Original paper

Polymerase Chain Reaction Testing in Comparison to Culture of Cerebrospinal Fluid for Diagnosis of Bacterial Meningitis in Children

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Abstract

Background: Childhood bacterial meningitis is a neurological exigency. Accurate, early, rapid diagnosis and treatment is essential to decrease its morbidity and mortality.
Aim of study: This is a retrospective study comparing real-time polymerase chain reaction (RT-PCR), with standard bacterial culture for the diagnosis of bacterial meningitis during cerebrospinal fluid (CSF) examination.
Materials and methods: RT-PCR was used for the detection of three most common causes of bacterial meningitis (*Streptococcus pneumonia, Haemophilus influenza type b*, and *Neisseria meningitides*) in 100 CSF samples from children aged 2 months to 12 years admitted to Al-Elwiya Pediatric Teaching Hospital during the period from January 2016 to January 2017
Results: Growth was detected in 6% CSF cultures of patients included in the study and *Streptococcus pneumoniae* was the most prominent isolated bacteria. RT-PCR was positive in 43% CSF samples from which *S. pneumoniae* was identified in all samples. PCR and culture showed concordance in 6% positive and 57% negative samples. Using culture as a reference method, the sensitivity, specificity, and positive and negative predictive values for PCR on CSF samples were 100 %, 60.64%, 13.93% and 100 %, respectively. RT-PCR was positive in 37% CSF samples whereas culture was negative.
Conclusion: RT-PCR is a rapid and sensitive test for the diagnosis of bacterial meningitis. The findings of this retrospective study recommend the use of RT-PCR for the diagnosis of children with a clinical suspicion of bacterial meningitis and as a complement to culture, especially, those who received previous antibiotic treatment before lumbar puncture.
Key words: Meningitis, Children, PCR, CSF, Bacteria, Culture.

Introduction

Meningitis is an acute inflammation of the meninges that covers the brain and spinal cord [(1)]. Generally, the inflammation may be caused by viruses or bacteria and less common by fungus and parasites [(1,2)]. Several factors have been associated with development of meningitis such as, immune status of the host, epidemiology of the pathogen, and age [(2)]. Bacterial meningitis is a medical emergency, which requires rapid diagnosis, and early, effective management. Meningitis has high mortality rate (100%) if untreated, and even with antibiotics treatment, it is approximately 5–10% [(3)]. Bacterial meningitis occurs in about 1.2 million people [(4)] and results in about 170,000 deaths worldwide each year [(5)]. The risk of

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developing neurological sequelae in patients following hospital discharge is approximately 20% worldwide [6], this include behavioral changes, mental retardation, hearing loss, and seizures [7]. Childhood vaccination schemes have changed the epidemiology of meningitis; nevertheless, it remains prevalent globally. Advances in clinical and laboratory tests (especially the combination of laboratory techniques) has improved the diagnosis of bacterial meningitis.

The morbidity and mortality rates of bacterial meningitis vary according to the age groups, geographical region, and the type of pathogen [8]. Common causes of bacterial meningitis in infants and children between 2 months to 12 years are *Streptococcus pneumoniae* (*S. pneumoniae*), *Haemophilus influenzae* type B (*H. infleunzae* Hib), and *Neisseria meningitis* (*N. meningitidis*) [2]. The clinical symptoms of childhood meningitis vary according to age and duration of disease. It is preceded by several days of fever with upper respiratory or gastrointestinal symptoms. Nonspecific signs include tachycardia, hypotension, fever, poor feeding, anorexia, vomiting lethargy, irritability, myalgia, and arthralgia [2,9]. Older children may develop neck stiffness, headache and photophobia. Seizures may occur in 20–30% of children infected with *S. pneumoniae* and Hib more than with *N. meningitidis* [2,10]. Meanwhile, fulminant sepsis is more common in meningococcal meningitis [11].

Diagnosis of meningitis is based on both clinical and laboratory findings. Laboratory investigations include, microscopic and chemical analyses of CSF and blood and CSF cultures. Antibiotic treatment should be started immediately based on the clinical findings. Meanwhile, bacterial antibiotic susceptibility test should be rapidly identified [12]. Culturing CSF sample is the gold standard method in the diagnosis of bacterial meningitis. However, this approach might have some disadvantages regarding rapidity and sensitivity. Culture takes up to 48 hours or more, and the bacterial recovery rates are low particularly in patients who received antibiotic treatment before lumbar puncture (LP), and meningitis may be caused by slow-growing or anaerobic or fastidious bacteria [13] [14] [15] [16]. This necessitate the use of rapid and accurate diagnostic methods [13,17] [18]. PCR is a nucleic acid amplification test that can detect small amounts of pathogen DNA in a specimen independently from culture [19]. PCR-based tests have become available for prompt diagnosis of bacterial meningitis [14]; it can detect as few as 10–100 CFU/ml of bacteria in CSF [20]. The advantage of this assay is to eliminate unnecessary antimicrobial therapy [21]. The purpose of this study was to compare the efficacy of NHS Meningitidis Real-TM, which is a rapid diagnostic PCR-based test to the standard culture method for the diagnosis of bacterial meningitis by retrospective review of the clinical and laboratory records of patients at Al-Elwiya Paediatric Teaching Hospital. Consequently, immunization programs can be arranged against the common causes of meningitis. We hypothesized that PCR may be more sensitive and provide rapid identification of bacteria that cause meningitis compared to culture.

Materials and methods

Patients and clinical specimens

This is a retrospective study comparing RT-PCR versus culture for the detection of bacterial meningitis in CSF samples from 100 patients aged 2 months to 12 years admitted to Al-Elwiya Paediatric Teaching Hospital in Baghdad during the period from January 2016 to January 2017. The criteria for requesting CSF sample for culture and PCR is clinical suspicion of meningitis. Patients' details and laboratory test results were obtained from the hospital

laboratory records for all cases tested by culture and PCR.

The suspected cases of bacterial meningitis were identified based on the clinical findings, CSF analysis results and laboratory tests. Clinical symptoms of bacterial meningitis were fever, anorexia, poor feeding, lethargy, irritability, seizures, and rash. Signs of increased intracranial pressure include headache, vomiting, bulging fontanel and widening of sutures (in children below one year), hypertension with bradycardia, altered mental status, abducens or oculomotor nerve paralysis, decorticate or decerebrate posture, and apnea or hyperventilation. Positive meningeal irritation signs (in older children) include nuchal rigidity, back pain, and Kernig and Brudzinski signs [(2)].

The physician under aseptic conditions did LP and CSF samples were collected. One patient had meningomyelocele and frontal tapping done by neurosurgeon. Part of the CSF sample was submitted for routine biochemistry, microbiology, and cytological testing. The remaining CSF sample was stored at −20◦C until transferred to the Central Public Health Laboratory for PCR analysis.

Patients were diagnosed according to their clinical and laboratory findings as follows:

Bacterial meningitis: CSF appearance is turbid, leukocytosis ≥ 100 cell / mm3 (PMN predominate), high CSF protein (>45mg/dL) usually 100-500 mg/dL, low CSF glucose ≤ 40 mg /dL (less than 50% serum glucose), with organisms seen on Gram stain and positive CSF culture for specific bacterial pathogen.

Partially treated bacterial meningitis: if there is a history of antibiotic treatment before LP, CSF leukocyte is between 5-10000 cell/mm3 (PMN predominate, ≥50% of total WBC count), protein >45mg/dL, normal or low CSF glucose, no organism seen on Gram stain and CSF culture.

No meningitis: if there are no abnormal laboratory findings, CSF leukocyte <5 cell/mm3, ≥75% lymphocyte, protein <45mg/dL (20-45 mg/dL), glucose >50 (or 75% serum glucose), no organisms seen on Gram stain and CSF culture.

Viral meningitis: if CSF shows leukocytosis, mainly lymphocyte, CSF protein is between 50-200 mg/dL, CSF glucose is normal.

While exclusion criteria include if LP was traumatic, presence of a chronic neurological disease (e.g. cerebral palsy) or known immunodeficiency. Age less than 2 months were also excluded because the clinical presentation and the laboratory findings of CSF are different in this age group.

Real-time PCR and culture

All patients' CSF samples for bacterial meningitis were tested by RT-PCR analysis at the Central Public Health Laboratory which is the only laboratory providing this test in Iraq. Briefly, DNA was extracted from CSF samples using Sorb-B purification kit (Sacace Biotechnologies, Como, Italy). Then the extracted DNA was tested using NHS Meningitidis Real-TM (Sacace Biotechnologies, Como, Italy) for the detection of three pathogens, *H. influenzae* Hib, *N. meningitidis*, and *S. pneumoniae*. The test was performed and results were evaluated according to the manufacturer's instructions.

CSF cultures were processed at Al-Elwiya Paediatric Teaching Hospital laboratories in line with their local practice and culture, results were confirmed at Central Public Health Laboratory. The CSF samples were cultured onto blood, chocolate, and MacConkey agar. Plates were incubated aerobically except chocolate agar incubated in carbon dioxide enriched atmosphere. All plates were incubated at 37 °C for up to 48 hours. A positive culture was defined as the growth of bacteria on agar plates, was confirmed by VITEK® 2 (bioMérieux, France) for bacterial identification and antibiotic susceptibility tests. Meanwhile, Gram-

negative bacteria were identified by Api Microsystems (bioMérieux SA, France).

Statistical analysis

The results of PCR and culture were compared using the Student's *t*-test and Fisher's exact test. Sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio were calculated, along with 95% confidence intervals, using GraphPad Prism software. Variables with a p-value ≤ 0.05 considered statistically significant.

Results

A total of 100 patients with presumptive diagnosis of meningitis from January 2016 to January 2017 were included in this study, based on clinical symptoms and CSF laboratory findings as described in materials and methods. The demographic characteristics of the patients are shown in Table 1. The males (73%) were significantly more (p = 0.0002) than females (27%), with male to female ratio of 2.7:1. The mean age of the patients was 9.46 ± 13.2 months (range: 2 months to 12 years). There were 91 patients (67 male and 24 female), who were 2 months to 2 years, 9 patients (6 males and 3 females) who were 2 years to12 years of age. Males were found to be more prominent in younger children with bacterial meningitis than older age groups (Table 1).

In six CSF samples, *S. pneumoniae* was both isolated from culture and detected in RT-PCR. Meanwhile, 43% CSF samples were positive when processed with RT-PCR, and all these cases were *S. pneumoniae* as well. 37% patients with positive RT-PCR, the culture was negative. The culture and RT-PCR were negative in 57% CSF samples. No sample was positive in CSF culture while the RT-PCR was negative. RT-PCR was more efficient (43%) than the culture (6%) in the detection of bacterial pathogens (p=0.048). Bacterial meningitis was found in 15% of patients (6 culture & PCR positive and 9 culture negative & PCR positive). Meanwhile, partially treated bacterial meningitis was found in 28% of patients (culture negative and PCR positive). Males affected more than females in bacterial and partially treated bacterial meningitis.

In order to assess the performance of RT-PCR, results were compared with CSF cultures as a gold standard method (Table 2). Accordingly, the diagnostic accuracy of RT-PCR including sensitivity, specificity, positive predictive value, and negative predictive value were 100%, 60.64%, 13.93%, 100%, respectively.

Clinical data was reviewed for discordant culture negative/PCR positive cases (37%) (Table 3). The mean CSF protein and sugar was 115.22 ±56.97 mg/dL (range 64-300 mg/dL), and 53.03 ±21.11 mg/dL (range 15-92 mg/dL), respectively. Mean blood WBC count was 15508 ±4199 cell/mm3 (range 11000 -25000 cell/mm3). While the mean CSF WBC count was 144.97 cell/mm3 (range 5 - >2000 cell/mm3). Most patients were treated with antibiotics prior to LP 33/37 (89.2%) (Table 3).

Table 1. Demographic characteristics of patients

Age (mean ± SD)	**9.46 ± 13.2 months**	
2mon –< 2yr	**Males (67%)**	**Females (24%)**
2yr – 12yr	**Males (6%)**	**Females (3%)**
Gender		
Male	73	
Female	27	
Antibiotic treatment before lumbar puncture		
Yes	53	
No	47	

Table 2. Diagnostic accuracy table comparing PCR against culture for cerebrospinal fluid samples

Diagnostic test	**Cerebral spinal fluid**	
Using bacterial culture only as the gold standard for comparison	Culture positive	Culture negative
PCR Positive	6	37
PCR Negative	0	57
Results		95% CI
Sensitivity (%)	100	51.68 – 100
Specificity (%)	60.64	50.00 – 70.40
Positive likelihood ratio	2.54	1.98 – 3.27
Negative likelihood ratio	0	N/A
Positive predictive value (%)	13.93	5.80 – 28.63
Negative predictive value (%)	100	92.13 - 100

Table 3. Clinical details for discordant culture negative and PCR positive results

Patient ID	**Gender**	**Age (months)**	**AB pre-LP**	**CSF appearance**	**Blood WBC count**	**CSF WBC count**	**PMN (%)**	**Lymph (%)**	**CSF Prot (mg/dL)**	**CSF sugar (mg/dL)**	**Diagnosis**
1	M	2	N	T	22000	>2000	90	10	300	17	Bacterial meningitis
2	M	24	Y	T	20000	1000	60	40	255	20	=
3	F	18	Y	T	23000	610	65	35	265	28	=
4	M	2	Y	T	21000	360	60	40	200	22	=
5	M	8	Y	T	19500	295	90	10	178	28	=
6	M	4	Y	T	16300	227	70	30	175	92	=
7	F	2	Y	C	13600	100	65	35	100	52	=
8	M	60	Y	C	15000	100	50	50	100	72	=
9	M	10	N	C	20000	95	75	25	115	25	=
10	M	3	Y	C	19300	90	80	20	100	54	Partially treated
11	M	5	Y	C	13700	50	70	30	98	70	Partially treated
12	M	4	Y	C	15000	40	50	50	91	58	=
13	F	5	Y	C	15500	35	60	40	111	42	=
14	M	5	Y	C	17000	30	80	20	110	38	=
15	M	6	Y	C	11000	30	50	50	80	63	=
16	M	9	Y	C	14300	30	70	30	150	41	=
17	F	4	Y	C	17000	30	50	50	117	60	=
18	M	10	Y	C	17200	27	60	40	122	73	=
19	M	4	Y	C	10000	25	60	40	90	88	=
20	M	2	Y	C	15000	20	50	50	87	76	=
21	F	9	Y	C	16000	20	90	10	92	55	=
22	M	7	Y	C	15000	15	80	20	85	68	=
23	F	2.5	Y	C	16200	15	60	40	73	77	=
24	M	3	Y	C	13700	15	75	25	85	55	=
25	F	36	N	C	25000	13	90	10	80	79	=
26	M	2	N	C	18000	12	100	0	100	36	=
27	M	4	Y	C	12000	10	70	30	70	54	=
28	M	3	Y	C	13200	10	50	50	90	15	=
29	M	4	Y	C	16000	10	90	10	88	50	=
30	M	9	Y	C	13800	10	60	40	95	37	=
31	M	5	Y	C	1100	8	50	50	67	55	=
32	M	24	Y	C	13000	7	75	25	122	65	=
33	M	4	Y	C	14000	5	80	20	72	80	=
34	F	4	Y	C	12000	5	100	0	86	51	=
35	M	2	Y	C	12800	5	60	40	70	83	=
36	M	3	Y	C	14000	5	100	0	80	50	=
37	F	5	Y	C	12600	5	80	20	64	33	=

AB Antibiotics, *CSF* Cerebral spinal fluid, *F* Female, *LP* Lumbar puncture, *Lymph* Lymphocytes, *M* Male, *N* No, *PMN* Polymorphonuclear leukocytes, *Prot* Protein, WBC White blood cells, *Y* Yes.

Discussion

Bacterial meningitis is a life-threatening disease; however, prompt diagnosis of the pathogen and the early treatment is essential for a favorable clinical outcome [22]. The rate of sequelae and mortality increases even in short delays in the diagnosis and treatment [23]. Although, CSF culture is considered to be the gold standard method for the diagnosis of bacterial meningitis. However, is not ideal in patients who received antibiotics before LP. In addition, to the 24-48 hours culture period which prohibit clinicians from making a prompt diagnosis and starting treatment [24], and laboratory insufficiencies to isolate microorganisms. Hence, it is necessary to look for non-culture assays such as, PCR that is more accurate and reliable, especially among patients who have used antibiotics before LP [7,25]. Furthermore, PCR does not require the presence of large numbers of organisms, provide rapid diagnosis (can be performed in 2 hours), use small amount of sample, and it may detect nonviable, slow-growing, or fastidious organisms [7,26]. Bacterial meningitis agents are currently identified by various PCR assays with high sensitivity and specificity. Nonetheless, these tests are not routinely used for diagnosis in hospitals in developing countries, except in referral centers since they require high cost equipment [27].

This retrospective study involved 100 patients with presumptive diagnosis of meningitis; with their CSF, samples were analyzed by culture and RT-PCR to determine the clinical advantage of such methods for prompt diagnosis and as a guide to the physicians in making decisions concerning antibiotic treatment. In the present study, we evaluated NHS Meningitidis RT-PCR assay for the diagnosis of three bacterial pathogens that cause meningitis by using specific primers. Our results showed that the PCR results were not in accordance with those of culture. Although some performance characteristics were in good correlation with culture results such us sensitivity (100%) and negative predictive value (100%). Meanwhile, the specificity (60.64%) and positive predictive value (13.93%) of PCR were low, which means that some culture results were false negative. Our results compare favorably with the results of other studies by Saravolatz *et al.,* 2003 and Sarookhani *et al.,* 2010 [21,28]. This specificity of RT-PCR does not reflect the true percentage, because the presence of fastidious bacteria, delay in CSF culturing, or consumption of antibiotics before LP, could result in such circumstance [14,15]. Since the negative predictive value of PCR was 100%, physicians may either not prescribe or cease antibiotic treatment [21,28]. This may lead to reduce the period of treatment for patients with WBC response in CSF before culture results are available. Furthermore, CSF laboratory findings in patients with non-infectious diseases or nonbacterial infections may result in the use of unnecessary antibiotics. Patients in the CSF WBC–positive group in our study represent these considerations. Antimicrobial treatment could be avoided for such patients, and the physicians could consider alternative diagnoses sooner.

It has been shown that the majority of culture-negative/PCR-positive discordant cases fit the clinical criteria for bacterial meningitis. There were no CSF culture positive cases that were not detected by PCR. This study highlights the importance of analyzing patient clinical details when assessing the efficacy of detection techniques. PCR assay has identified more positive children with bacterial meningitis than culture (43 cases versus 6 cases). The low percentage of positive CSF bacterial culture may be due to delay in culturing the CSF samples sent overnight have caused loss of some of the fastidious bacteria or administration of antibiotics before LP.

Thus, PCR is useful for the diagnosis of bacterial meningitis, especially when patients are on antibiotics. PCR can detect low amounts of bacteria in CSF. PCR results may be positive despite pre-treatment with antibiotics. PCR has the advantage of being rapid, results are reported within the same day in contrasts with the longer time needed for culture. For cases with bacterial meningitis, PCR provide early diagnosis, while for those cases with negative results, PCR gives an additional confirmation that antibiotics could be stopped.

Our study shows that most cases of meningitis were under two years of age (91%). The decreased ability to produce antibodies against polysaccharide capsular antigen in children less than 2 years of age may explain in part the increased susceptibility to *S. pneumoniae*. It was also found that bacterial meningitis occurs more in males than in females, and most often between 2 months and 2 years of age. This is also noted by other studies [29] [30] [31]. Male gender is one of the risk factors for meningitis [2].

NHS Meningitidis Real-TM kit used in this study is manufactured to test three pathogens most frequently associated with bacterial meningitis. The only detected pathogen in our study was *S. pneumoniae* (43%). Similar incidence was also found in other studies [14,32] [33] [34]. Pneumococci are the leading cause of childhood mortality worldwide, causing approximately one million deaths among children aged > 5 years [35]. The burden of pneumococcal disease is largely under investigation in developing countries [36]. PCV13 (pneumococcal polysaccharide conjugate vaccine 13-valent) has been included into the Iraqi vaccination program since 5/3/2017. The development of this vaccine has led to decrease the incidence of pneumococcal meningitis in countries with an active immunization programs. However, pneumococcus remains the most frequent cause of bacterial meningitis in children, due to many distinct serotypes of pneumococcus that have been identified and not covered by PCV13 vaccine [34]. *H. influenza* (Hib) is a highly virulent strain that cause the majority of *H. influenzae* meningitis cases was not detected among our group of patients this might be due to the introduction of Hib conjugate vaccine into the routine immunization schedule in Iraq since January 2012. Low incidence of Hib was reported in other studies due to Hib vaccination for infants [34] [37] [38] [39]. In UK, because of the introduction of *H. influenzae* vaccine in 1992, number of cases of *H. influenzae* meningitis in children under five years has fallen by 87% [40]. Meanwhile, meningococcus is the leading pathogen of meningitis in young adults [41].

The above information demonstrated that CSF culture could not be a considered as a method for early and exact diagnosis of bacterial meningitis. In conclusion, PCR has high sensitivity for the detection of bacterial pathogens such as *S. pneumoniae* in the CSF. This assay is useful for the diagnosis of bacterial meningitis, especially when results of CSF culture are negative and when patients had received antibiotics prior to LP. Due to the need to antimicrobial susceptibility testing, PCR should be considered as a complement to culture and antimicrobial susceptibility testing and other clinical and laboratory findings.

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