the diagnosis of "closed" tuberculosis of the urinary tract. Preliminary study]. Minerva Urol Nefrol 1999;51:197-201.
11. Aziz A. Tuberculosis: Past, present and future. Presented at SAARC Conference on Tuberculosis and Respiratory Diseases Lahore Pakistan 1993.
12. Khader K, Fassi J, Karmouni T, Tazi K, Ibnattya A, Hachimi M, Lakrissa A. Urogenital tuberculosis. Apropos of 40 cases. Ann Urol 1997; 31:339-43.
13. Garcia-Rodriguez JA, Garcia Sanchez JE, Munoz Bellido JL, Montes Martinez I, Rodriguez Hernandez J. Genitourinary tuberculosis in Spain: review of 81 cases. Clin Infect Dis 1994; 18:557-61.
14. Chattopadhyay A, Bhatnagar V, Agarwala S, Mitra DK. Genitourinary tuberculosis in pediatric surgical practice. J Pediatr Surg 1997; 32:1283-86.
15. Bernaschina CP, Cabrera M, Cardona P, Colon B. Genitourinary tuberculosis; the importance of early diagnosis and management. Case presentation. Bol Asoc Med P R 1994; 86:75-80.
16. Buchholz NP, Salahuddin S, Haque R. Genitourinary tuberculosis: a profile of 55 in-patients. JPMA J Pak Med Assoc 2000; 50: 265-9.
17. Bukhari SNH. Current concepts in diagnosis of tuberculosis. Specialist Pak J Med Sci 1999; 16:39-46.
18. Moussa OM, Eraky I, El-Far MA, Osman HG, Ghoneim MA. Rapid diagnosis of enitourinary tuberculosis by polymerase chain reaction and non-radioactive DNA hybridization. J Urol 2000 164:584-8.
19. Abdul Razic MM, el-Morsy FE. Genitourinary mycobacteria in infertile Egyptian men. Fertil Steril 1990;54:713-7.
20. Pfyffer GE, Cieslak C, Welscher HM, Kissling P, Rusch-Gerdes S. Rapid detection of mycobacteria in clinical specimens by using the automated BACTEC 9000 MB system and comparison with radiometric and solid-culture systems. J Clin Microbiol 1997; 35:2229-34.
21. Otal I, Samper S, Asensio MP, Vitoria MA, Rubio MC, Gomez-Lus R, Martin C. Use of a PCR method based on IS6110 polymorphism for typing Mycobacterium tuberculosis strains from BACTEC cultures. J Clin Microbiol 1997;35:273-7
22. Gengvinij N, Pattanakitsakul SN, Chierakul N, Chaiprasert A. Detection of Mycobacterium tuberculosis from sputum specimens using one-tube nested PCR. Southeast Asian J Trop Med Public Health 2001; 32:114-25.
23. Patnaik M, Liegmann K, Peter JB. Rapid detection of smear-negative Mycobacterium tuberculosis by PCR and sequencing for rifampin resistance with DNA extracted directly from slides. J Clin Microbiol 2001; 39:51-2.