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Sensitivity and Specificity of TaqMan Real Time PCR, PCR, Microscopy and Culture in Diagnosis of Tuberculous Meningitis in a High Incidence of Tuberculosis Province in Southeast of Iran

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Background: The most dangerous form of extra-pulmonary tuberculosis is tuberculous meningitis (TBM). Diagnosis of TBM has special problem due to its paucibacillary. Also, sensitivity and specificity of routine microscopy and culture in the diagnosis of this disease is controversial. So, faster and more accurate laboratory test is required. Polymerase chain reaction (PCR) and real time PCR may be good candidates for this purpose.

Objectives: We did this study to compare sensitivity and specificity of TaqMan real time PCR, PCR, microscopy and culture in diagnosis of TBM.

Patients and Methods: We had 49 patients with primary diagnosis of TBM during January 2007 and January 2008 in Bou-Ali University Hospital, Southeast of Iran. Combining and using the definite and probable TB as a gold standard, 29 of these patients had a final diagnosis of TBM. The extracted DNA of samples was applied for conventional PCR and TaqMan real time PCR.

Results: Our study showed that the sensitivity and specificity of TaqMan real time PCR was 96% and 95% respectively. These values were 89% and 90, 38% and 100%, 6% and 100% for PCR, culture and microscopy, respectively.

Conclusions: Our study showed that sensitivity of TaqMan real time PCR was higher that PCR, culture and microscopy but specificity of culture and microscopy was more than PCR and even TaqMan real time PCR.

Keywords: Sensitivity; Specificity; Real-Time Polymerase Chain Reaction; Polymerase Chain Reaction

1. Background

Tuberculosis is one of the oldest known diseases of human society (1). Its incidence in developing countries is 160/100000 (2). Annual incidence of tuberculosis in 2011 in Iran was 19/100000. The incidence was even higher in Sistan & Baluchestan (40-135/100000), the biggest and most contaminated province in southeast of Iran (3). Previous studies have shown that prevalence of extra pulmonary tuberculosis and the most dangerous form of it, tuberculous meningitis (TBM) differs in different countries (4, 5). On the other hand, diagnosis of TBM has special difficulties which arise from low sensitivity in microscopy and culture (6, 7) and relatively long time required for obtaining culture results. Many efforts have done to develop faster and more accurate laboratory tests for diagnosis of TBM. Polymerase chain reaction (PCR) is one of these testes. Application of PCR as a diagnostic test for TBM has been controversial. In some studies apparent false negative results has been reported for PCR in diagnosis of TBM (8) and in some others, low sensitivity has been reported for this test (9, 10). In the meantime, there are several studies in which high specificity and sensitivity has been reported for PCR in diagnosis of TBM (11-13). Also different studies have shown that application of real time PCR in many instances has increased sensitivity and specificity of PCR technique (14).

2. Objectives

In this study we evaluated sensitivity and specificity of TaqMan real time PCR and PCR in comparison with routine microscopy and culture in diagnosis of TBM.

3. Patients and Methods

During January 2007 and January 2008 we had 49 patients with primary diagnosis of tuberculous meningitis in Bou-Ali University hospital and among them with

Implication for health policy/practice/research/medical education:
Results of this study help the health care provider and clinician in selection proper and precise laboratory test for evaluation of tuberculous meningitis.

combining and using the definite and probable TB as a gold standard, 29 had a final diagnosis of TBM. One CNS sample from each patients underwent cell count and biochemistry and the other was centrifuged and aliquoted into three 200 μL for microscopy, culturing and PCR respectively. All the CNS underwent Auramin staining and culture on Lowenstein-Jensen media. DNA extraction and PCR:DNA was extracted from 200 μl of centrifuged resuspended deposit of CNS sample using DNA Extraction kit (Fermentas, Tehran, IRAN) and amplification was done with the Mycobacterium tuberculosis PCR kit (Cinnagen, Tehran, IRAN) in a Mini MJ Thermal Cycler apparatus (Bio Rad) with the following program: 93°C for 60 seconds and 72°C for 30 seconds followed by 37 cycle 93°C for 20 seconds,72°C for 30 seconds and ended by one cycle 93°C for 20 seconds and 72°C.

Amplified DNA was detected by electrophoresis of 10 μL of amplified product on 1% agarose gel with 0.1% ethidium bromide. The amplified product at 160 bp region was detected. Gel Doc (UVP, USA) was used for documenting the gel picture. On the basis of the kit's instruction, positive control and DDW of the kit were used as positive and negative control respectively. TaqMan real time PCR: We used Primer Design Mycobacterium tuberculosis kit (Primer Design co, UK). The target of the amplification is IS 6110 and the related probe labeled at the 5′ end with the reporter dye (R) FAM (6-carboxyfluorescein) and at the 3′ end with the quencher dye (Q) TAMRA (6-carboxytetramethylrhodamine). The supplied Internal standard is labeled with VIC. Real-time PCR was performed using an ABI Prism 7500 System (Applied Biosystems, Foster City, CA). Each 20 μL reaction mixture consisted of 10 μL of PCR TaqMan mastermix (Applied Biosystems, Foster City, CA) combined with 2.5 μL of internal standard control (Primer Design, UK), 2.5 μL of Probe/Primer mixture (Primer Design, UK) and 5 μL specimen DNA or DDW (in the case of negative control) or Mycobacterium tuberculosis extracted DNA (supplies in the kit in the case of positive control). The test was read when control negative had not peak and positive control showed related peak and internal control had a CT about 31 cycles. Amplification program was as follow: After 2 minutes at 50°C and 10 minutes at 95°C, there were 45 cycles (95°C for 15 seconds and 60°C for 1 minute) of PCR amplification for IS6110 gene detection. AmpErase and dUTP within the master mix provided carryover contamination control.

4. Results

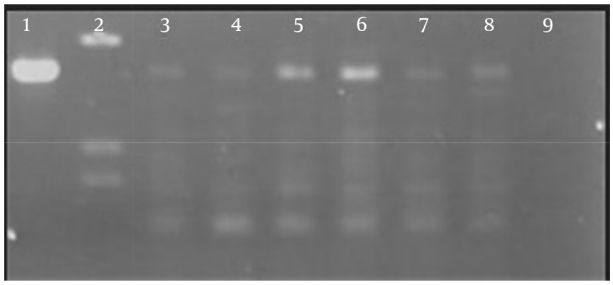
In all, we had 48 patients with primary diagnosis of tuberculous meningitis of which 2 had positive smear staining report, 11 had positive culture for mycobacterium tuberculosis, 26 had PCR positive results and 28 had TaqMan real time PCR positive test results. On the basis of microscopy, culture, PCR, TaqMan real time PCR and response to anti TB drugs, a final diagnosis of tuberculous meningitis was made for 29 patients (Table 1). Agarose gel electrophoresis of the PCR products is illustrated in Figure 1 and TaqMan real time PCR amplification plot were shown in Figure 2. PCR and TaqMan real time PCR results of TBM patients were shown in Table 2. In our study, the sensitivity and specificity of real time PCR, PCR, culture and microscopy in diagnosis of tuberculous meningitis was 96% and 95%, 89% and 90%, 38% and 100%, 6% and 100% respectively.

Table 1. Real Time PCR, PCR, Culture and Microscopy Results in TBM and Non-TBM Group and Their Sensitivity and Specificity of Each Test [a]

Teat Results	Final Diagnosis		Sensitivity, %	Specificity, %
	TBM	Non-TBM		
TaqMan Real Time PCR			96	95
Positive	28	1		
Negative	1	19		
PCR			89	90
Positive	26	2		
Negative	3	18		
Culture			38	100
Positive	11	0		
Negative	18	20		
Microscopy			6	100
Positive	2	0		
Negative	27	20		
Total	29	20		

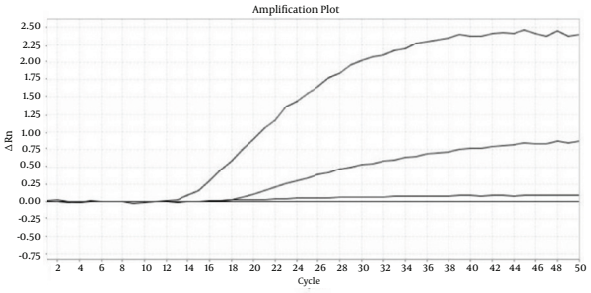
[a] Abbreviation: PCR, polymerase chain reaction.

Figure 1. Polymerase Chain Reaction Results of CNS Samples of TBM Patients



Line 1, control positive (a 166bp band); line 2,1 Kbp DNA ladder; line 3-8, TBM patients; line 9, control negative.

Figure 2. TaqMan Real Time PCR Amplification Plot



A) Positive control; B) Patient's sample; C) Negative control.

Table 2. Results of Different Criteria Used in Our Final Diagnosis of Tuberculous Meningitis [a]

Sample Number	Real Time PCR	PCR	Culture	Microscopy	Response to Anti TB Treatmen
1	+	+	+	-	improved
2	+	+	-	-	NA
3	+	+	+	-	died
4	+	+	-	-	NA
5	+	+	+	-	improved
6	+	+	+	-	improved
7	+	+	-	-	NA
8	+	+	-	+	improved
9	+	+	-	-	improved
10	-	-a	+	-	improved
11	+	+	-	-	improved
12	+	+	-	-	improved
13	+	+	+	-	died
14	+	+	+	-	improved
15	+	+	-	-	improved
16	+	+	-	-	improved
17	+	+	+	-	improved
18	+	+	-	-	improved
19	+	+	-	-	improved
20	+	+	-	+	died
21	+	+	+	-	improved
22	+	+	-	-	improved
23	+	+	+	-	improved
24	+	+	-	-	improved
25	+	+	-	-	NA
26	+	+	+	-	improved
27	+	-	-	-	improved
28	+	-	-	-	improved
29	+	-	-	-	Improved

[a] Abbreviation: NA, not available.

5. Discussion

Different studies have been shown that global incidence of tuberculosis is increasing (5). One of the consequences of this increasing of tuberculosis is increasing in extra pulmonary tuberculosis (5). Prevalence of extra pulmonary tuberculosis and the most dangerous form of it, tuberculous meningitis (TBM) is different by countries (15) and because of their paucibacillary, extra-pulmonary tuberculosis, cannot be diagnosed by routine microscopy and culture methods efficiently. Taking these points in mind, there is an emergency to establish and evaluate more rapid and sensitive laboratory tests in diagnosis of tuberculous meningitis.

In this study we used TaqMan real time PCR assay with IS6110 target in diagnosis of TBM in accompanying with PCR, culture and microscopy for evaluation and comparison of the sensitivity and specificity of these tests with each other. Our findings showed that TaqMan real time PCR has greatest sensitivity in diagnosis of TBM followed by PCR, culture and microscopy. This results is higher than what Sun's study reported in which the reported sensitivity of microscopy, culture and PCR were 3.3%, 26.7% and 66.7%, respectively, but both experiments showed the same order of sensitivity and specificity among tests (16). The sensitivity of our PCR test was more than what Ahuja and Bhigjee reported, i.e. 89%, 75% and 87.5%, respectively (5, 17) but PCR specificity in this experiment was less than what Sun's study showed, i.e. 90% against 97% respectively (16). In the present study real time PCR sensitivity and specificity were 96% and 95% respectively but the reported sensitivity and specificity in Bhigjee's study for real time PCR were 70.5% and 87.5% (5). Collectively, our study showed that real time PCR could be used in the diagnosis of TBM as a fast and reliable test.

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Author's Contribution

Taghi Naserpour Farivar , designed the research, performed the experiment and analyzed the data; Pouran Johari, performed the experiment; Mohammad Hashemi Shahri, worked in clinical evaluation of the patients; Mohammad Naderi, worked in clinical evaluation of the patients; Roya Alavi, worked in clinical evaluation of the patients; Batoul Sharifi-Mood, worked in clinical evaluation of the patients.

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