Utility of Polymerase Chain Reaction in rapid and reliable diagnosis of tuberculosis in high endemic rural settings.

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ABSTRACT

Objective: To evaluate the efficacy of conventional Polymerase Chain Reaction using clinical specimens collected directly from suspected tuberculosis patients by targeting IS 6110 sequence gene and to compare it with conventional microscopy and culture inoculation.

Study Design: A cross sectional observational study.

Place and Duration: At Fatima Jinnah TB Hospital, Quetta from 1st Sept 2014 to 28th Feb 2015.

Methodology. In total 200 clinical samples (Pulmonary=180; extra pulmonary=20) were collected from suspected patients visiting hospital. All the samples were screened through ZN Microscopy, culture and nucleic acid based Polymerase Chain Reaction was applied directly to the clinical samples.

Results: Overall, 52.5 % samples were found positive by PCR, followed by 35.5% with Culture and 24.5 % with ZN microscopy, respectively. Furthermore, PCR also detected more extra pulmonary samples than conventional bascilloscopy. However culture detected non significantly higher number of extra pulmonary samples than PCR.

Conclusion: PCR is more reliable for the confirmation of the Mycobacterium tuberculosis complex directly from clinical samples than ZN microscopy and less time consuming than culture technique.

Keywords: Tuberculosis, Diagnosis, Endemic area, Culture, Sputum, Extrapumonary, PCR, IS 6110 gene

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INTRODUCTION

Tuberculosis (TB) is the 2nd lethal infectious disease after Human Immunodeficiency Virus (HIV). In most of the cases, Mycobacterium tuberculosis is the causative agent for TB in humans. Myco tuberculosis is a member of Mycobacterium tuberculosis complex (MTC) which comprises of different strains of this bacterium i.e. M. tuberculosis, M. africanum, M. canetti, M. ovis, M. bovis, and M. microtti[1].

Zeihl Neelsen (ZN) stain based microscopy is the mostly used test for the detection of M. tuberculosis. Although it is a quick and economical method but lacks specificity and sensitivity. This test can only detect the infection if the yield is between 5000 – 10,000 bacteria/ ml of sample[2]. Culturing is another technique for detection of M. tuberculosis. Although it is very specific but can only be performed by highly trained people and also requires prolog incubation of the organism. Early diagnosis of TB is the key for its effective treatment and control[3].

With the induction of new molecular based techniques in clinical laboratories the problems associated with ZN based microscopy and culture techniques for diagnosis have been improved. PCR can be used for the diagnosis of TB instead of microscopy even in the acute phase of the disease[4,5]. It has been reported that as few as 10 genome copies in a starting material for PCR based detection in multiplex PCR setup. It can also be performed

directly on clinical TB samples without culturing. This molecular based technique has a better sensitivity and specificity and can also be helpful in early diagnosis of the disease as compared to the ZN based microscopy and culturing techniques and thus can also lead to early treatment of the TB patients[6]. Different gene like 16S rDNA, IS6110 and hsp 65 genes of MTC are targeted for amplification using PCR[7]. However, IS6110 is the mostly used gene for epidemiological and clinical studies of tuberculosis[8]. Pakistan is the 5th most infected country with tuberculosis. It has been estimated that around 48000 TB patients die each year, while only 8 % patients receive proper treatment[9].

Balochistan is the largest province of Pakistan but, with the least population. It has the lowest per capita income and literacy rate as compared to other provinces of the country. Quetta is the provincial capital with more than 2.8 million population situated at high altitude. It shares two international borders with Afghanistan in north- East and with Iran in South. The city also harbors more than 0.3 million afghan refugees[9]. Most of the people in Balochistan live in joint family system in rural and remote areas. They have limited access to health care centers for proper treatment. Effective TB control programs have already been established by the government but the disease is still prevailing and is on the increasing trend in this province. This study was aimed to evaluate and compare traditional conventional and molecular based PCR techniques directly from clinical samples for the early diagnosis of tuberculosis.

METHODOLOGY

A total of 200 samples (pulmonary=180; extra pulmonary = 20) were collected from both male and female patients, with 20-80 years age visiting from different areas of the province.

All the samples were collected randomly from the patients with the history of prolonged cough, fever, chest pain and were referred by clinician as TB suspects. Sputum samples were collected from outdoor and indoor patients while extra pulmonary samples were collected from indoor patients only. Patients below 20 years and overage (≥ 80 Y) population were excluded from the study.

Sputum samples were collected randomly from patients in sterile container early in the morning mixed in 10 ml sample digestion NALC solution for 15 minutes. The samples were homogenized and equal quantity of Phosphate Buffer Saline (PBS), were added, and followed by centrifugation at 3500 g for 15 minutes. The supernatant was discarded and pellet was dissolved in sterile distilled water.

Lowenstein Jensen (LJ) medium (Vivantis, USA) with glycerol was prepared for culture inoculation, as proposed by the manufacturer. The slides were prepared for ZN microscopy as proposed[2]. A drop of sample was inoculated onto LJ medium followed by incubation at 37 ^{0}C for upto 8 weeks.

Similarly, for PCR reaction the Sediment was heat killed by thermolysis and DNA was collected in a separate tube. Total of 50 µl reaction volume was prepared alongwith Reverse and Forward IS 6110 primer. The reaction mixture was denatured at 98^0 C for 5 minutes followed by 35 cycles of PCR reaction as suggested[5].

Data Analysis: The data collected was subjected for statistical analysis using Pearson Chi square test in the SPSS-16 version and significant results were obtained with 1.200 E3 value while(P< 0.05) indicating high reliability of PCR over conventional techniques. Similarly, Pulmonary and Extra Pulmonary samples were also compared and analyzed using Mann-Whitney Test.

RESULTS

A total of 200 clinical samples were screened from patients for the detection of tuberculosis. Overall, 24.5% samples were found positive with centuries old traditional Zeihl Neelsen bascilloscopy followed by culture 35.5 %. However PCR detected 52.5 % samples positive for tuberculosis directly from clinical samples (Table-I). Although stating and PCR was done on same day while Culture growth of the organism was detected 6-8 weeks. Some fast growing contaminated samples were discarded and typical growth (buffy and rough) colonies of Mycobacterium tuberculosis complex were selected for culture. The ZN based detected 24.66 % and 25 % samples in pulmonary and extra pulmonary samples, respectively. Similarly Culture detected 33.3 % and 55% samples positive from pulmonary and extra pulmonary specimens. However, PCR detected 54.4 % and 35% samples, correspondingly.

Table-I: Comparison Mycobacterium tuberculosis in ZN Stain, Culture and PCR*(N=200).

Samples	No of Samples	Smear Positive n (%)	LJ Positive n (%)	PCR Positive n (%)
Pulmonary	180	44 (24.44 %)	60 (33.33%)	98 (54.4%)
Extra Pulmonary	20	5 (25 %)	11 (55%)	7 (35%)

PCR* = Polymerase Chain Reaction; LJ = Lowenstein Jensen.

DISCUSSION

Tuberculosis still remains a catastrophic global threat to the world population of low income countries. In the present study, 200 clinical samples were screened detection of Mycobacterium tuberculosis.

Out of which 24.5%, samples were found positive through smear bascilloscopy, 35.5 % (Culture) and 52.5% (PCR), respectively. Our these results corroborate results to some other studies in which they have also recognized and validated PCR as more sensitive than old traditional techniques[5,10-12]. Different advanced techniques can be used for early diagnosis of tuberculosis even in MDR resistant cases using Cepheid Gen Expert and Line Probe assay (LPA). These techniques have promising results with more specificity and higher sensitivity and detects in short time[13].

Similarly, PCR was found equally suitable for detection of pulmonary and extra pulmonary samples for TB diagnosis. Polymerase Chain Reaction detected relatively higher 54.4% and 35 % pulmonary and extra pulmonary samples than ZN smear 24.4 % and 25%, However culture detected 33.3 % pulmonary and relatively higher 55% positive extra pulmonary samples.

These findings are in line with some earlier studies that PCR has promising results in detection of the IS 6110 genes in pulmonary and extra pulmonary samples[12,14,15]. Similarly, in another study from Hyderabad Pakistan, PCR was also recognized as more sensitive and fast technique for the early diagnosis of tuberculosis[16]. Molecular based diagnosis using conventional PCR has strategic potential in early diagnosis of tuberculosis. It could be the best possible alternative with higher accuracy as compare to smear microscopy and time consuming culture method.

In our study culture was found with more positive cases than PCR in extra pulmonary samples. It may possibly be due to the presence of PCR inhibitors in direct clinical samples as used in this study. These results are similar to the findings of Iqbal et al., who also indicated the presence of PCR inhibitors in direct detection of clinical samples[2]. Human errors also play important role in the performance of smear microscopy and cause differences in term of sensitivity as compare to culture technique. Similarly Sinha et al. (2017) and Kyaw et al. (2018) also declared PCR equally suitable for the detection of Mycobacterium tuberculosis in different extra pulmonary samples[17,18].

Balochistan has scattered population with low socioeconomical status and living standards. Most of the populations live in remote mountainous rural areas with least access to health care centers. This could be the best cost effective reliable diagnostic test especially in high endemic areas. Although ZN microscopy is time consuming and with very nominal charges. The culture is again very time consuming for weeks and patients can't wait for their results. However, PCR is the ultimate choice for clinician to replace the centuries old bascilloscopy and culture techniques.

CONCLUSION

PCR is more reliable for the confirmation of the Mycobacterium tuberculosis complex directly from clinical samples than ZN microscopy and less time consuming than culture technique.

CONTRIBUTION OF AUTHORS

Shafee M: Conceived idea, Designed research methodology, Data analysis
Naeem M: Statistical analysis, Final review of manuscript
Mandokheil K: Manuscript writing, Data interpretation
Umar M: Data collection and compilation
Rehman FU: Data Interpretation, Final approval of manuscript
Khan N: Manuscript writing

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