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Evaluation of Molecular and Serological Techniques for the Detection of *Mycobacterium tuberculosis* in Urinary Infections

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ABSTRACT

This work was conducted to evaluate the methods frequently used for the diagnosis of Tuberculosis (TB). The study was run on 175 subjects in two groups including 150 cases suspected to have urinary TB (patients group) and 25 healthy subjects (control group). The following investigations were performed: Z–N staining smears, culture on L–J medium, Amplicor–PCR for urine specimens and detection of IgM antibodies against *M. tuberculosis* in serum samples. Considering the culture results as the golden standard for diagnosis of TB, the sensitivity, specificity, positive and negative predictive values and accuracy for (i) Ziehl–Neelsen (Z–N) staining smears were 71.4, 31.8, 57.1, 46.7 and 54%, respectively, (ii) Amplicor– PCR were 83.3, 72.7, 79.5, 77.4 and 78.7%, respectively, and (iii) *M. tuberculosis*–IgM antibodies were 27.4, 77.3, 60.5, 45.5 and 49.3%, respectively. On taking the consensus analysis as the golden standard, the sensitivity, specificity, positive and negative predictive values and accuracy for (i) Z–N staining smears were 75.8, 40, 68.6, 48.9 and 62.7%, respectively, (ii) Lowenstein–Jensen (L–J) culture were 85.3, 94.5, 96.4, 78.8 and 88.7%, respectively, (iii) Amplicor–PCR were 86.3, 89.1, 93.2, 79 and 87.3%, respectively, and (iv) *M. tuberculosis*–IgM antibodies were 31.6, 85.5, 78.9, 42 and 51.3%, respectively. It was concluded that (i) PCR is more rapid, sensitive, and specific than the currently available techniques, but more expensive, (ii) PCR can distinguish cases with positive smears or cultures due to atypical mycobacteria from tuberculous cases, (iii) PCR is a useful and rapid method in monitoring the efficacy of anti–TB treatment. Thus, PCR may be suggested to be the golden standard for the diagnosis of TB.

Key Words: *Mycobacterium tuberculosis*; Tuberculosis; Urinary infection; Amplicor– PCR; ELISA

INTRODUCTION

Tuberculosis (TB) infection with *Mycobacterium tuberculosis* is rising in both the industrialized and the developing countries (Tortoli *et al*., 2001). Factors contributing to the resurgence of tuberculosis include the epidemic human immunodeficiency virus (HIV), the immigration of people from countries with a high incidence of TB and the increase in the medically underserved population. Consequently, rapid and accurate identification of the etiologic agent is necessary both to decide measures needed to prevent its diffusion and to make therapeutic choices (Maria *et al*., 2000; Tortoli *et al*., 2001).

Diagnosis of TB is based on the microscopic detection of Acid Fast Bacilli (AFB) in secretions or tissue samples. However, about 75% of patients with extra–pulmonary disease are smear negative and TB culture which takes several weeks to give a result has to be done (Wilkins, 1998). Therefore, a number of alternative diagnostic tests that use immunological and molecular techniques have been developed.

The immunological methods use the specific humoral or cellular immune responses of the host to infer the presence of the disease. Various modifications of ELISA have been developed to detect different antibody classes against various antigens of the tubercle bacilli (Bothamley, 1995).

The Polymerase Chain Reaction (PCR) as a powerful *in vitro* tool has been developed**.** Some PCR tests have been reported for the identification of *M. tuberculosis* by Eisenach *et al.* (1990), Herman *et al.* (1990) and Patel *et al.* (1990). These tests were highly sensitive, the amplified genomic fragments are common to the *M. tuberculosis* complex (*M. bovis*, *M. microti* and *M. africanum*) and have produced widely different results with regard to the sensitivity of the assay with different types of clinical samples (Noordhoek *et al.,* 1993; Kox *et al.,* 1994; Seth *et al*., 1996).

This work was carried out to isolate *M. tuberculosis* from the urine of patients suffering from Chronic Renal Insufficiency (CRI), Chronic Renal Failure (CRF), Haemodialysed patients (HD) and Renal Transplant recipients (RTX) and to evaluate the diagnostic value of various methods widely used in microbiological diagnosis of TB.

MATERIALS AND METHODS

The study was performed at the National Institute of Urology and Nephrology– Matareya– Cairo during the

period from January 1999 to December 2002.

Patients. The study enrolled 150 patients [88 males and 62 females] suffering from renal troubles. Their age ranged from 13 to 75 years [mean & S.D.=36.3±11.8]. They were all suspected to have urinary TB. They included 21 CRF patients, 76 CRI patients, 24 HD patients and 29 RTX patients.

Controls. Twenty–five healthy individuals were included in the study as controls (10 females and 15 males). Their age ranged from 15 to 50 years old (mean±S.D.= 31.7±7.9). Patients and controls were subjected to the following investigations, (i) Ziehl–Neelsen (Z–N) staining of urine samples for AAFB, (ii) Culture of urine specimens on Lowenstein–Jensen (L–J) media and identification of *M. tuberculosis*, (iii) PCR for urine specimens, and (iv) Detection of IgM antibodies against *M. tuberculosis* in serum samples.

PROCEDURES

I. Sample Collection

1. Blood samples. Five mL of venous blood samples were collected from both patients and controls under strict sterile conditions. The blood samples were allowed to clot and sera were separated by centrifugation at 3000 rpm for 5 min. and stored in aliquots of 0.5 mL at –80°C.

2. Urine samples. Early morning voided urine samples were collected in sterile containers daily for three days. Urine processing was carried out by N–acetyl L–cystine–sodium hydroxide decontamination and concentration method**.** The sediment was used to prepare slides for Z–N staining, culture on L–J medium and for PCR.

II. Ziehl–Neelsen Staining Technique. This technique and composition of stains and reagents was according to Cheesbrough (2000).

III. Culture of TB. 250 µL of the resuspended deposits of each urine specimen were inoculated on the entire surface of L–J medium slope and incubated at 37°C in a horizontal position with loosen screw cap for two days and then placed upright with tightened cap and inspected every week till the appearance of mycobacterial colonies or contaminated growth or up to two months when no growth appeared. The identification of *M. tuberculosis* isolate was carried out according to morphology of the colony, Z–N staining smears, pigment production test and growth at 25ºC (Cheesbrough, 2000).

IV. PCR. The AMPLICOR *M. tuberculosis* PCR test (Roche diagnostic system, USA) is based on three major processes: PCR target amplification, hybridization of the amplified product to a specific nucleic acid probe and detection of the amplified product by color formation using ELISA. This test permits the simultaneous amplification of *M. tuberculosis* target DNA and *Mycobacterium* internal control (IC) DNA that has been added to the Amplicor tests to identify specimens containing inhibitory substances that may interfere with PCR amplification. The test was carried out according to the procedure indicated by the manufacturer.

V. Detection of IgM antibodies to *M. tuberculosis.* Pathozyme–myco kits (Omega Diagnostics Limited, Scotland, UK) were used for detecting IgM antibodies (Abs) to *M. tuberculosis* in patients' sera. The test was carried out according to the instructions of the manufacturer. A color would develop in wells indicating the presence of human anti–*Mycobacterium* species antibody. The absorbance was measured at 450 nm. A positive result should have an O.D. greater than the O.D. of the low positive control X 1.5**.**

VI. Statistical analysis. Statistical analysis of the data was performed using statistical medical program. The tests used were, (i) Mean and standard deviation, and (ii) Validity of the test was clarified using sensitivity, specificity, PPV and NPV of tests.

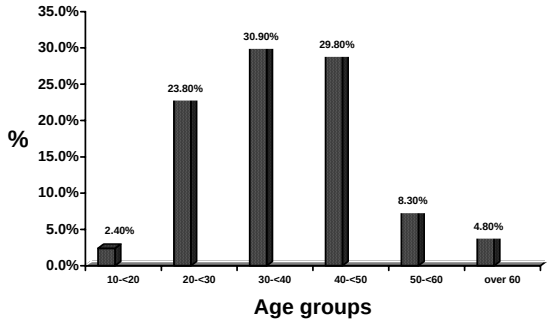
RESULTS

According to the results of culture shown in Fig. 1, the highest percentage of tuberculous patients in relation to the total number of diseased patients was within 30 to 40 years age group (30.9%) followed by 40 to 50 years age group (29.8%), while the least percentage was detected in the age group 10 to 20 years (2.4%). It was found also that 53 males (63.1%) and 31 females (36.9%) were infected with the disease.

Four laboratory assays were carried out for the detection of *M. tuberculosis* on both patients and control groups. The urine samples of control group were negative for TB using Z–N stain, L–J culture, PCR and their serum samples were also negative for *M. tuberculosis* IgM. Results presented in Table I show that on testing 150 suspected urine specimens of the patients group, the highest number of TB positive samples was detected on using Z–N smears (105), followed by PCR (88) (Plate 1) and L–J cultures (84). On the other hand, *M. tuberculosis* IgM Abs were detected in 38 (25.3%) serum samples only (Plate 2). The difference between the patients and control groups was highly significant (P value for all assays was < 0.01).

When L–J culture is considered as the golden standard test for detecting *M. tuberculosis* infections (Table II), 60

Fig. 1. Distribution of tuberculous patients among different age groups



Z–N smears out of 105 positive Z–N specimens were also positive for culture and 24 out of 45 negative smears were positive for culture. The sensitivity and specificity of Z–N smears were 71.4 and 31.8%, respectively. The PPV and NPV were 57.1 and 46.7%, respectively.

As regard to PCR, out of 88 PCR positive specimens, 70 were also positive on using culture, while 14 positive cases for culture were recovered from 62 negative specimens by PCR. The sensitivity and specificity of Amplicor– PCR were 83.3 and 72.7%, respectively with PPV and NPV of 79.5 and 77.4%, respectively.

As regard to *M. tuberculosis* IgM Abs, out of 38 positive cases for *M. tuberculosis* IgM, only 23 were positive for culture while 61 cases were positive for culture out of 112 negative cases. The sensitivity and specificity of *M. tuberculosis* IgM assay were 27.4 and 77.3%, respectively with a PPV and NPV of 60.5 and 45.5%, respectively.

Plate 1. An agarose gel containing PCR products of some patients' urine samples (Lane 1 contains DNA-marker. Lanes from 2 to 16 contain PCR products of patients. Lanes 5, 6 & 16 show positive results (DNA band of approximately 317 bp)).

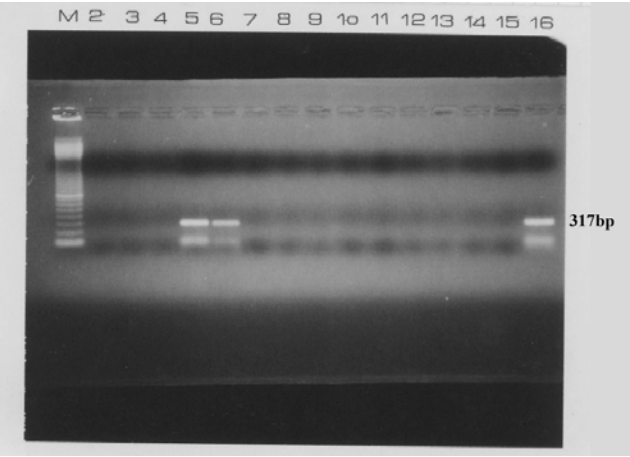
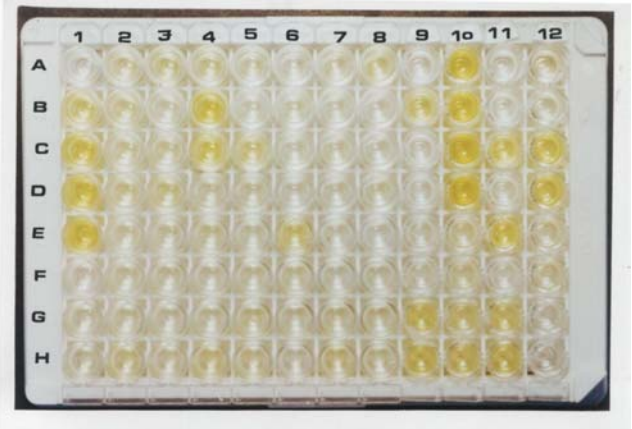


Plate 2. Pathozyme ELISA plate for detection of *M. tuberculosis* IgM in some patients' sera with negative (colorless) and positive (yellow) reactions



The relationships between the results of the different assays used were evaluated using McNemar–corrected Chi–square (Tables III–V).

Results of Z–N smear method were studied versus PCR assay among the patients group (Table III). Out of 105 positive cases detected on using Z–N smear, only 59 cases were positive for Amplicor–PCR and out of 45 negative cases, 29 were positive for Amplicor–PCR. The difference was statistically significant ($*x^2$ = 2.1, P <0.05) i.e. PCR technique was more specific than Z–N smears.

When the results of Z–N smears were compared with those of *M. tuberculosis*–IgM assay among the patients group (Table IV), out of 105 positive cases detected on using Z–N smear, only 26 had IgM antibodies for *M. tuberculosis*, while within 45 negative cases using Z–N smears, 12 patients had IgM antibodies against *M. tuberculosis.* The difference was highly significant ($*x^2$ = 6.1, P< 0.001) i.e. Z–N smears were more specific than *M. tuberculosis* IgM assay.

Also, when the results of PCR were compared with that of *M. tuberculosis*–IgM assay among the patients group (Table V), among 88 cases positive for Amplicor–PCR, only 26 patients had IgM antibodies against *M. tuberculosis*. On the other hand, within 62 negative cases, only 12 had IgM antibodies in their sera. The difference is statistically significant ($*x^2$ = 5.6, P <0.001) i.e. PCR technique was more specific than *M. tuberculosis* IgM assay.

The consensus analysis test was used to clarify which assay is better for the diagnosis of *M. tuberculosis* (Tables VI–IX). It was found that (i) The Z–N smears' sensitivity and specificity were of 75.8 and 40%, respectively with PPV of 68.6% and NPV of 48.9% while the accuracy of the test was 62.7% (Table VI), (ii) The L–J culture had sensitivity and specificity of 85.3 and 94.5%, respectively with PPV of 96.4% and NPV of 78.8% .The accuracy of the test was 88.7% (Table VII), (iii) The PCR assay had sensitivity and specificity of 86.3 and 89.1%, respectively. The PPV and the NPV were of 93.2 and 79%, respectively with accuracy of 87.3% (Table VIII), and (iv) Lastly, *M. tuberculosis* IgM assay recorded sensitivity and specificity of 31.6 and 85.5%, respectively with PPV of 78.9% and NPV of 42%. The accuracy of the test was 51.3% (Table IX).

The performance of the laboratory assays for detection of urinary tuberculosis used in our study was summarized in table (X). The PCR assay showed the best sensitivity (86.3%) followed by L–J culture (85.3%) and Z–N smear (75.8%) while, the lowest one was *M. tuberculosis* IgM assay (31.6%). The L–J culture assay was the most specific (94.5%) followed by PCR (89.1%) and *M. tuberculosis* IgM assay (85.5%). The least specific one was Z–N smear (40%). The highest accuracy was that got by L–J culture (88.7%) followed by PCR (87.3%) and Z–N smear (62.7%)

while the least accuracy was got by *M. tuberculosis* IgM antibodies (51.3%).

DISCUSSION

Tuberculosis remains a major public health problem in the developing countries, as it is the largest cause of death in the world from a single infectious disease. Despite the decline in incidence seen in the 1980s, resurgence has occurred. The appearance of multiple drug resistant strain of *M. tuberculosis* has intensified the need for the use of rapid methods for its detection (Hemal *et al.*, 2000).

The genitourinary system follows the respiratory system as the most common site of tuberculosis. Worldwide, the genitourinary form of the disease accounts for 14% of the non–pulmonary tuberculosis. In the western world, only 8–10% of patients with pulmonary tuberculosis develop renal tuberculosis, meanwhile the incidence in the under–developed countries was ranging between 15–20% (Omar *et al*., 2000).

The laboratory diagnosis of tuberculosis is based on traditional methods i.e. examination of Z–N staining smear and culture on L–J medium, while the diagnostic criterion for genitourinary tuberculosis has based on the isolation of *M. 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, 1995).

There is an urgent need for a reliable diagnostic test that meets all the requirements of rapidity, sensitivity and specificity. This was the motivation for the development of the PCR as a diagnostic tool for tuberculosis. This technique is capable to amplify an extremely small amount of a specific genomic sequence rapidly. Thus, the presence of an extremely small number of bacteria can be detected within 24–48 h (Hemal *et al*., 2000).

Therefore, the aim of the present study was to evaluate four laboratory techniques for the diagnosis of genitourinary tuberculosis in our locality. These techniques are: Z–N staining smears, culture on L–J medium, PCR technique and finally ELISA technique for the detection of tuberculosis antibodies (IgM), finding the relationship between them and their validity on using L–J culture as the golden standard for diagnosis.

The percentage of detected tuberculous patients in the present study was 84 (56%) out of 150 patients. This rate of infection might be higher when compared to that of Hemal *et al*. (2000) and Omar *et al*. (2000) who found a rate of infection of 30.9 and 36.3%, respectively. The incidence of tuberculosis infections is significantly higher in end stage patients and renal transplant recipients than in normal individuals as these patients were suffering from defects in CMI that decrease host resistance to infection (Tushar *et al.*, 2000). Also, delayed presentation with advanced disease might be another explanation.

Regarding the distribution of the tuberculous patients among the age groups, this study revealed that the highest rate of infection was within the age group 30 to 40 (30.9%). This is in agreement with the results of Assad (1999) who reported that tuberculosis in the community is mainly a

Table I. Detection of *M. tuberculosis* using different laboratory assays among patients and control groups

Laboratory assay		Patients group (Total No. 150)		Control group (Total No. 25)		X^2	P value
		No.	%	No.	%		
Z-N Smear	Positive	105	70	0	0	43.8	< 0.001
	Negative	45	30	25	100		
L-J culture	Positive	84	56	0	0	14.03	< 0.001
	Negative	66	44	25	100		
PCR	Positive	88	58.7	0	0	29.5	< 0.001
	Negative	62	41.3	25	100		
IgM Abs	Positive	38	25.3	0	0	8.1	< 0.01
	Negative	112	74.7	25	100		

Table II. The validity of Z-N smear method, PCR assay and IgM assay versus L-J culture results in the patients group

Laboratory assay	L–J culture		Total
	Positive No.	Negative No.	No.
No. of positive Z-N smears	60 (true + ve)	45 (false + ve)	105
No. of negative Z-N smears	24 (false – ve)	21 (true - ve)	45
No. of positive PCR samples	70 (true + ve)	18 (false + ve)	88
No. of negative PCR samples	14 (false - ve)	48 (true - ve)	62
No. of positive IgM samples	23 (true + ve)	15 (false + ve)	38
No. of negative IgM samples	61 (false - ve)	51 (true - ve)	112

Table III. The relationship between Z-N smear and PCR assay among the patients group

Z-N Smear	PCR				McNemar $*x^2$	P Value
	Positive		Negative			
	No.	%	No.	%		
Positive	59	39.3	46	30.7	2.1	<0.05
Negative	29	19.3	16	10.7		

Table IV. The relationship between Z-N smears and *M. tuberculosis* IgM assay among the patients group

Z-N Smear	M. tuberculosis IgM				McNemar $*x^2$	P value
	Positive		Negative			
	No.	%	No.	%		
Positive	26	17.3	79	52.7	7.1	<0.01
Negative	12	8.0	33	22		

Table V. The relationship between PCR and *M. tuberculosis* IgM assay among the patients group

PCR	M. tuberculosis IgM				McNemar $*x^2$	P Value
	Positive		Negative			
	No.	%	No.	%		
Positive	26	17.3	62	41.3	5.7	<0.05
Negative	12	8.0	50	33.3		

disease of young adults.

For sex distribution, results showed an infection ratio of 1.7:1 as males to females. This is comparable to the results of Tushar *et al*. (2000) who found a ratio of 2:1. However, Taskapan *et al*. (2000) reported a ratio of 1:1. This difference might be due to the group of patients selected to run the study on them and/or the presence of predisposing factors such as defects in CMI.

The rapid diagnosis of the genitourinary tuberculosis is based on the detection of AAFB in urine by Z–N staining smears. The sensitivity of the microscopic examination depends on the clinical presentation. More than 10^4 bacilli per mL of urine are necessary to secure microscopic positivity. However, the success of microscopy is highly variable (Barnes, 1997). Although the NALC–NaOH method was used in the present study as it is reported to yield the best recovery rate of mycobacteria when compared with different liquifaction and decontamination procedures (Tortoli *et al*., 1993), the sensitivity of Z–N smears was only 71.4% (using culture on L–J medium as the golden standard for the diagnosis of tuberculosis). Also, the specificity was 31.8%. On considering the consensus test as the golden standard, these values were raised up to 75.8 and 40%, respectively. This is consistent with the results of Omar *et al*. (2000) in Mansoura city who revealed sensitivity and specificity of 52.6 and 96.7%, respectively. However, Hamdey *et al*. (2000) showed a higher percentage of 90 and 71.4%, respectively in a study carried out in Zagazig city. On assuming the clinical diagnosis as the golden standard, Herrera and Segovia (1996) recorded a sensitivity of 69% and a specificity of 87%. These results show wide variability between the different studies in the yield of smear positivity. The variation might be attributed to the difference in the included subjects, the method of processing and staining of the specimens and the number of the examined smears. Anyway, all of these results assure the limited sensitivity of smear examination in detecting AAFB. On the other hand, the specificity of Z–N smear depends on the specimen type rather than the staining technique used. In the sterile materials, e.g. the spinal fluid, a positive AFB smear is diagnostic for mycobacterial meningitis. However, the presence of AFB in urine, sputum or stool does not necessarily allow the identification of pathogenic mycobacteria with the same certainty since environmental e.g. *Nocardia* and diphtheroids might contaminate such samples and microscopic examination alone does not discriminate between them. Therefore, confirmation of the microscopic finding and identification of the mycobacteria present in the specimens are mandatory (Fox *et al.*, 1982; Richeldi *et al*., 1995).

AFB culture is the reference standard for the diagnosis of tuberculosis and it is considered to be the most accurate test because of its high sensitivity and specificity (Jonas *et al*., 1993). Eighty four of the total 95 L–J medium cultures (84/95, 88.4%) were identified as *M. tuberculosis*, thus typical *M. tuberculosis* was detected in 56% (84/150) of suspected tuberculous patients in this study. In accordance, Hamdey *et al*. (2000) reported that the percentage of typical *M. tuberculosis* positive cultures was 58.3% for suspected patients. On the other hand, Hemal *et al*. (2000) and Omar *et al*. (2000) recorded low percentages of 36.3 and 37.14%, respectively. On considering a consensus test as the golden standard, the sensitivity and specificity of L–J medium culture were 85.3 and 94.5%, respectively.

Assuming the clinical diagnosis as the golden standard, Herrera and Segovia (1996) reported that the

Table VI. The validity of Z-N smear method versus consensus analysis in the patients group

Z-N Smears	Consensus analysis (L-J culture, PCR & IgM)		Total No.
	Positive No.	Negative No.	
Positive	72 (true +ve)	33 (false +ve)	105
Negative	23 (false –ve)	22 (true -ve)	45
Total	95	55	150

Table VII. The validity of L-J culture versus consensus analysis in the patients group

L-J culture	Consensus analysis (Z-N smears, PCR & IgM)		Total No.
	Positive No.	Negative No.	
Positive	81 (true + ve)	3 (false + ve)	84
Negative	14 (false - ve)	52 (true - ve)	66
Total	95	55	150

Table VIII. The validity of PCR assay versus consensus analysis in the patients group

PCR	Consensus analysis (Z-N smears, L-J culture & IgM)		Total No.
	Positive No.	Negative No.	
Positive	82 (true + ve)	6 (false +ve)	88
Negative	13 (false – ve)	49 (true – ve)	62
Total	95	55	150

Table IX. The validity of *M. tuberculosis* IgM assay versus consensus analysis in the patients group

M. tuberculosis IgM	Consensus analysis (Z-N, culture & PCR)		Total No.
	Positive No.	Negative No.	
Positive	30 (true + ve)	8 (false + ve)	38
Negative	65 (false – ve)	47 (true – ve)	112
Total	95	55	150

Table X. Performance of the four laboratory assays for detection of AAFB

Laboratory assay	Sensitivity	Specificity	PPV	NPV	Accuracy
Z-N smear	75.8%	40%	68.6%	48.9%	62.7%
L-J culture	85.3%	94.5%	96.4%	78.8%	88.7%
PCR	86.3%	89.1%	93.2%	79%	87.3%
M.tuberculosis IgM	31.6%	85.5%	78.9%	42%	51.3%

sensitivity and the specificity of tuberculosis cultures were 73 and 87%, respectively. Also, Cohen *et al.* (1996) reported 44.5% positive cultures out of 101 patients suspected to have extra–pulmonary tuberculosis.

It is obvious that culture must be done simultaneously with the smear as it is more confirmatory. The definitive diagnosis of mycobacterial disease demands isolation and identification of the causative agent. The culture is more sensitive than microscopy, being able to detect as few as 10 bacilli/mL of digested concentrated material. It helps in the diagnosis of cases with negative Z–N smears. In addition, the growth of the organisms is mandatory for species identification.

In the present study, smear positive, culture negative results were reported in 37 specimens. This might be due to intake of anti–tuberculous drugs that may impact the viability of the mycobacteria to great extend (Yajko *et al*., 1995).

The introduction of nucleic acids amplification technology has opened new merits in the diagnostic possibilities of infectious disease, and their application for the diagnosis of tuberculosis is currently under evaluation. In the present study, the PCR technique was used for the detection of *M. tuberculosis* DNA in urine specimens collected from patients suspected to have genitourinary tuberculosis. Several studies on the Amplicor–PCR were carried out since the introduction of the Amplicor system in 1992. Fritz *et al*. (1998) carried out a study using the Amplicor system on 870 specimens (545 respiratory and 325 non–respiratory). The sensitivity and specificity values were found to be 78.5 and 93.5%, respectively. Also, among the 190 cases of clinically diagnosed extra–pulmonary TB described by Claudio *et al*. (2000), 25 specimens were smear and culture positive and out of them 23 were Amplicor positive. Seven out of eight negative smears, culture positive specimens and four out of eight smears and culture negative were Amplicor–PCR positive. The sensitivity and specificity were 85 and 100%, respectively. In addition, Boris *et al*. (2001) carried out a study on 655 cases of clinically diagnosed TB (273 were respiratory and 382 were non–respiratory). They found Amplicor–PCR positivity in 30 out of 42 positive cultures and 6 out of 531 negative cultures leading to sensitivity and specificity of 71 and 99%, respectively. Recently, in a study performed by Kamyshan *et al*. (2003) the urinary PCR test confirmed tuberculous etiology of the disease and corresponded to *M. tuberculosis* detection in 27 (60%) of 45 patients with urinary TB. *Mycobacterium tuberculosis* DNA detection rose significantly in patients with mycobacteriuria (71%) in examination of aspirates from blocked kidney, epididymis, prostate and seminal vesicles.

However, the sensitivity in culture–negative and/or smear–negative patients was recorded to be limited (40 to 70% in most reports). The lowered PCR sensitivity in bacteriologically negative group could be due to many reasons. Firstly, Marks (1993) claimed that due to technical or sampling error, even if the technology evolves to the level of reliably amplifying one genome equivalent (one organism/sample), the possibility of a false–negative result couldn't be excluded. Indeed the sensitivity and specificity of detecting the presence of an infectious agent will improve but not reach the theoretical 100% mark. However, more searches are required to correlate clinical information with the increased sensitivity of PCR. Secondly, the presence of inhibitory substances might affect the efficiency of PCR. These substances are either exogenous such as anticoagulants and detergents, or endogenous as found in urine or in other samples. Systemic inclusion of the internal control would greatly contribute to the accuracy of the assay and also provide important information when testing non–approved types of samples as stool (Claudio *et al*., 2000). Fritz *et al*. (1998) revealed a percentage of inhibition of 14.2% for non–respiratory specimens. However**,** Claudio *et al*. (2000) found a low percentage of inhibition (2.6%), while the recent study of Boris *et al*. (2001) showed the percentage as high as 18.6%. In this context, removal of the inhibitory substances may be an alternative approach to improve sensitivity. However, it seems unlikely to find a routine–fitting procedure able to remove all inhibitory substances because the nature of inhibition is still unclear and probably affects amplification technique unevenly (Della Latta & Vivian, 1999).

This lack of agreement between smear, culture and PCR results may be due to their difference in sensitivity. Smear sensitivity is greater than 10^4 organisms/mL; culture sensitivity in L–J medium is greater than 10^2 organisms/ mL (Kim *et al*., 1984), while PCR could detect 1 to 10 mycobacterial organisms. In addition, culture permits only the growth of viable bacteria, while positive PCR might in the presence of non–viable bacteria due to the intake of anti–tuberculous treatment (Eisenach *et al.*, 1990).

Since the introduction of ELISA in 1972 and the availability of monoclonal antibodies as well as purified antigens, the serological diagnosis of TB has become more promising (Daniel, 1996). The 38–KDa antigens, a phosphate–binding protein, have been identified as the immunodominant antigen in smear–positive pulmonary and extra–pulmonary TB and a potential reagent for use in screening for infectious TB (Sudha *et al*., 2000).

Mycobacterium tuberculosis IgM assay sensitivity and specificity were found to be 27.4 and 77.3%, respectively on using culture results as the golden standard. On considering the consensus test, these values were found to be 31.6 and 85.5%, respectively.

The sensitivity and specificity of the serological tests varied widely in other reports, ranging from 20 to 60% for sensitivity and from 65 to 100% for specificity. Sudha *et al*. (2000) reported a sensitivity of 18% with 80% specificity. A higher rate of seropositivity for the smear–positive group compared to that for the smear–negative group has been attributed to the higher bacillary loads in smear–positive patients, resulting in a greater exposure to antigen and thus a

more vigorous antibody response (Alifano *et al*., 1997; Sudha *et al*., 2000). The diagnostic value of a given test in clinical practice depends on its positive and negative predictive values. These values vary markedly with the prevalence of the disease in a given community (Chinag *et al*., 1997). Thus, these serological tests could potentially be used for those subgroups of patients with TB from whom specimens are difficult to obtain i.e. those with extra–pulmonary and childhood TB and smear–negative patients, to aid in clinical decision–making. Investigators have recommended improving the performance values of the tests by either adjusting the cutoff values or combining the results of different tests (Wilkins, 1998).

The inter–relationships between the results of the assays were evaluated using McNemar's Chi–square test. No significant statistical difference was observed between different assays except for Amplicor–PCR assay, which was significantly sensitive than Z–N stain, and *M. tuberculosis* IgM antibody assays ($P < 0.05$ for both). However, Z–N stain smear results were significantly sensitive than *M. tuberculosis* IgM antibody assay ($P < 0.01$).

On applying consensus analysis the Amplicor–PCR assay appeared to have the highest sensitivity (86.3%) followed by L–J medium culture (85.3%) and Z–N stain smear (75.8%); while, the lowest sensitivity was for *M. tuberculosis* IgM antibody (31.6%). On the other hand, L–J medium culture had the highest specificity (94.5%) followed by the Amplicor–PCR assay (89.1%). So, it is concluded that although, at present, PCR assay cannot replace culture technique, it is rapid and sensitive for the detection of *M. tuberculosis* in clinical samples as its protocol is easy to perform and suitable for a routine microbiology laboratory's workflow (Claudio *et al*., 2000). In the beginning of application of PCR technique, it was expensive in comparison with the currently used techniques, but with the progressive advancement in the field of molecular biology, the cost of different reagents is markedly decreased. Although, the price of PCR is still higher than that of other conventional methods, the isolation, identification and drug–resistance properties of mycobacteria can be determined directly from clinical specimens and the results are available in a matter of days rather than weeks. Also, the cost of conventional culture, drug susceptibility determinations, unnecessary periods of isolation, delay in recognition of resistant isolates and deaths before the availability of laboratory information, all are high prices to pay (Marks, 1993). Therefore, PCR has the potential to become the golden standard for the detection of *M. tuberculosis* in clinical specimens and much effort should be exerted to improve the sensitivity of the amplification procedure for smear–negative, culture–positive non– inhibitory specimens.

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