The Utility of Polymerase Chain Reaction in the Diagnosis of Pulmonary Tuberculosis in Smear-Negative, Culture-Negative Patients

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Objective: This study was conducted to assess the use of polymerase chain reaction (PCR) amplification in diagnosing pulmonary tuberculosis (PTB) in smear-negative, culture negative (SNCN) patients.

Method: Fourteen patients with suspected PTB and SNCN status were identified. All of them underwent bronchoscopy to obtain bronchoalveolar lavage (BAL). All BAL’s were examined with auramine stain for acid fast bacilli (AFB), mycobacterium culture and PCR to detect mycobacterium tuberculosis (MTB). All patients were prospectively followed-up to assess response to therapy and final outcome.

Result: Seven patients had positive results with PCR. Follow-up showed that in six of these patients PTB was the most likely diagnosis while one (14.3%) out of seven was considered as false-positive. The remaining seven patients had negative results with PCR. These were considered as true-negative as their clinical course did not support the diagnosis of PTB.

Conclusion: PCR test in BAL to detect MTB is a useful test in suspected cases of PTB with SNCN results. However, the final interpretation of this test should always be subjected to the clinical course of the patient because of the recorded rate of false-positive results in such group.


Tuberculosis (TB) was predicted to be obsolete by the year 2000, but now is on the increase around the world. World Health Organization (WHO) estimates show that in the next decade 300 million will become infected with TB, 90 million people will develop the disease and 30 million will die from it. TB currently kills more adults each year than AIDS, malaria and tropical diseases combined.

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One of the problems with PTB is the relatively high rate of SNCN cases. Sputum specimen requires high number of mycobacteria (50,000-100,000/ml) for a positive result2. Culture in the other hand is associated with problems primarily because mycobacterial grow slowly and negative culture in strongly suspected cases continued to exist3.

Recently, however, methods have been described which permit the amplification of specific DNA sequences, which can be used to increase even a single copy of a given DNA sequence by 10^12 fold4. In these new methods, based on polymerase chain reaction (PCR) technology, target sequences of MTB are specifically amplified and easily detect either directly, on agarose gels, or using hybridization to non-radioactive probes5. This newly developed technology is thought to be able to detect mycobacteria that cannot be cultured and it can
overcome the potential difficulties of the conventional microscopic testing of sputum smears.

Several primer pairs have been used for PCR amplification of specific conserved target sequences in MTB DNA for better sensitivity and specificity. One of the most frequently used primers amplifies 123 bp fragments in the insertion sequence IS6110. This insertion sequence is highly conserved and consequently specific for MTB complex, and is repeated several times in the chromosomes. The amplification product, 123 bp DNA fragment is detected using 3% agarose gel electrophoresis.

Several studies have reported using methods based on PCR amplification to detect MTB. However, as pointed out recently by a US public Health Service report, the usefulness of the PCR approach to diagnose MTB is still unsettled.

We conducted this study to assess the use of this technique in diagnosing MTB in Asir province of Saudi Arabia in cases with SNCN results.

METHOD

a. Patients and clinical samples

A total of 14 patients with suspected PTB and SNCN status were identified. All patients underwent fiberoptic bronchoscopy to obtain bronchoalveolar lavage (BAL). All BAL specimens were evaluated with auramine stain, for AFB mycobacterial culture and PCR. PCR amplification was performed by the second author who did not know about the results of auramine stain and cultures or the clinical presentation of the patient.

All patients were followed-up clinically and radiologically to assess response to therapy.

b. PCR technology

MTB was detected and identified in BAL using PCR-TB kits manufactured by Sino-American Biotechnology Co. (SABC), Henan, and PR China. The cell pellets were collected from samples by centrifugation at 14,000 rpm for 5 min. as recommended by the manufacturer.

The cell pellets were washed twice with phosphate buffered saline (PBS), pH 7.2 and centrifuged, as before, to collect cells. The cell pellets were lysed by inoculation with lyses buffer for 2-4 hrs at 550C then centrifuged for few seconds and heated at 950C for 10 min.

PCR amplifications were done according to the manufacturer's recommendations: 10 ml of cell lysate were mixed with 41 ml of PCR amplification mix (containing Tag polymerase) in sterile 0.5 ml micro centrifuge tube and 50 ml paraffin oil added. The amplifications were done using Perkin Elmer Thermal Cycles 480 according to the following thermal profile parameters:

- 940C for 5 min. pre-denaturation
- 940C for 1 min. Denaturation
- 680C for 2 min. Primers' A realign
- 720C for 1 min. Primer Extension

35 cycles

The products of PCR amplifications were electrophoresed on 3% agarose gels, stained with ethidium bromide and photographed. The positive results were identified by the presence of 125 bp DNA fragments on the stained agarose gels.
RESULT

Seven patients were PCR positive for MTB. Follow-up showed that in six of these patients PTB was the most likely diagnosis. They responded only to antituberculous (ATB) medication with good recovery clinically and radio logically. One patient with SNCN results had positive PCR. This patient had an infiltrate in the superior segment of the left lower lobe with persistent fever and cough. He eventually responded to broad spectrum antibiotics with disappearance of fever and cough with good resolution of the left sided infiltrate. He did not require ATB medications. In this case the PCR test was considered as false-positive.

DISCUSSION

This is the first study in Saudi Arabia which analyzes the utilization of PCR in the diagnosis of PTB. In this study we limited the analysis to SNCN cases, because this is the situation where a more useful diagnostic test is definitely needed. At the same time SNCN cases continue to be a problem in the daily care of TB clinics. Our study showed that SNCN cases were 14 out of 19 consecutive patients with clinical suspension of PTB (74%) This is a higher rate than the rates reported in the literature. This is most likely related to the fact that our study was a hospital-based study in a tertiary care centre where more difficult situations are usually met.

In this setting we found PCR test a very useful test in clinically-suspected PTB. It showed true-positive result in 6 out of 7 suspected cases with SNCN results (86%). Querol JM, et al reported that PCR detected MTB genome in 102 (97%) of 105 patients diagnosed clinically as having PTB. This higher rate of true-positive result in their published study could be related to the fact that this study included all patients with PTB and not only the SNCN cases. Overall, there is agreement in the literature that the availability of this new diagnostic tool can add more strength to the clinical diagnosis. But the rates of false negative, false positive and true negatives results vary significantly between different studies.

In our study we found only one false-positive result out of 14 consecutive patients with clinical suspicion of PTB and SNCN results (7%). In the published studies about PCR in suspected cases of TB, the false positive results continued to exist and it ranged between 0.8-10%. The possible explanation for the false-positive result is the crossover contamination between specimens and the products from previous amplifications of the same target sequences. This form of contamination can be avoided by using careful techniques and observing stringent quality control practices.

Our study showed no evidence of false-negative results. In another study false negative results existed but in a very low rate (3 out of 105 patients) REF. This fact makes the diagnosis of PTB most unlikely in the presence of a negative PCR test. It is clear that total dependence on PCR test cannot be recommended at this stage. This is because false negatives and false positive results continued to exist. The clinical background and the follow up should be the ones that make the final decision.

CONCLUSION

The study indicates that PCR test is a very useful tool to support the clinical diagnosis of PTB in SNCN cases. The finding of a negative PCR test in SNCN patients makes PTB most unlikely. But a positive result with PCR may strengthen and hasten the clinical diagnosis in SNCN patients. Different studies reported different rates of false negative, false positive and true negative results. For this reason studies with larger number of patients are needed in this field.
REFERENCES