

**Accuracy of universal polymerase chain reaction (PCR) for detection of bacterial meningitis among suspected patients**Ali Reza Moayedi<sup>1</sup>, Abdolazim Nejatizadeh<sup>2</sup>, Maryam Mohammadian<sup>3</sup>, Mohammad Bagher Rahmati<sup>4</sup>, Vahideh Namardizadeh<sup>5</sup>

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**Type of article:** Original**Abstract**

**Introduction:** Central nervous system (CNS) infections are life-threatening diseases caused by viral, bacterial, parasitic and fungal microorganisms. The aim of this study was to determine the accuracy of universal polymerase chain reaction (PCR) for the detection of bacterial meningitis among patients who were referred to Koodakan Hospital in Bandar Abbas because they were suspected of having the disease.

**Methods:** This study was conducted in 2013 on the patients who were admitted to Bandar Abbas' Koodakan Hospital because they were suspected of having meningitis. A questionnaire, including demographic data, was completed for each patient. Universal PCR, Cerebrospinal fluid (CSF) analysis, and gram staining and cultures were done for all the patients. The data were analyzed using SPSS software.

**Results:** Among the 100 patients studied 59 (59%) were male and 41 (41%) were female. No patient in our study had a positive smear and culture for meningitis. Among the patients with negative smears and cultures six (6%) had positive universal PCR, and 94 (94%) had negative universal PCR. Based on these results, PCR had 95% specificity and 100% negative predictive value for the prediction of meningitis. In 30 patients (30%), the biochemical analysis of CSF were in favor of meningitis. Among the 30 patients, six patients (20%) had positive universal PCR and 24 patients (80%) had negative universal PCR.

**Conclusion:** Based on our results, the universal PCR test is useful in the diagnosis of bacterial meningitis in children. We recommend using it in combination with other tests, such as CSF analysis, for diagnosis of bacterial meningitis.

**Keywords:** meningitis, universal PCR, gram stain and culture

**1. Introduction**

Infections of the central nervous system (CNS) are life-threatening diseases caused by viral, bacterial, parasitic, and fungal microorganisms (1-4). In spite of advances in the diagnosis and management of acute bacterial meningitis, it is still a life-threatening condition. However, with proper antibiotic therapy and supportive care, the mortality is lower than 10% (5, 6). The symptoms of CNS infections vary among different age groups. Therefore, fast and accurate diagnostic tests are very important for the rational use of antibiotics and for lowering the treatment costs (7-

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11). The usual diagnostic method for meningitis is gram staining and culture, which is dependent on previous use of antibiotics by the patient and is unable to fully differentiate between viral and bacterial infections. This method has a high rate of false negatives, which causes controversy concerning the selection and duration of appropriate treatment. Antibiotic therapy does not affect pleocytosis with neutrophil dominance, protein level, or Cerebrospinal fluid (CSF) glucose level (11). Therefore, the diagnosis of bacterial meningitis can be made even when the culture is negative by this method, because these changes are not affected by antibiotic therapy during the first days after treatment. In this situation, molecular assessment can be helpful. Patients are very frequently diagnosed with CNS infection and negative CSF culture. The general objective of the study was to determine the accuracy of universal polymerase chain reaction (PCR) for the detection of bacterial meningitis among patients who were referred to Koodakan Hospital in Bandar Abbas because they were suspected of having the disease. The specific objectives of the study were to compare the results of the PCR test with the results of gram staining and culture for the detection of bacterial meningitis among patients suspected of having the disease; and to compare the results of the PCR test with CSF fluid analysis for the detection of bacterial meningitis among patients suspected of having the disease

## **2. Material and Methods**

### *2.1. Study setting and ethics*

This study was conducted in 2013 on the patients who were admitted to Bandar Abbas Koodakan Hospital because they were suspected of having meningitis. The study was approved by the Ethics Committee at Hormozgan University of Medical Sciences. All of the patients' data were kept confidential.

### *2.2. Measurement tools*

A questionnaire that included demographic data was completed for each patient. Sample volumes of 500-1000 ml of the CSF samples were kept in sterile conditions at -80 °C for the next PCR test. Also, the samples were sent for gram staining and culture. For DNA extraction, 500 µl of CSF were placed in a microtube, and gene extraction was done with a DNA minikit (Kimia Co.). The extracted solution was kept at -20 °C. The supernatant DNA of each microtube was measured by a Nano Drop machine by comparing the absorbance at wavelengths of 260 and 280 nm. A 10<sup>8</sup> concentrations of *E. coli* and *acintrobacter* species were prepared using 0.5 Mc Farlen standard tube and DNA contents were used as pattern in PCR process. The sequences of the primers were: U1(F):5'-CCAGCAGCCGCGGTA ATACG-3' and U2(R) :5'-ATCGG(C/A)TACCTTGTTACGACTTC-3'. The PCR reaction was conducted by mixing 2 µL of extracted DNA, 1 µL of forward and reverse primer, 8.5 µL of sterile water, and 12.5 µL of master mix. All of the products were purchased from Gen-Fan-Avaran Company. The PCR reaction was conducted using a thermocycler device (SENSOQUEST). The thermal cycle for the PCR reaction was 1 min at 95 °C, then 25 cycles of 20 seconds each at 95 °C, 1 min at 63 °C, 1 min at 72 °C, and 5 min at 72 °C. The PCR products were detected using 1% agar gel and UV transluminator device. Then, the molecular weight of the proliferated segments were measured and found to be about 1500 base pairs.

### *2.3. Statistical analysis*

SPSS version 16 software was used for the statistical analysis and calculation of specificity and sensitivity of the tests. Chi-squared test and the independent samples t-test were used for data analysis.

## **3. Results**

### *3.1. Demographic information*

Among 100 patients studied, 59 (59%) were male and 41 (41%) were female. The ages of the participants ranged from 2 days to 11 years, and their mean age was 19.17 months. Among the patients 71 (71%) were less than 18 months old, 22 (22%) were 1.5 to 6 years old, and 7 (7%) were 6 to 12 years old.

### *3.2. Comparison of PCR results with gram staining and culture results*

No patient in our study had a positive smear and culture for meningitis. Among the patients with negative smears and cultures, 6 (6%) had positive universal PCR and 94 (94%) had negative universal PCR. Based on these results, PCR had 95% specificity and 100% negative predictive value for the prediction of meningitis.

### *3.3. Comparison of PCR results with CSF analysis results*

Analytical sensitivity of the methods was determined with two specific species, i.e., *E. coli* and *acintrobacter*, with concentrations of 10<sup>8</sup> colonies per milliliter of CSF. Among the 100 patients studied, none had a positive gram staining or culture. PCR was positive for 6 (6%) patients. In 30 patients (30%), the biochemical analyses of CSF

were in favor of meningitis. Among them 6 (20%) patients had positive universal PCR and 24 (80%) had negative universal PCR.

#### **4. Discussion**

Early diagnosis and treatment of meningitis is vital, and research is ongoing to identify accurate methods for the diagnosis of meningitis. Among the efforts to meet this goal, different PCR-based methods have been designed (12). Simple PCR methods are specific only for detection of a single specific antigen of a single specific microorganism and therefore cannot be used in laboratory. Also, some PCR methods are complex and costly, so they are not used in clinical laboratories. Designing methods based on health issues and available equipment can be helpful. Some molecular methods have been designed based on universal primers. In this study, we used conserved primers of the 16srDNA gene, which is found in the genome of all bacteria (13). Detection of specific species of bacteria is of no help to physicians at the beginning of the disease. Physicians are able to detect a wide range of bacteria without using complex devices, and they easily can distinguish between bacterial and non-bacterial meningitis.

##### *4.1. Demographic information*

The mean age of the participants in this study was about 19 months, and a large percentage of the participants were less than 6 years old. Based on the patients' ages, different microorganisms can cause meningitis. Therefore, the results of our study cannot be applied for different age groups.

##### *4.2. Comparison of PCR results with gram staining and culture results*

Few gram staining and culture results are positive in our Center. In this study, we had no positive smear and culture tests. Therefore, this is an important limitation for interpretation of the smear and culture results for diagnosis of meningitis. Using universal PCR, we diagnosed six cases of meningitis. Therefore, considering the limitation of gram staining and culture in this study, using universal PCR is very helpful to increase the probability of correctly diagnosing bacterial meningitis. Also, other studies have reported that PCR and staining are highly accurate for the detection of bacterial meningitis (14).

##### *4.3. Comparison of PCR results with CSF analysis results*

Based on our results, all universal PCR positive cases of meningitis can be diagnosed by CSF analysis. Other researchers have confirmed that CSF analysis is a strong tool for the detection of bacterial meningitis and that it can be used to diagnose meningitis (15). However, we recommend using universal PCR in combination with CSF analysis for more accurate diagnoses.

#### **5. Conclusions**

Based on our results, the universal PCR test is useful in the diagnosis of bacterial meningitis in children. We recommend using it in combination with other tests, such as CSF analysis, for the diagnosis of bacterial meningitis. Future research should focus on the accuracy of the combination of these tests in diagnosing bacterial meningitis.

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#### **Conflict of Interest:**

There is no conflict of interest to be declared.

#### **Authors' contributions:**

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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