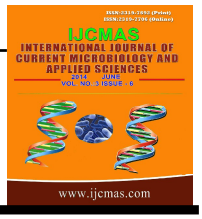
Int.J.Curr.Microbiol.App.Sci (2014) 3(6) 408-414

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 6 (2014) pp. 408-414**
http://www.ijcmas.com



Original Research Article

Comparison between polymerase chain reaction and Ziehl–Neelsen stain for detection renal tuberculosis

Mohammed Abdul-Daim Saleh[1*], Khalid J. Khaleel[2] and Lina Abdulameer Salman Alsaadi[3]

[1]Department of Biology, College of Science, Diyala University, Iraq
[2]Iraqi center for cancer researches, Al-Mustansryiah University, Iraq
[3]Department of Biology, College of Science, Diyala University, Iraq
Corresponding author

Keywords

PCR , Ziehl-Neelsen stain and renal tuberculosis

A B S T R A C T

The aim of the present study was to evaluate the diagnostic value of polymerase chain reaction in renal tuberculosis compared with Ziehl–Neelsen stain. In total 44 urine samples from suspected cases of urinary tuberculosis were collected during the period from November 2009 to August 2011, they included 24 males and 20 females with age range 11 to 61+ year. For each patient, three urine samples collected on three consecutive days as early morning urine. In this study Ziehl–Neelsen stain was negative in all urine specimens, while polymerase chain reaction was positive in 34.1% (15/44) of urine specimens with highly significance difference was noticed between two methods. According to age, the 15 positive patients were 21 to 61+ year (mean 42.37±19.61), Also it was found that more than half of patients were located within fourth and fifth decade, with a percentage of 66.8%, moreover as regard to sex, 9 male (37.5 %) out of 24 were positive and 6 females (30.0%) out of 20 were positive for renal tuberculosis. In this study, renal tuberculosis patients constitutes 15 (34.1%) out of the 44 patients according to the positive results of PCR. In conclusion, PCR is a rapid, sensitive and specific technique to detect *Mycobacterium tuberculosis* in urine samples for urinary tuberculosis patients.

Introduction

Mycobacterium tuberculosis (MTB), the etiologic agent of tuberculosis (TB), is estimated to have infected one-third of the world's population and annually causes 8 million new TB cases and >2 million deaths (Corbett, *et al.*, 2003). Tuberculosis is still a major health hazard in both developed and developing countries (Kafwabulula, *et al.*, 2002).

Although MTB is mainly affecting the lungs, however, kidneys are the second target organ for the bacterium (Lenk and Schroeder, 2001). According to a recent report, extrapulmonary tuberculosis (EPTB) constitutes up to 20% of the total cases of the disease, and with the involving rate of 14%, the urogenital system is one of the most common

affected sites (Yazdani, *et al.,* 2008). According to (Warren, *et al.,* 2002) statement, "While renal TB is uncommon in developed countries, as many as 15 to 20% of TB patients in developing countries are found with *M. tuberculosis* in the urine". The infection almost always affects the kidneys during the primary exposure to infection but does not present clinically. The course of renal tuberculosis may be indolent, with the appearance of few, if any, symptoms. Presentation is usually late and symptoms usually occur as a result of nonspecific urinary tract infection (Nawaz Khan and Chandramoha, 2004). Genitourinary TB (GUTB) is usually caused by metastatic spread of organisms through the blood stream during the initial infection. Active disease results from the reactivation of the initial infection (Cek, *et al.,* 2005). The diagnostic criterion for GUTB 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 (Menzies, *et al.,* 2007).

Direct smears are often negative and do not differentiate tuberculosis from non-tuberculous mycobacterium. Culture, which is more sensitive, takes 6 to 8 weeks because of the slow growth rate of mycobacterium (Verhagen, *et al.,* 2011). Although the Ziehl–Neelsen (Z.N.) stain is rapid and inexpensive, it lacks sensitivity (Leung, *et al.,* 2010). The advent of polymerase chain reaction (PCR) as a diagnostic tool has opened new possibilities for diagnosing TB (Figueiredo, *et al.,* 2009). PCR is thought to be a potential tool to overcome the limitations of sensitivity and specificity of mycobacterial conventional diagnostic methods (Dochviri, *et al.,* 2005).

The current study was performed aiming to compare between the PCR technique and other conventional method such as Ziehl–Neelsen stain (Acid fast stain) for diagnosis of renal tuberculosis.

Materials and Methods

Forty four patients clinically suggested to have renal tuberculosis were chosen from the Nephrology Department in Baghdad Teaching Hospital, during period from November 2009 to August 2011. They presented with dysuria, hematuria, flank pain and pyuria. They included 24 males and 20 females with age range 11 to 61+ year. For each patient, three urine samples collected on three consecutive days as early morning urine. The specimens were pooled and centrifuged at 3000 g for 20 min. Supernatant was removed and the pellet was divided into two part. One part was used for acid fast staining by the Ziehl–Neelsen method and other part was used for PCR analysis.

Ziehl–Neelsen stain (AFB)

Purpose: Used in the demonstration of acid-fast bacteria belonging to the genus 'mycobacterium', which include the causative agent for tuberculosis (Brown, *et al.,* 2007).

Procedure

1. Prepare smear . 2. Air dry and heat fix it. 3.Rinse in carbol fuchsin .4. Light a cotton swab and hold it underneath until steam appears. 5.Wash with dilute hydrochloric acid until a faint pink color remains. 6. Counterstain with Methylene Blue Chloride for a minute .7. Wash with gentle water till violet becomes faint 8. Blot dry .9. View under oil immersion lens (Brown, *et al.,* 2007).

DNA extraction and 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-EDTAbuffer (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 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). 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 (2). 15 µl of the amplification products were analyzed by electrophoresis in an ethidium bromide stained 2% agarose gel (Sambrook, *et al.*, 1989).

Statistical analysis

Data were collected and analyzed by using SPSS version 20 for windows, which were included statistical tables (frequencies, percentages and Pearson correlation).

Results and Discussion

Renal tuberculosis is the most common site of extra-pulmonary tuberculosis. This infection can result in cessation and destruction of renal mass and healing can lead to strictures, obstruction and infection causing renal functional loss and failure (3).In fact, age at infection seemed to be the most influencing factor in prognosis, table (1) showed the distribution of suspected TB patients according to age. It was found that the age of suspected TB patients ranged between (11-61+) year, with a mean age of 48.45 ± 21.15 year, as shown in table (1). Moreover, regarding suspected TB patients, the males (54.6 %) are more than females (45.4 %) with the ratio of (1:0.83) as shown in table (2).

Table.1 Distribution of suspected TB patients according to age

	suspected patients	
Age groups (years)	**Number**	**%**
11-20	**1**	**2.3**
21-30	**3**	**6.8**
31-40	**6**	**13.6**
41-50	**14**	**31.8**
51-60	**11**	**25.0**
61+	**9**	**20.5**
Total	**44**	**100**
Mean age (years)	**48.45±21.15**	

Table.2 Sex distribution of studied group

	suspected TB patients	
Sex	**Number**	**%**
Male	**24**	**54.6**
Female	**20**	**45.4**
Total	**44**	**100**
M/F ratio	**1:0.83**	

Renal tuberculosis is still one of the major health problems in the many countries including Iraqi (Bates, *et al.*, 1993). One

of the principle reasons for the failure of T.B control programs, is inability to detect infectious cases early enough (Moussa, *et al.,* 2000). Mycobacteria are usually present in relatively small number in most clinical samples including urine (16). There were two methods were used for diagnosis of renal tuberculosis in this study. The first method by using Ziehl–Neelsen stain for detection of *Mycobacterium tuberculosis*. In this study Ziehl–Neelsen stain was negative in all urine specimens (table 3).

Direct smears are often negative and do not differentiate tuberculosis from non-tuberculous mycobacterium. Although the Ziehl–Neelsen stain is rapid and inexpensive, but it lacks sensitivity. The laboratory diagnosis of tuberculosis is based on traditional methods i.e. examination of Z–N staining smear and culture on Lowenstein– Jensen (L–J) medium, while the diagnostic criterion for genitourinary tuberculosis has based on the isolation of *Mycobacterium tuberculosis* from urine. However, this is not easy to achieve as the discharge of organisms into the urine is sporadic and more importantly involves few organisms. Therefore, single specimen was likely to be false negative and at least 3 first–morning specimens should be collected to give the highest yield (Chain, *et al.,* 1995). Another advanced method such as polymerase chain reaction (PCR) has been applied in this study.

PCR was positive in 34.1% (15/44) of urine specimens with highly significance difference was noticed between two methods as shown in table (3). Additionally, the results of this study agreed with (Kamyshan, *et al.,* 2003) who reported that a significant difference has been noticed between PCR and Ziehl–Neelsen stain for detection TB infection. Moreover (Van-Vollenhoven, *et al.,* 1996) has found that a considerable difference among PCR and Ziehl–Neelsen stain for investigating renal tuberculosis and the percentage of positivity were lower when using Ziehl–Neelsen method.

Table.3 Comparison between polymerase chain reaction and Ziehl–Neelsen stain according to suspected TB patients

Methods	**Number of suspected TB patients**	**Frequency of positive cases**	**%**	**significance**
Ziehl–Neelsen stain (AFB)	**44**	**------**	**0**	**0.001 HS**
Polymerase chain reaction	**44**	**15**	**34.1**	

Table.4 Distribution of infected TB patients according to age

	infected patients	
Age groups (years)	**Number**	**%**
11-20	**0**	**0**
21-30	**1**	**6.6**
31-40	**2**	**13.3**
41-50	**4**	**26.6**
51-60	**6**	**40.2**
61+	**2**	**13.3**
Total	**15**	**100**
Mean age (years)	**42.37±19.61**	

Table.5 Distribution of infected TB patients according to sex

	Number of patients	infected TB patients	
Sex		**Number**	**%**
Male	**24**	**9**	**37.5**
Female	**20**	**6**	**30.0**
Total	**44**	**15**	**34.1**

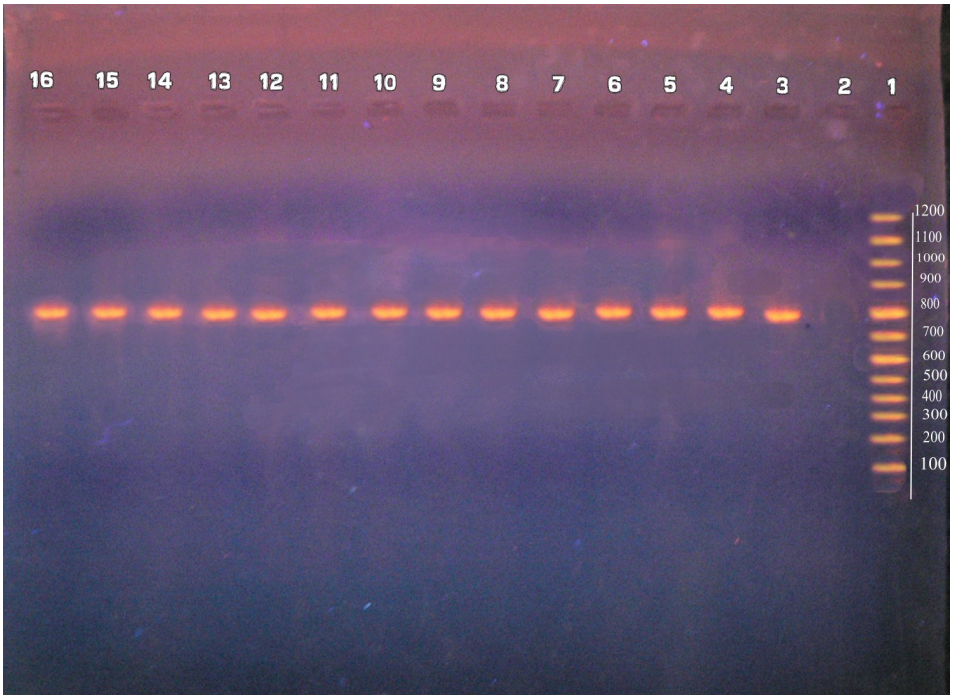


Figure.1 Gel electrophoresis for PCR product of 786bp (1.5%)agarose for 90 minutes at 60 volt.
1) DNA marker; 2) Negative control ;
3) Positive control; 4 to16) TB positive sample (786 bp band)

According to age, the 15 positive patients were 21 to 61+ year, Also it was found that more than half of patients were located within fourth and fifth decade, with a percentage of 66.8%, moreover as regard to sex, 9 male (37.5 %) out of 24 were positive and 6 females (30.0%) out of 20 were positive for renal tuberculosis as revealed in table (4 and 5). As regard to age, in our study the mean age of positive cases was 42.37±19.61 year. This is in accordance with (Hemal, *et al.*, 2000) who stated that the mean age of patients with renal tuberculosis was 43.12 year. As regards to sex in our study 37.5% of males were positive for T.B while 30.0% of females were positive. This was supported by (Aslan, *et al.*, 2007) who stated that incidence of renal tuberculosis in males is more than females due to their excessive exposure to the sources of tuberculous infections. In this work, renal tuberculosis patients constitutes 15 (34.1%) out of the 44 patients according to the positive results of PCR (table 5), these result agreement with (Fontana, *et al.*, 1997) who found that 33.6 % of suspected patients were found to be positive for TB by PCR technique.

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 few hours, the high sensitivity of PCR is particularly useful in paucibacillary situations such as non-pulmonary tuberculosis (renal tuberculosis) (Hemal, *et al.*, 2000). Springer, *et al.*, (1996) also found that PCR was not only rapid but also more accurate than traditional methods for the detection of *M. tuberculosis* ,therefore, PCR is a rapid and sensitive tool to detect Mycobacterium tuberculosis in urine samples for Urinary tuberculosis (UTB) patients.

References

Aslan, G. Doruk, E. Emekda, G. Serin, M.S. Direkel, S. Bayram, G. and Durmaz, R. 2007. Isolation and identification of *Mycobacterium tuberculosis* from the urine samples by conventional and molecular methods. *Mikrobivol. Bull*. 41:185-192.

Bates, J.H. and Stead,W.W. 1993.The history of tuberculosis as a global epidemic. *Med. Clin. Nor. Am.* 77:1205-1217.

Brown, M. Varia, H. Bassett ,P. Davidson, R.N. Wall, R. and Pasvol, G.2007. "Prospective study of sputum induction, gastric washing, and bronchoalveolar lavage for the diagnosis of pulmonary tuberculosis in patients who are unable to expectorate". *Clin Infect Dis.* 11: 1415–1420.

Cek, M. Lenk ,S. Naber, K.G.Bishop, M.C. Bjerklund, T.E. Botto ,H. Grabe ,M. Lobel ,B. Redorta, J.P. and Tenke,P. 2005. EAU guidelines for the management of genitourinary tuberculosis. *Eur. Urol.* 48: 353-362.

Chain, K.1995. Clinical microscopy. *In*: Murray, P.R. Baron, M.A. Pfuller, F.C. Tenover and R.H. Yolken Eds.: *Manual of Clinical Microbiology*, pp: 33–51, 6th ed., American Society for Microbiology, Washington DC.

Corbett, E.L. Watt, C.J. Walker, N. Maher ,D. Williams ,B.G. Raviglione, M.C. and Dye, C. 2003. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch. Int. Med.* 163: 1009-1021.

Dochviri, T.Z. Katsitadze ,V.A. Khosiashvili, G.Z.and Chigogidze, T.G. 2005.Detection of *Mycobacterium tuberculosis* in patients with Urogenital tuberculosis

by PCR method. *Georgian Med. News.* 9: 14-17.

Figueiredo, A.A. Lucon, A.M. Júnior, R.F. Ikejiri, D.S. Nahas, W.C. and Srougi, M. 2009. Urogenital tuberculosis in immunocompromised patients. *Int. Urol. Nephrol.* 41: 327-333.

Fontana, D. Pozzi, E .and Morra, I. 1997. Rapid identification of *Mycobacterium tuberculosis* com-plex on urine samples by Gen probe amplification test. *Urol Res.* 25:391-394.

Hemal, A.K.Gupta, N.P. Rajeev, T.P. Kumar, R. Dar, L. and Seth, P. 2000. Polymerase chain reaction in clinically suspected genitourinary tuberculosis: comparison with intravenous urography, bladder biopsy and urine acid fast bacilli culture. *Uro.* 56: 570-574.

Kafwabulula, M. Ahmed ,K. Nagatake, T. Gotoh ,J. Mitarai, S. Oizumi, K. Zumla, A. 2002. Evaluation of PCR-based methods for the diagnosis of tuberculosis by identification of mycobacterial DNA in urine samples.*Int. J. Tuber. Lung Dis*. 6: 732-737.

Kamyshan, I.S. Stepanov, P.I. and Ziablitsev,S.V. 2003. Role of polymerase chain reaction in diagnosing tuberculosis of the bladder and male sex organs. *Urol*., 3: 36–39.

Lenk, S. and Schroeder, J. 2001. Genitourinary tuberculosis. *Curr. Opin. Urol.*11: 93-98.

Leung, C.C. Yam, W.C. Yew, W.W. Ho, P.L. Tam, C.M. and Law, W.S. 2010.T-Spot.TB outperforms tuberculin skin test in predicting tuberculosis disease. *Am J Respir Crit Care Med*. 6:834-40.

Menzies ,D. Joshi ,R. and Pai, M. 2007.Risk of tuberculosis infection and disease associated with work in health care settings. *Int J Tuberc Lung Dis*.11:593-605.

Moussa, O.M. Eraky, I. El-Far, M.A. Osman, H.G. and Ghoneim, M.A .2000. Rapid diagnosis of genitourinary tuberculosis by polymerase chain reaction and non-radioactive DNA. *J. Urol.* 164: 584-588.

Nawaz Khan A, Chandramoha M 2004. Tuberculosis, Genitourinary Tract. *Medicine*. 10: 76-79.

Sambrook, J. Fritsch, E.F. and Maniatis T.1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory, New York.

Springer, B.Stackman, L. and Teschner, K. 1996. Two laboratory study on identification of mycobacterium: molecular versus phenotypic methods. *J.Clin. Microbiol.*34:296-303.

Van-Vollenhoven, P. Heyns, C. F. de-Beer, P. M. Whitaker, P. Van-Helden, P. D. and Victor, T.1996 .Polymerase chain reaction in the diagnosis of urinary tract tuberculosis. *Urol Res.* 24:107-110.

Verhagen, L.M. Hof, S. Deutekom, H. Hermans, P.W. Kremer, K. and Borgdorff, M.W. 2011 .Mycobacterial factors relevant for transmission of tuberculosis. *J Infect Dis*. 9 :1249-1255.

Warren, D. Johnson, J.R. Johnson, C.W. Franklin, C. and Lowe, F.C. 2002.Genitourinary Tuberculosis Campbell's Urology. 8th ed. Saunders.

Yazdani, M. Shahidi, S. and Shirani, M. 2008. Urinary Polymerase Chain Reaction for Diagnosis of Urogenital Tuberculosis. *Urol. J.* 5: 46-49.