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Determining the Diagnostic Value of Mycobacterium Tuberculosis DNA in the Differentiation of Blood Samples of Patients with Active Pulmonary Tuberculosis and Healthy Controls Using Polymerase Chain Reaction

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Abstract

***Background*:** Tuberculosis (TB) is now a major cause of mortality and morbidity in the world. Nowadays, different methods are used to diagnose tuberculosis. Although classical microbiological methods (such as sputum smear) are specific, they have little sensitivity and the culture is also time-consuming. Using Polymerase Chain Reaction (PCR) in blood samples in terms of *Mycobacterium tuberculosis* DNA, this study examines diagnostic power of this test in the diagnosis of pulmonary tuberculosis compared with other standard methods.

***Materials and Methods*:** In a cross-sectional descriptive-analytic study, blood samples were taken from 40 TB patients and 40 non-TB cases. Following DNA extraction by the commercial kit QIAGEN, the PCR assay was performed using IS6110 primer.

***Results*:** In this study, there were 80 people in two groups of TB and non-TB cases. Each group composed of 14 men (35%) and 26 women (65%). Sensitivity, specificity as well as positive and negative predictive values obtained 37.5, 100, 100 and 61.5%, respectively.

***Conclusion*:** Despite high costs of using PCR for TB diagnosis, sensitivity of this method is low due to various factors and cannot replace current standard methods for TB diagnosis such as smear and culture. It can only be used as a complementary method to confirm diagnosis in strongly suspected cases of tuberculosis.

Introduction

Tuberculosis (TB) is one of the oldest known human diseases. More than 50% of TB patients will die after five years, if untreated [1]. In 1993, the World Health Organization (WHO) called it a global health emergency [2]. 90% of TB cases are based in developing countries [1]. According to the WHO in June 2005, the prevalence of TB is 37 per 100,000 in Iran and its mortality rate is 3.3 per 100,000 people annually. In Iran, according to the information of Office of Disease Prevention and Combating, the average incidence rate is 37 cases per 100,000 people [3].

Key factors in the control of tuberculosis are rapid diagnosis, appropriate treatment and contact tracing to prevent further transmission of the disease [4]. 95% of disease cases and 28% of deathes from tuberculosis occur in developing countries, 75% of which is in the economically active age group, i.e. 15 to 25 years old [5]. Sistan and Baluchestan province is a region with high prevalence of TB in the country. In a study during 1998 to 2002, 2729 patients with pulmonary TB were identified in Zabol. The prevalence of smear-positive TB in the population of this province is 76 per 100,000 people [6]. Conventional diagnostic methods for tuberculosis are direct sputum smear test (for this purpose, at least three sputum samples are collected from TB suspects within two to three days and are tested microscopically) [7, 8], TB lab culture (confirmation by culture takes several weeks and its acid-fast staining (Ziehl-Neelsen) lacks specificity and sensitivity) [7, 8], chest radiography, skin tuberculin test result (it has very little importance and lacks specificity) [8, 9] and blood test (e.g. T-SPOT). The basis for the diagnosis of pulmonary tuberculosis (PTB) is microscopy test results of sputum for suspected patients [8, 9]. Classical microbiological methods for PTB diagnosis lack sensitivity and specificity [10].

New and conventional medical technologies for the diagnosis of TB and tuberculosis antibodies are methachromatic test, polymerase chain reaction, chromatographic mass spectrometry and enzyme safety test. Molecular biology and safety reviews may be used only for early detection and screening, and following a thorough physiological study as TB markers and tuberculosis antibodies [10, 11]. PCR is a technique used for determining the sequence of a special DNA genetic fragment by which the presence of an extremely small number of bacteria is specified. Although sensitivity and specificity are high in patients with smear negative and culture positive, it becomes positive in 70% of those

exposed to tuberculosis and not suffered from active disease so it makes disorder in TB diagnosis as a false positive test [12]. Given that tuberculosis bacillus is put in circulatory at the time of the disease activity, determining *Mycobacterium tuberculosis* DNA by PCR can help in early detection in easy-to-take samples, such as blood and urine.

Materials and Methods

This study is of descriptive-analytic type. The studied population composed of 80 patients: 40 cases with smear positive pulmonary TB and 40 cases (the control group) without TB entered into the study after completing informed consent form. The sample size in each group was calculated 20 according to statistical formulas. Due to the availability of patients, 40 cases for each group entered into the study.

The groups were matched in terms of sex and geographic area. Although tuberculosis is higher in men, we decided that in this study, gender distribution in the control group is based on the experimental group so that there were 14 men and 26 women in each group. Since tuberculosis is more common in people living in this province due to specific boundary and geographic conditions, non-native or immigrants from neighboring provinces were excluded. Moreover, given the influence of age and immune conditions, the studied subjects were selected from over-30 age group. Inclusion criteria were as follows: people with confirmed TB disease who had smear and culture positive fell in the first group and healthy people with no clinical and laboratory evidence of TB fell in the second group. Exclusion criteria were considered as persons hospitalized with suspected tuberculosis but without smear and culture positive or persons who did not consent to continue collaboration in the research. PCR was performed on blood samples from TB and non-TB groups.

Descriptive statistics were used to determine mean, standard deviation, and frequency of percentage. Necessary statistical methods were used to determine sensitivity, specificity, positive and negative predictive value and the *t*-test was used to determine the presence or absence of differences in Mycobacterium PCR in blood samples from TB and non TB groups. The implementation method was as follows: to prepare serum samples, 2 ml blood samples were taken from suspected patients, and samples were transferred to clot tubes. Then, the patients serums were separated by centrifugation and poured into 1.5 ml microtubes and kept in the freezer until tested.

The serum samples were prepared for DNA extraction. Extraction of DNA was done using Qia gene kit. To check the purity of DNA, we mixed 1 µLit of DNA with 5 µLit of loading buffer and loaded on the gel. Next, we examined the purity of DNA through the resulting band. To perform primer, IS6110 designated by Takapoo Zist Co. was purchased. Other necessary materials were purchased from Neday-e-Fan and Takapoo Zist companies. To perform the PCR test for primers, we added polymerase DNA and template DNA to the reaction mixture in a 0.2 ml microtube. Next, we gave the PCR specific application to the thermocycler device and we entered the microtube into the device to perform the PCR test. Once PCR was performed, the resulting product was stored at 4°C until electrophoresis. We prepared the 1.5% gel and loaded a certain amount of PCR product on the gel. Once electrophoresis completed, we checked the presence or absence of gel with the Gel Doc. For data analysis, descriptive statistics were used to determine mean, standard deviation, and frequency of percentage; and statistical methods were used to determine sensitivity, specificity, positive and negative predictive value and LR. SPSS-16 statistical software was used for data analysis. The significance level of results was considered $p \leq 0.05$. The sampling method and the work procedure were approved by the ethics committee of Zahedan University of Medical Sciences.

Results

In this study, there were 80 people in two TB and non-TB groups. The mean age of TB group was 59.7±18.2 years and that of non-TB group was 56.2±16.7. TB and non-TB groups were gender-matched so that each group contained 14 men (35%) and 26 women (65%). Attempts were also made to geographically match TB and non-TB groups. Therefore, in the TB group, 29 cases (72.5%) were Iranian and 11 cases were Afghani; in the control group, 27 cases (67.5%) were Iranian and 13 cases (32.5%) were Afghani.

The sensitivity of *Mycobacterium tuberculosis* DNA in the diagnosis of pulmonary tuberculosis using PCR obtained 37.5% in this study. Given that PCR of all the patients in the control group was reported negative, the specificity of *Mycobacterium tuberculosis* DNA in the detection of pulmonary tuberculosis using PCR obtained 100% in this study. The positive predictive value for *Mycobacterium tuberculosis* DNA in the diagnosis of pulmonary tuberculosis using PCR was 100% in this study using table 1.

Table 1. PCR results in patient and non-patient subjects

Disease / PCR	-	+
15	0	+
25	40	-

The negative predictive value for *Mycobacterium tuberculosis* DNA in the diagnosis of pulmonary tuberculosis using PCR was obtained 61.5% in this study. The positive and negative likelihood ratio value of *Mycobacterium tuberculosis* DNA in the diagnosis of pulmonary tuberculosis using PCR obtained 24.2% in this study and p=0.01

Discussion

Based on this study, the sensitivity of peripheral blood PCR test in the diagnosis of pulmonary tuberculosis

obtained 37.5%. Although the obtained sensitivity is low, it can be very helpful due to its 100% specificity in suspected cases.

Studies conducted to date in this field are very heterogeneous in terms of the number and type of disease (either pulmonary or extrapulmonary, either smear-positive or smear-negative and with or without immunocompromised, especially HIV/AIDS), which make coming to a conclusion difficult. The only study that used IS6110 Primer was a study by Ahmed et al. They excluded HIV and AIDS patients and performed it only on 16 patients with pulmonary tuberculosis and reported the positive result of 43.57% [13]. In this study, 15 (37.5%) out of 40 cases of tuberculosis, had positive PCR and 25 cases (62.5%) had negative PCR. It can be said that it is consistent with our results [14]. In another study, Abdulbaqi examined 69 pulmonary TB cases and 26 extrapulmonary TB cases in which 32 cases (33.7%) were PCR-positive and 63 cases (66.3%) were PCR-negative. Moreover, PCR sensitivity in peripheral blood mononuclear cells was 44.1% for pulmonary TB, 19.2% for extrapulmonary TB and 10% for disseminated TB. The overall result was 33.7%, which is consistent with our results. It is also consistent with the study by Taci in which 16 out of 40 patients (40%) were positive and 24 out of 40 patients (60%) were negative. None of the controls had positive PCR. Sensitivity, specificity and overall accuracy of the PCR method were respectively 40, 100 and 60% [15].

Furthermore, the sensitivity of the PCR method obtained in our study (37.5%) is consistent with a study by Honore on 90 hospitalized patients and 50 healthy controls. In that study, 23 patients received the diagnosis of tuberculosis; culture was positive in 20 patients with TB, and smear was positive in seven patients in terms of acid-fast bacilli. Sensitivity of smear, culture and nested PCR were 30.4, 87 and 30.4, respectively. Specificity of smear and culture was both 100%. Sensitivity of nested PCR in patients with pulmonary TB was low, but that of patients with pulmonary-extrapulmonary TB and extrapulmonary TB and disseminated TB reached 50, 33 and 33%, respectively. In a study by Condos, IS6110 primer was used; 26 patients of those with negative sputum smears were detected by the PCR method. In five patients who received the diagnosis of TB by the PCR method, the final clinical diagnosis did not prove TB, and 2 out of 44 cases with PCR-negative results were ultimately false negative [23]. But in this study, all smear-negative cases were PCR-negative. Based on the results obtained from this project and the obtained sensitivity (37.5%), it seems that the peripheral blood PCR sensitivity is low for the diagnosis of tuberculosis, so it is not considered reliable as an alternative method for the detection of *Mycobacterium tuberculosis* in cases where there is a serious suspicion of the disease.

The quality of extracted DNA, inhibiting products of PCR, selecting the target sequence and the skill of the operator performing PCR are the main elements affecting the sensitivity of genomic amplifier. Given its high cost, until this method is more standardized and a method is found to increase its sensitivity and specificity, it is better to use acid-fast staining of clinical samples for rapid diagnosis and to use culture as the gold standard test in suspected cases, and this method is only used as an additional and complementary method for diagnosis or confirmation of diagnosis in critically ill patients suspected to tuberculosis, particularly in types of extrapulmonary TB that it is difficult or impossible to obtain samples for diagnosis. It is suggested to re-examine peripheral blood PCR in a specific clinical situation, such as disseminated, miliary and cavitary TB to be used in critical, suspected cases, if useful.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Longo DL, Fauci AS, Kasper DL, editors. Harrison's principles of internal medicine. 18th ed. New York: McGraw-Hill; 2011: 1340-59.
2. Hass DW, Mandell GL, Bennett JE, editors. Principles and practice of infectious diseases. 6th ed. New York: Churchill Livingstone; 2005: 2852-86.
3. WHO. Global tuberculosis control. Report 2005. Available from: http: //www.who.int/ globalatlas/ predefinedReports/.
4. Hajiabdolbaghi M, Allishah HA, Rasoolinejad M, et al. Peripheral blood mononuclear cell mycobacterium tuberculosis PCR sensitivity in diagnosis of tuberculosis. Tehran Univ Med J 2008; 65(11): 6-12.
5. Mirhaghani L, Nasehi N. Managing and monitoring tuberculosis. In: Salek A, Mohraz M, Siadati A, editors. National tuberculosis manual. Tehran: Ceneter for Disease Control, Ministry of Health; 2002: 104-113.
6. Kha1zaei HA, Rezaee N, bagheri GR, et al. Epidemiogy of tuberclusis in the southeastern Iran. Iran J Epidemiol 2005; 20(10): 879-83.
7. Green C, Huggett JF, Talbot E, et al. Rapid diagnosis of tuberculosis through the detection of mycobacterial DNA in urine by nucleic acid amplification methods. Lancet Infect Dis 2009; 9(8): 505-11.
8. Singh SK, Bhargava R. Urine as an adjunct specimen for the diagnosis of active pulmonary tuberculosis. Int J Infect Dis 2010; 14 Suppl 3: e345.
9. Committee on the elimination of tuberculosis in the United States, division of health promotion and disease prevention.

Ending neglect: The elimination of tuberculosis in the United States. 1st ed. Washington DC: National Academies Press; 2000: 154-163.
10. Torrea G, Van de Perre P, Ouedraogo M, et al. PCR-based detection of the Mycobacterium tuberculosis complex in urine of HIV-infected and uninfected pulmonary and extrapulmonary tuberculosis patients in Burkina Faso. J Med Microbiol 2005; 54(Pt 1): 39-44.
11. Kamyshan IS. [Current methods in diagnosis of the kidney tuberculosis] Russian [Abstract]. Urologiia 2006; (4): 57-61.
12. Singh S, Saluja TP, Kaur M and Khilnani GC. Comparative evaluation of FASTPlaque assay with PCR and other conventional in vitro diagnostic methods for the early detection of pulmonary tuberculosis. J Clin Lab Anal 2008; 22(5): 367-74.
13. Ahmed N, Mohanty AK, Mukhopadhyay U, et al. PCR-based rapid detection of Mycobacterium tuberculosis in blood from immunocompetent patients with pulmonary tuberculosis. J Clin Microbiol 1998; 36(10): 3094-5.
14. Taci N, Yurdakul AS, Ceyhan I, et al. Detection of mycobacterium tuberculosis DNA from peripheral blood in patients with HIV-seronegative and new cases of smearpositive pulmonary tuberculosis by polymerase chain reaction. Respir Med 2003; 97(6): 676-81.
15. Tan Y, Li Y, Zhang Y. [Quantitative detection of mycobacterium tuberculosis DNA in peripheral blood from patients with pulmonary tuberculosis by ampliSensor-PCR technique] Chinese [Abstract]. Zhonghua Jie He He Hu Xi Za Zhi 1999; 22(8): 481-3.
16. Schluger NW, Condos R, Lewis S and Rom WN. Amplification of DNA of mycobacterium tuberculosis from peripheral blood of patients with pulmonary tuberculosis. Lancet 1994; 344(8917): 232-3.

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