

The study of 16S rRNA in meningitis by molecular biology assay

Review Article

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Summary

In order to treatment of patients with meningitis rapid diagnosis of agent is very important. Now all of researchers have approved qualification and efficiency of molecular tests. Detection of bacteria from cerebrospinal fluid (CSF) and blood is big cumbersome as atmosphere condition and usage of antibiotics by patients. We explored on CSF samples by PCR test and used DG74 and RDR80 primers for 16S rDNA sequence. Our cases are children with meningitis symptoms that had referred to hospitals at Tehran. This samples are different from culture, cell counter and protein glucose amounts. After researching we reached to these results that 23.5% of case were positive as bacterial culture and 41.1% of them were positive as PCR test. So sensitivity of PCR was 95.23%, specificity of PCR was 96.66% and efficiency of PCR was 96%.

I. Introduction

Rapid identification of bacterial meningitis is very important. Now, Isolation of bacteria from CSF or blood in 24 hours incubation is routine method, but some bacteria are fastidious, some patients have received antibiotic before sampling, so culture will be negative. Growth of bacteria depends on sampling and transfer condition too. Treatment of cell culture for identifying of viruses in some sample is very troublesome, expensive and requires to long time. Therefore we need a sensitive method to solve above problems. Meningitis is an acute life-threatening infection. The mortality rate is approximately 10-15% (depending on the bacteria involved), even with appropriate anti microbial therapy. The incidence of disease decreases 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 (neckal rigidity and Brudzinski and Kernigs signs are infrequent and often minimal when found early signs include temperature instability, poor feeding and vomiting.

In children 1-18 month of age signs and symptoms are often nonspecific and include fever, irritability,

drowsiness, Vomiting, poor feeding, crying when handled, bulging fontanels (due to increased intravascular pressure) and febrile seizures. So, rapid identification is very important. Chemical tests and cell count of CSF in bacterial and viral meningitis is not 100% specific. The molecular methods in identification of microorganisms in clinical specimens have developed. One of these methods is PCR, we can use aseptic primer to multiply of unknown DNA. In our research we used 16S rDNA gene sequence for PCR. As 16srRNA sequence was constant during the evolution than to 23s and 5srRNA and approximately is identical in all of prokaryotes.

II. Material and Methods

This research is descriptive. Sampling is done in Tehran pediatric hospitals from children with meningitis. Sampling method was lumbar puncture. All of tests such as bacteriologic, biochemistry cell count and PCR was done on sample in sterile condition. 200 µl of each sample in a micro tube is kept in -20°C. On remaining of CSF, the first is done gram staining, bacterial culture, cell count with hemacytometer, cell typing, considering protein and Glucose.

Bacteriologic culture is blood agar, EMB and chocolate agar In PCR we use 2 type primers that are specific for 16S rRNASequence:

DG 74: AGGAGGTATCCAACCGCA
 RDR 80: AACTGGAGGAAGGTGGGGAG
 PCR is done in Automatic Thermocycler.

- PCR has 3 process:
- i. Denaturation in 94°C
 - ii. Annealing in 60°C
 - iii. Extension in 72°C

These processes are repeated 30- 35 times. For each sample in micro tube, we use dNTP mixture, PCR buffer, MgCl₂, 2pair primers, Taq polymerase and production of PCR electrophoresis on 2% gel.

III. Results

Finding a rapid and specific test for identifying of bacterial meningitis, 51 CSF samples from children under 6 years in Mofid hospital from July to March were received 44.7%.

Patients with meningitis were suspected to meningitis, 55.3% were negative for PCR. 34.2% of 44.7% suspected to bacterial meningitis and 10.5% suspected to viral meningitis.

We studied about culture, cell count of CSF in children with meningitis that has been shown in Table 1. We found the positive culture of CSF in children with meningitis was 23.5%, **Table 2**. We resulted the frequency of positive PCR in CSF of children with meningitis 41.1%, **Table 3**.

IV. Discussion

In this research, we use 16srRNA gene sequences of bacterial to identify bacterial infection on CSF specimens from children who refer to Tehrans hospitals. David Fredrics in 1999, used PCR method and 16srRNA sequence in sterile specimens such as blood, spinal fluid, specificity and sensitivity was more than 97%. In 1998 Dagan et al, used PCR for identifying DNA of pneumococci in children and sera. Blood culture was positive 30% and sensitivity of PCR was 100%. In 1997 Tang et al, used this method for identifying of infectious disease such as gold standard. In 1996 Newcombe et al, used PCR for identifying meningococci in peripheral blood. In 1993 Greisen et al, used PCR for identifying of 102 bacteria species. Specificity and sensitivity was more than 96%. It is important to know that sterile fluid such as patient specimen that treatment with antibiotic, number of bacteria were low, some of bacteria were fastidious and need to an enrichment media and specific atmosphere, (for example CO₂ or anaerobic) and some of this bacteria Sensitive to transport conditions. Therefore, we can t identify all of bacteria by culture and isolation of bacteria and we can t reach to desire results.

In first group 8 specimen were positive PCR (88.8%). In second group, all of 12 specimens were positive PCR (100%).

Table 1. Result of culture, cell count in children with meningitis that refer to Mofid hospital in 2000

Method (%)	Cell count in LP	Culture	Manifestation of meningitis
9	10.7	N > L	----
12	23.5	N > L	+
		Meningococcus	+
		Pneumococcus	
8	10.5	L > N	+
		Haemophilus influenza	
22	55.3	----	+

51	100	N: Neutrophil, L: Lymphocyte	

Table 2. The frequency of positive culture in children with meningitis refer to Mofid hospital in 2000.

Frequent Culture	Number	Percent
Positive	12	23.5
Negative	39	46.5
Total	51	100

23.5% of 51 specimens suspected to bacterial meningitis.

Table 3. The frequency of positive PCR in CSF of children with meningitis refer to Mofid hospital

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

In third, 8 specimens suspected to viral meningitis, only one case was positive PCR, so it had bacterial agent. In fourth group, all of 22 specimens were negative PCR. There fore sensitivity and spesitivity of PCR test with 16S rDNA A gene sequence in identification of bacterial agent in CSF was 95.23% and 96.66%.

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