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DIAGNOSIS OF MYCOBACTERIUM TUBERCULOSIS BY USING PCR AND COMPARISON IT WITH ZIEHL NEELSEN STAINING AND MANTOUX TEST

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ABSTRACT

Nucleic acid amplification and detection methods developed in the past decade are useful for the diagnosis and management of a variety of infectious diseases. The most widely used of these methods is the polymerase chain reaction (PCR). Commercial PCR assays for the diagnosis of tuberculosis are now routinely used in many diagnostic laboratories. 126 sputum samples were collected from 126 subjects who went through tuberculin skin test. The samples were processed for detection of Mycobacterium tuberculosis by ZN staining and PCR. The results showed that the rate of positivity with PCR was 105/126 (83.33%), while it was 75/126 (59.5%) with microscopy, and the TST showed very low percentage of positivity in comparison to other tests performed for detection of tuberculosis i.e. 30/126 (23.8%). The results of the present study provided the similar evidence about the importance of PCR in detection of Mycobacterium tuberculosis. This also suggests that the use PCR can be significant in the early diagnosis of pulmonary and extra pulmonary tuberculosis.

KEY WORDS

M.tuberculosis, PCR, TST Test, ZN Staining and Sputum.

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INTRODUCTION

Tuberculosis is one of the major air borne pathogen and a major threat to the people of developing countries. It has been estimated 1.6 to 2.4 million people are dying globally because of this infection, and the problem became more devastating with the emergence of drug resistant mycobacterium tuberculosis bacterium[1]. So the question rises can early diagnosis of this bacterium can solve this

problem? Yes early diagnosis can play a pivotal role in stopping the transmission of this bacterium, and it can be helpful in starting the treatment of the patients at the early stage of the infection.

In many of the developing countries still microscopy is in use for the diagnosis of this bacterium even it is having its own shortcoming like low sensitivity and specificity. Culturing of this bacterium which is a gold standard method of diagnosis is also one of the recommended techniques for diagnosis. But culturing requires a well standardized lab, well experienced lab personnel to identify the specific micro-organism from a sample, nevertheless lot of patience for getting the results because its takes weeks to get the culture of this bacterium.

Timely detection of *mycobacterium tuberculosis* is important for starting antitubercular treatment, management of patients, prophylaxis etc. The patient's outcome could be improved with the development of fast, simple, specific and sensitive technique.

During the last decade several major advances have been made in studying the genetic structure of mycobacteria. Based on this knowledge about newer gene sequences, several gene amplification systems for tuberculosis have been developed.

Diagnosis of TB by PCR is one the technique which is now routinely used in many laboratories, because of its high specificity, sensitivity, and cost effective nature. PCR under optimum conditions are expected to detect 1-10 organisms. If care of contamination have been taken, these PCR plays are very helpful in detection of *Mycobacterium tuberculosis* bacterium in many paucibacillary samples and can be proved very useful in early diagnosis[2-11].

MATERIALS AND METHODS

Sputum samples were obtained from 126 subjects who have gone through Tuberculin Skin Test (TST) through random sampling. The status of the subjects was confirmed with their strong clinical and radiological evidence including clinical response to antitubercular treatment referred from different hospitals of Hyderabad like Owaisi Hospital, Esra Hospital and DOT Centers. This criterion of selection of subjects is considered as a gold standard for classifying cases of TB. All the necessary clinical details were obtained from the referring hospital in the prescribed format. The sputum specimens collected, were examined by using conventional ZN staining and PCR technique. Extraction of mycobacterial DNA from sputum samples was done by using modified C-TAB (Cetyl Trimethyl Ammonium Bromide) method. Primers used in the study showed in Table No.1.

PCR Conditions

The reaction cycles are as follows: The temperature profile consisted of an initial denaturation step at 94°C for 5 min, followed by 40 cycles at 94°C for 90 s (denaturation), 62°C for 120 s (annealing), and 72°C for 180 s (extension). And Final Extension of 10 min, five micro liters of the reaction mixture was further analyzed by agarose gel electrophoresis according to standard protocols.

RESULTS

A total of 126 subjects cases were evaluated of age group 20-60. All 126 cases were probable of TB infection. Sputum was collected as a specimen of diagnosing Tuberculosis and TST was performed on all the 126 subjects. Of the total 126 subjects 30 were positive for TST, 75 for Microscopy and with PCR it was 105, i.e. 24%, 59.5% and 83.3% respectively. No other specimens were collected for the detection of this bacterium in this study. The comparison of three tests used in the present study is shown in the Table No.2.

DISCUSSION

Rapid detection of *Mycobacterium tuberculosis* complex is important in patient management in terms of initiating appropriate antimycobacterial therapy as well as controlling the spread of this pathogen. *Mycobacterium tuberculosis* is a major threat to the health in third world countries. Tuberculosis management is always been a great task because of slow detection rate of *Mycobacterium* by culture which is a gold standard method of diagnosis. Other techniques are available like Microscopy and Tuberculin skin test, but their case detection is very

less, in other words they are less specific and sensitive. The other alternate method for early detection of this bacterium is the nucleic acid amplification. The use of PCR has proved its importance because of its high sensitivity and specificity, affordability and its time consuming quality.

In the present study we used sputum specimens, and we found with the use of PCR in less than a day we can find the results of tuberculosis infection, while it will be 48-72 hrs, with TST and with microscopy we can find the results early but its limitations make it less effective in a countries where there is high burden of this infection.

The case detection of mycobacterium with PCR has shown to be a sensitive as much (88% to 100%) and specific (>90%) for pulmonary TB in adults, while with AFB microscopy it was only 60% of all tuberculosis cases[6, 7]. In our study we found, the case detection rate of tuberculosis is more accurate and specific with PCR, where 83% of the cases have been detected, while other diagnostic test which we have used has shown low sensitivity and specificity as shown by others, but the results of our study are still inconclusive because of the absence of Mycobacterial culture test, which is still the gold standard method of diagnosis, and the limited number of samples.

Table No.1: Primers used in the study

S.No	Primers	Product Size
1	INS1 (5′-CGTGAGGGCATCGAGGTGGC-3′) INS2 (5′-GCGTAGGCGTCGGTGACAAA-3′)	121 bp

Table No.2: Sensitivity of different tests conducted from suspected cases of tuberculosis

S.No	Tests Performed	No of Samples Tested	Results		Sensitivity %
			Negative	Positive	
1	Mantoux Test	126	96	30	24%
2	ZN Staining	126	51	75	59.5%
3	PCR	126	21	105	83.33%

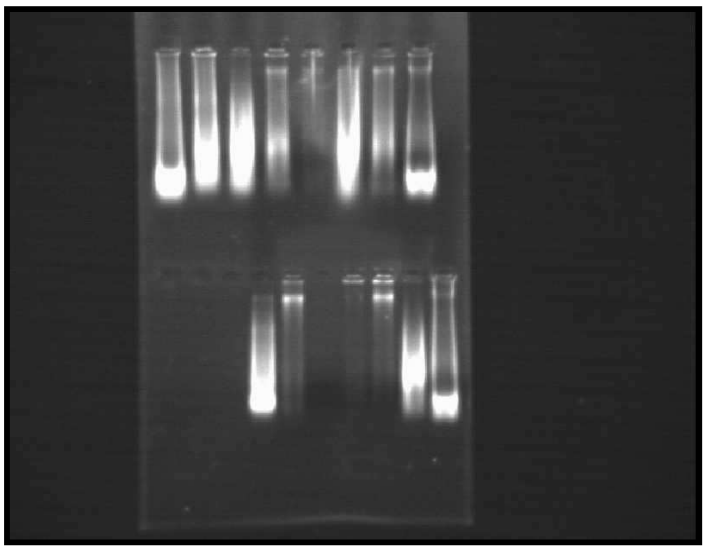

Figure No.1: Genomic DNA isolated from Sputum Samples of *Tuberculosis* Patients

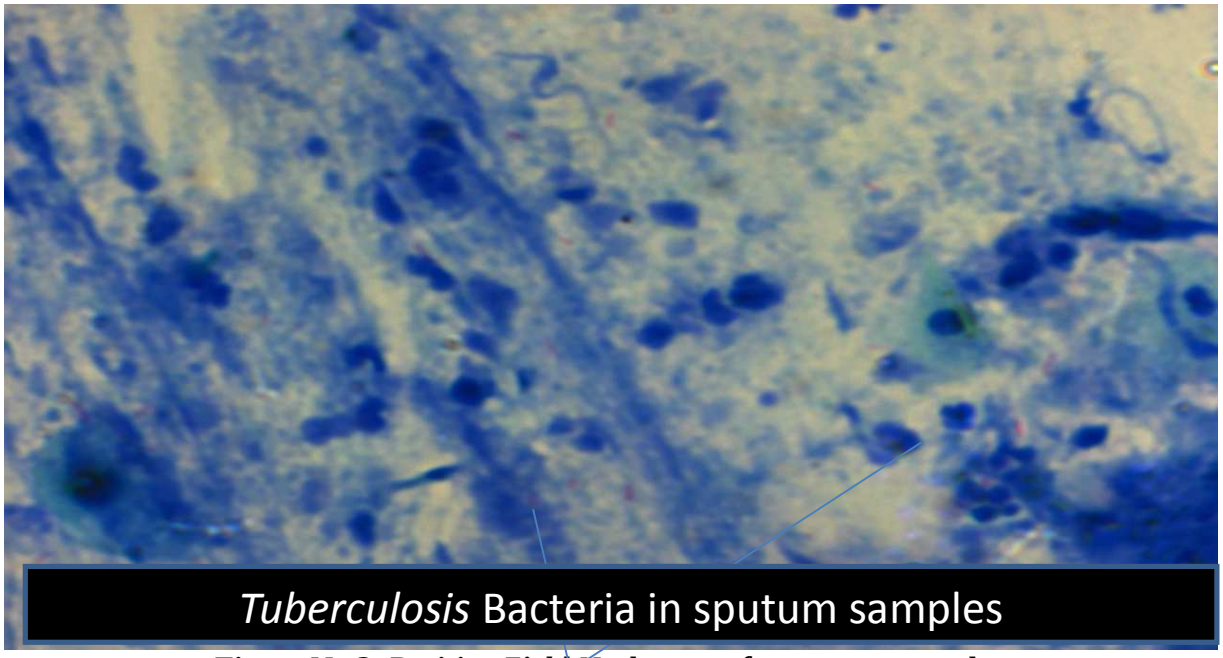

Figure No.2: Positive *Ziehl Neelsen* test for sputum samples

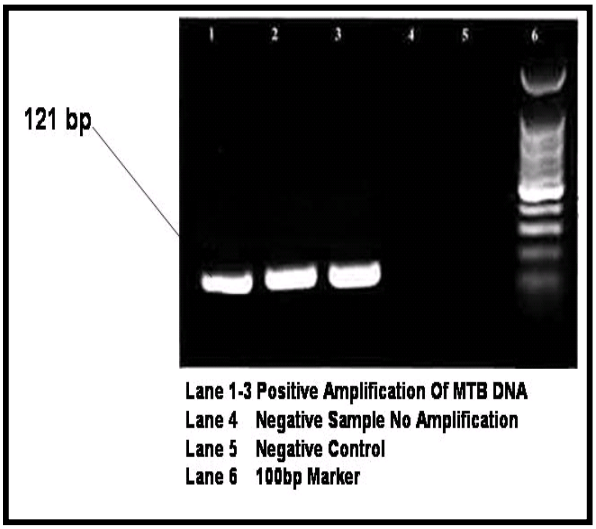

Figure No.3: Positive Amplification of MTB DNA using INS1 and INS2 Primers

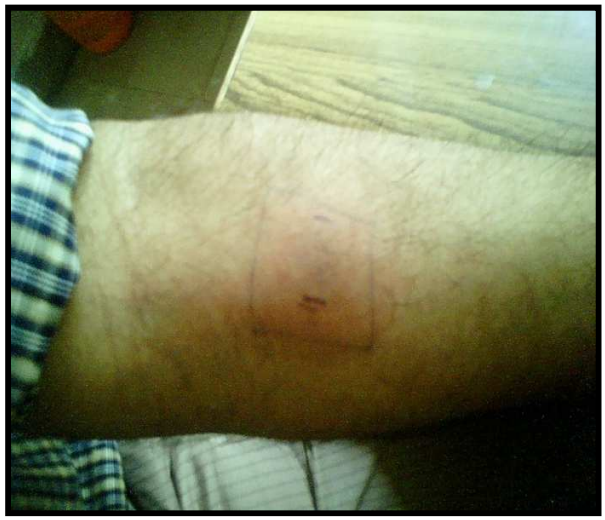

Figure No.4: Positive Mantoux test with 20mm in duration

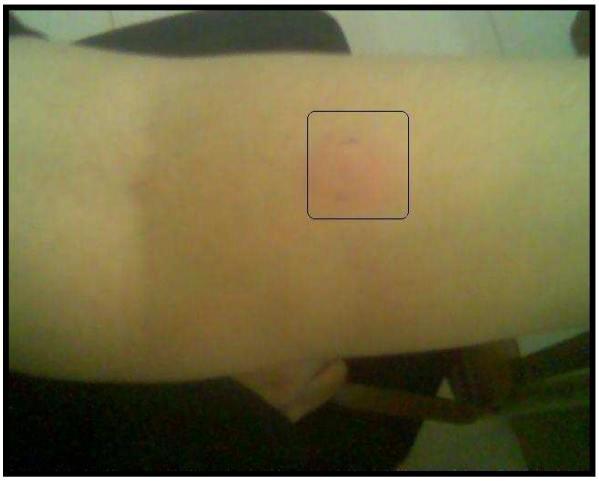

Figure No.5: Positive Mantoux test with 15mm in duration

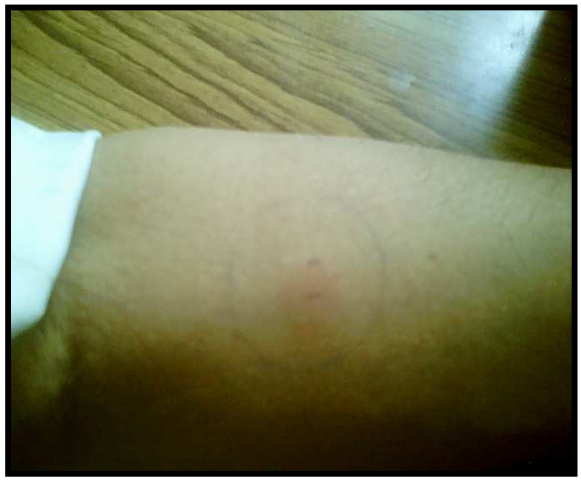

Figure No.6: Negative Mantoux test with 5mm in duration

CONCLUSION

In conclusion, we can advocate the use of PCR which can be a reliable method, and will have a great potential role in helping clinicians for early detection of tuberculosis. This early detection will ensure early treatment to control this infection from transmission. However, further more work is required to prove the sensitivity, specificity and affordability of PCR with more number of samples, and comparing it with other diagnostic tests available.

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