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Research Article

Role of Polymerase Chain Reaction in diagnosis of Tuberculosis as compared to routine tests

Dr Haris Memon[1], Dr Muhammad Haroon Mujtaba Memon[2], Dr Mahum Shahab[3], Dr Mahmood Iqbal[4], Dr Ghulam Murtaza Memon[5]

[1]House officer Liqauat University, Hospital , Jamshoro
[2]Medical Trainee William Harvey hospital, Ashford UK
[3]Isra University
[4]Public Health Consultant, Govt of Sindh
[5]Epidemiologist, Directrate General, Hyderbad

Abstract:
Introduction: There is a need for rapid and sensitive detection of *Mycobacterium tuberculosis* in clinical samples. A study was conducted in which the target for the amplification being a segment of IS6110 in the *M. tuberculosis* chromosome was evaluated using real time PCR and its results were compared with routine tests, using pulmonary and extra-pulmonary specimen.
Methods: In this descriptive cross-sectional retrospective study, specificity and sensitivity of PCR were analyzed. A total of 293 clinical samples were processed at a tertiary care hospital of Peshawar, during the time period of 2016-2018, from patients suspected of having pulmonary and extra-pulmonary tuberculosis and Follow up patients with DOTS treatment and MDR treatment that are referred by tertiary hospital were also included in this study after taking their informed consent. Patients not willing to participate in the study were excluded. For identification specimens were stained by Ziehl Neelsen staining (ZN), cultured on *Lowenstein–Jensen (*LJ) medium and then confirmed by PCR for the detection of Mycobacterium tuberculosis (MTB).
Results: Of the 293 samples, 165(56.3%) were from males and 128(43.7%) females. Mean age was 44 years (2-85 years). Specimen types included: CSF (30.4%), pleural fluid (4.1%), sputum (15%), urine (2.4%), synovial fluid (2.4%), other fluids (33.1%) and biopsies (12.6%). Only 3.1% of specimens were ZN-smear positive for (MTB). LJ culture identified 7.2% whereas PCR method detected (MTB) in 15% of the total specimens. Using PCR as gold standard, ZN microscopy correctly identified 20.5% of total (MTB) positive specimens and LJ culture detected 47.7%.Specimen types showed significant association with PCR test: 42.9% of synovial fluid samples and 41.7% of pleural fluid samples; 28.6% of urine samples were positive for (MTB) by PCR method. This indicates that PCR analysis of these specimens' exhibit greater positivity rates for (MTB) as opposed to CSF and other fluids and biopsies
Conclusions: TB PCR is a rapid and reliable test in the diagnosis and management of tuberculosis.

Keywords: polymerase chain reaction, Mycobacterium tuberculosis, clinical evaluation

Introduction:

Tuberculosis is a global disease affecting one third of worldwide population. Every year 8 million new cases are diagnosed and of which 3 million people dies (1). In America, from last two decade there is an increase in TB incidence number and it is increasing day by day (2). Pakistan is the 5th amongst 22 countries with highest burden of TB (3). In Pakistan, approximately 430,000 people of which 15,000 children get Tuberculosis (TB) and 70,000 deaths occurs every year due to the disease (3). Without proper treatment, up to two thirds of people with TB will die (4). Tuberculosis is a bacterial infection. Various species cause tuberculosis, among them mycobacteria is the most common specie (5). Mycobacteria tuberculosis is an acid fast bacilli and is slow growing and facultative microbe (6). Usually tuberculosis is diagnosed by routine tests like ZN smear chest X-rays and culture examination. These routine tests are not that effective due to low levels of mycobacterium and prolong time consuming procedures (8). Of which, Z N smear is easy and simple test to perform; but it has low specification and sensitivity (9). Mycobacteria Culture usually takes 4-6 weeks to make diagnosis and it gives 20-30% of false negative results (10). This makes the diagnosis and treatment slow and compliance of TB patient difficulty which leads to emergence of MT drug resistance (11). There are various factors which increase TB incidence especially when it co-exist with HIV as well as in low socioeconomic countries where it spreads easily and especially where facilities are insufficient (12) or in state of overcrowding and in hospital setting when diagnosis is either delayed or initiation of its treatment is delayed (13). Even in developed countries where culture takes long, non-tuberculosis mycobacteria were detected. (14)(15) For rapid and cost-effective diagnosis of tuberculosis, new techniques are needed. (4) Polymerase chain reactions are the most

suitable option for the diagnosis of MTB and PCR can even detect MTB in negative samples with high degree of sensitivity and specificity (16)(17), in both pulmonary and extra-pulmonary cases. PCR detects tuberculosis within 1-2 hrs with one sputum. Even in developing countries, PCR has a promising future in diagnosis of tuberculosis. (15) Previous studies showed that PCR sensitivity and specificity ranges from 77% to >95% of smear positive specimen (19) (18). Mycobacteria DNA was prepared from microscopic slide (20) to find out about the effectiveness and performance of PCR and its comparison with ZN microscopy and LJA culture was done in detecting (MTB).

Materials and Methods:

This is descriptive cross-sectional retrospective study to compare sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Ziehl Neelsen (ZN) microscopy and Lowenstein Jensen (LJ) medium culture in detecting Mycobacterium tuberculosis ((MTB)) and comparing with PCR assay as the gold standard.

A total of 293 samples of various specimen types were collected at main tertiary teaching hospital at Peshawar from patients suspected of having pulmonary and extra-pulmonary tuberculosis. Specimens underwent Ziehl Neelsen staining, were cultured in LJ medium and processed by PCR for the detection of (MTB).

The data was analyzed using computer statistical package of social sciences (SPSS) version 22.0. Fisher exact test was used to determine statistical significance.

Results:

Of the 293 samples, 165(56.3%) were from males and 128(43.7%) were of females. Mean (± SD) age was 44 (±20) ranging from 2 years to 85 years. Specimen types included: CSF (n=89), pleural fluid (n=12), sputum (n=44), urine (n=7), synovial fluid (n=7), other fluids (n=97) and biopsies (n=37).

Gender	Male	165(56.3%)
	Female	128(43.7%)
ZN smear	Positive	9(3.1%)
	Negative	284(96.9)
LJ culture	Positive	21(7.2%)
	Negative	23(92.8%)
PCR	Positive	44(15%)
	Negative	249(85%)
Specimen types	CSF	89(30.4%)
	Pleural fluid	12(4.1%)
	Sputum	44(15%)
	Urine	7(2.4%)
	Synovial fluid	7(2.4%)
	Other fluids	97(33.1%)
	Other biopsies	37(12.6%)

Only 3.1% (n=9) of specimens were ZN-smear positive for *(MTB)*. LJ culture identified 7.2% (n=21) whereas PCR method detected *(MTB)* in 15% (n=44) of the total specimens. Using PCR as gold standard, ZN microscopy correctly identified 20.5% of total *(MTB)* positive specimens and LJ culture detected 47.7%.

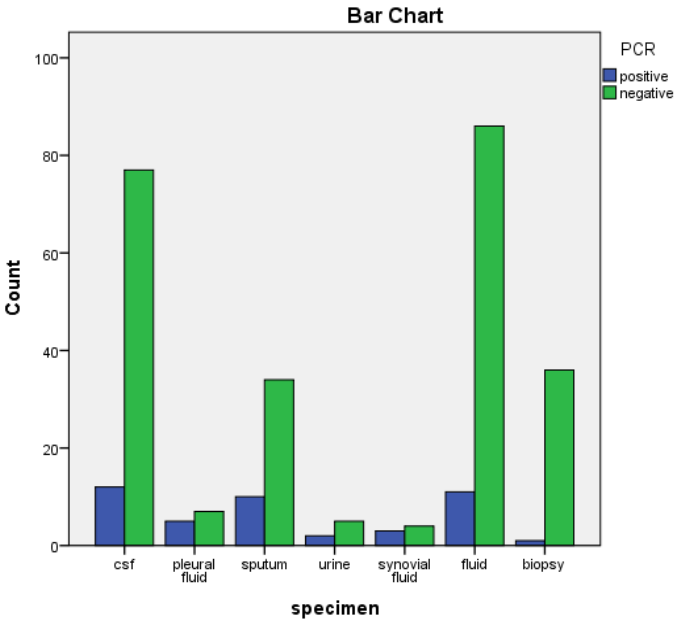
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
PCR	100%	100%	100%	100%
ZN staining	20%	100%	100%	88%
LJ culture	47%	100%	100%	91%

Neither ZN microscopy nor LJ culture identified a truly negative patient as a positive patient therefore exhibit 100% specificity. Both tests did not give false positive results and thus exhibit 100% PPV. However ZN smearing failed to detect 34 true positive patients and LJ culture also failed to detect *(MTB)* in 23 specimens thus both have low sensitivity and low NPV as opposed to PCR method.

Fisher exact tests were performed to detect any association between gender, specimen type and laboratory test. There is no significant difference (P-value = 0.408) between males and females in the detection of *(MTB)* by PCR, LJ culture medium nor by ZN microscopy.

Specimen types showed significant association with PCR test: 42.9% of synovial fluid samples and 41.7% of pleural fluid samples; 28.6% of urine samples were positive for *(MTB)* by PCR method. This indicates PCR analysis of these specimens' exhibit greater positivity rates for *(MTB)* as opposed to CSF and other fluids and biopsies.

		PCR Result		**P value**
		Positive	Negative	
Specimen type	CSF	12(13.5%)	77(86.5%)	<0.005 (0.002)
	Pleural fluid	5(41.7%)	7(58.3%)	
	Sputum	10(22.7%)	34(77.3%)	
	Urine	2(28.6%)	5(71.4%)	
	Synovial fluid	3(42.9%)	4(57.1%)	
	Other fluids	11(11.3%)	86(88.7%)	
	Other biopsies	1(2.7%)	36(97.3%)	
Gender	Male	26(15.8%)	139(84.2%)	>0.05(0.408)
	Female	18(14.1%)	110(85.9%)	



Discussion

Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis (21).Tuberculosis is the second leading cause of death. Every year tuberculosis is effecting 10.4 million people around the world. In United States, during 1985-1992, an increase by 20% was reported in the incidence of tuberculosis to the centre for disease control cases. Since 1992 Tuberculosis, awareness became more and TB control became more manageable. (22-27)

In Southeast, including Pakistan, Tuberculosis kills more people than any other infectious disease. Pakistan is 22nd among the countries according to world health Organization. Pakistan have high rate of MDR-TB and XDR-TB strains have been found in this Southeast Asia than any other infectious disease. According to World Health Organization (WHO) reports, rapid TB control followed by adequate treatment are important in prevention of TB transmission and misdiagnosis(28)(29)(30)

In the local population where TB prevalence is high, our study has shown PCR performed better than the current routine diagnostic processes of ZN smear microscopy and LJ culture in detecting Mycobacteria tuberculosis in various specimen.

In this study, ZN showed least sensitivity (20%) of the 3 diagnostic methods concurrent with previous studies. From their research, Chakravorty et al found that the conventional smear method to have 3.9% sensitivity which was increased to 21.1% by universal sample processing technique (31) reported ZN smearing to have 50% sensitivity(32)(33). This is due to ZN sensitivity being directly influenced by the HIV status of the patient as reported by Lydia et al (34). Moreover, the sensitivity of ZN is dependent on the type and quality of the specimen (34). This is similar finding to our study as ZN mostly detected (MTB) in sputa as opposed to other specimens (p value <0.001). One of the reasons for low sensitivity is reported to be due to the fact that 104/ml is required for AFB to be seen using smear microscopy (35) (36).

In this research, LJ culture method demonstrated sensitivity of 47%. Chakravrty et al reported that conventional culture detected zero cases of MTB but universal sample processing culture method demonstrated 7.9% sensitivity (31). In the past (MTB) culture as a gold standard with estimated sensitivity and specificity rates of 96% and 81%. However, a meta-analysis carried out in 2009, states (MTB) culture has limited value in clinical diagnosis as its sensitivity specificity rates have varied significantly from study to study. (37) A previous study in Pakistan reported a sensitivity rate of (MTB) culturing to be 15%-20% on over 50,000 specimens received from different geographical areas of the country. (38) Our data revealed that PCR analysis showed 100% specificity and sensitivity. Bainomugisa et al showed PCR to have 100% sensitivity and 99% specificity (39). A Study conducted in Lusaka, using low-cost in-house one –tube nested PCR, showed 55% of sensitivity (40) (7) (20). Cheng et al reported TB PCR to have overall sensitivity of 78.3% and a specificity of 100 %(41).In our study PCR positivity rates were higher in specimens such as synovial fluid and pleural fluid as opposed to other specimens. This is a statistically significant with a p-value of < 0.005 (0.002). This may be because of larger volume of bodily fluid as opposed to that of sputa or other tissues specimens. This is similar finding to another study in Karachi, Pakistan where Amin et al reports PCR assay to demonstrate positivity rates of 70% in Bronchial Alveolar Lavage, Pleural fluid specimens (42). This is concurrent with study by Chakravorty et al where PCR efficiencies were significantly high in samples of pleural fluid (31).

In Pakistan due to insufficient facilities it is difficult to control (MTB) transmission. However routine tests like ZN smear, culturing and PCR methods are used in diagnosis of TB. ZN staining is simple and fast test but has low sensitivity and specificity. Culturing tuberculosis has greater sensitivity but is time consuming, it takes many weeks to give results. PCR facilitates prompt detection of infectious agent in various specimen types, thus is appropriate for both pulmonary and extra pulmonary tuberculosis.

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