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**Research**

# Molecular Methods (16s-rRNA) Compared to Bacteriological Diagnosis of Meningitis

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**Abstract**

**For treatment of patients with meningitis, rapid diagnosis of the agent is very important. Nowadays all of researches have approved qualification and efficiency of molecular tests.**

**Detection of bacteria from CSF and blood is the major problem as a result of usage of antibiotics by patients. So, we researched on CSF samples by PCR test and used DG74 and RDR80 primers for 16s rRNA sequence. Our cases were 51 children with meningitis symptoms that had referred to Mofid Hospital in Tehran. These samples were different from culture, cell counter and protein glucose amounts.**

**After researching we reached to these results that 23.5% of cases were positive for bacterial culture and 41.1% of them were positive for PCR test. So sensitivity of PCR was 95.23%, specificity of PCR was 96.66% and efficiency of PCR was 96%.**

**In first group 8 specimen were PCR positive (88.8%). In second group, all of 12 specimens were PCR positive (100%). In third, 8 specimens were suspected for viral meningitis, only one case was PCR positive, so it had bacterial agent. In fourth group, all of 22 specimens were PCR negative. Therefore sensitivity and specificity of PCR test with 16s rRNA gene sequence in identification of bacterial agent in CSF was 95.23% and 96.66% respectively.**

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## Introduction

Rapid identification of bacterial meningitis is of a high importance. Nowadays, isolation of bacterias from CSF or blood within 24 hours incubation is routine method, but some bacteria are fastidious, some patients have received antibiotics before sampling, so culture will be negative. Growth of bacteria depends on sampling and transfer condition too. Treatment of cell culture for identifying viruses in some samples is very troublesome, expensive and requires long time. Therefore, we need a sensitive method to solve above problems.

Meningitis is an acute life – threatening infection. The mortality rate is approximately 10 to 15% (depending on the bacteria involved), even with appropriate antimicrobial therapy. The incidence of disease decrease with age. The prevalence of a particular etiologic agent is also related to patients age. Clinical manifestations vary considerably depending on the virulence of the organism and the age of patient. In neonates the signs of meningeal irritation (nuchal rigidity and Brudzinski and Kernig signs) are frequent and often minimal

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after finding signs including temperature instability, poor food taking and vomiting.

## Patients and Methods

This research is a descriptive kind. Sampling was performed in Mofid Pediatric Hospital over children with meningitis from July to March of 2000. Sampling method was lumbar puncture. All tests such as bacteriologic, cell counts and PCR was performed on samples in sterile condition. 200λ of each sample were kept in a micro tube in 20°C. On the remaining CSF, the first gram staining, bacterial culture, cell count with hematocytometer, cell typing, considering protein and glucose was performed. Bacteriologic culture was blood agar, EMB and chocolate agar. In PCR we used 2 type primers that were specific for 16s rRNA sequence:

*DG 74: AGGAGGTATCCAACCGCA*

*RDR 80: AACTGGAGGAAGGTGGGGAG*

PCR was performed in Automatic – Thermocycler and PCR has 3 processes:

1. Denaturation in 94°C
2. Annealing in 60°C
3. Extension in 72°C

These process were repeated 30 to 35 times, for each sample. In micro tube, we used dNTP mixture, PCR buffer, $MgCl_2$, 2 pair primers, Taq polymerase, and production of PCR electrophoresis on 2% gel.

## Results

To find a rapid and specific test for identifying bacterial meningitis, 51 CSF samples obtained from children under 6 years in Mofid hospital from July to March 2000 were studied. Fourty four percent point seven of patients with meningitis were suspected to meningitis, 55.3% had negative PCR. Thirty four point two percent of 44.7% suspected to have bacterial meningitis and 10.5% suspected to have viral meningitis.

**Table-1: Result of Culture and Cell Count in Children with Meningitis**

| No. | Method percent | Cell count in LP | Culture | Manifestation of meningitis |
|---|---|---|---|---|
| 9 | 10.7 | N>L | ---- | + |
| 12 | 23.5 | N>L | Meningococcus Haemophilus influenza | - |
| 8 | 10.5 | L>N | ---- | + |
| 22 | 55.3 | ---- | ---- | + |
| 51 | 100 | | | |

*N: Neutrophil L: Lymphocyte*

**Table-2: The Frequency of Positive Culture in Children with Meningitis**

| Frequency Culture | Number | Percent |
|---|---|---|
| Positive | 12 | 23.5 |
| Negative | 39 | 46.5 |
| Total | 51 | 100 |

**Table-3: The Frequency of Positive PCR in CSF of Children with Meningitis**

| PCR | Number | Percent |
|---|---|---|
| Positive | 21 | 41.1 |
| Negative | 30 | 58.9 |
| Total | 51 | 100 |

## Discussion

In this research, we used 16s rRNA gene sequences of bacteria to identify bacterial infection on 51 CSF specimens of children who referred to Mofid hospital. David Fredrics in 1999, used PCR method and 16s rRNA sequence in sterile specimens such as blood, spinal fluid, with specificity and sensitivity more than 97%. In 1998 R. Dagan used PCR for identifying DNA of pneumococci in children. Blood culture was positive in 30% and sensitivity of PCR was 100%. In 1977 YL – Wei targe used this method for identifying of infectious disease

as a gold standard. In 1996 Jane New combel used PCR for identifying meningococci in peripheral blood. In 1993 K. Greisen used PCR for identification of 102 bacterial species, specificity and sensitivity was more than 96%. It is important to know that sterile fluid such as patients specimen that were treated with antibiotics, number of bacteria are low, some of bacterias were fastidious and need an enrichment media and specific atmosphere, (Co2 or anaerobic) and some of this bacterias are sensitive to transport conditions. Therefore, we couldn't identify all by culture and isolation of bacteria and we couldn't reach to desire results. In children of 1 to 8 months signs and symptoms are often non-specific and includes fever, irritability, drowsiness, vomiting ,poor feeding, crying while handled, bulging fontanels (due to increased intravascular pressure) and febrile seizures. Chemical tests and CSF cell count of CSF in bacterial and viral meningitis is not 100% specific. A variety of molecular methods for identification of microorganisms in clinical specimens have been developed. One of these methods is PCR. In our research we used 16s-rRNA gene sequence for PCR, as 16sr-RNA sequence was constant during the evolution comparing to 23s and 5s rRNAs and approximately is identical in all of prokaryotes.

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