ORIGINAL ARTICLE

Clinical characteristics and molecular epidemiology of children with meningitis in Tehran, Iran: a prospective study

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Abstract

The molecular epidemiology of meningitis in children is unclear in Iran, and data are scarce. We aimed to characterize its clinical and paraclinical features as well as to determine the distribution of genotype/capsular types of common bacterial meningitis agents in children in Iran. All children suspected to have meningitis aged 4 days to 15 years were enrolled onto a prospective cross-sectional study from January 2015 to September 2017. Diagnostic values of clinical features, cerebrospinal fluid and serum parameters were evaluated independently and in combination with each other by multivariate logistic regression to develop a diagnostic rule. Genotype/capsular types of all the isolates were determined by targeting serotype-specific genes with uniplex or multiplex PCR. Among 119 patients suspected of having meningitis, 43 had bacterial meningitis, 19 aseptic and one tuberculous; and there were 56 nonmeningitis cases (NMC). Presentation of four features at the same time—cerebrospinal fluid white blood cell count, protein, polymorphonuclear leukocytes and serum C-reactive protein—revealed 100% sensitivity and 86.4% specificity for diagnosis of bacterial meningitis. *Haemophilus influenzae* type b (60%), *Streptococcus pneumoniae* serotype 3 (28.5%) and *Neisseria meningitidis* B (63.5%) were the most prevalent serotypes. This study demonstrated that a well-designed combination of clinical and paraclinical features is useful, but these combinations are not good enough to be relied on as stand-alone exclusionary tests for the diagnosis of bacterial meningitis. In addition, public immunization of infants with the most prevalent bacterial meningitis serotypes is recommended.

Keywords: Children, diagnosis, genotype, meningitis, molecular epidemiology
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Introduction

Meningitis is a medical emergent illness which leads to devastating neurologic sequelae and is almost always life-threatening without treatment [1,2]. Its prognosis depends heavily on rapid diagnosis and early sufficient treatment [3]. Children and neonates are at a high risk of neurologic disorders, especially meningitis [4]. There are many different types of meningitis, including bacterial, viral, fungal, parasitic, noninfectious and aseptic meningitis. Here we use the term 'aseptic meningitis' to describe both confirmed and presumed cases of viral meningitis [5]. Many studies previously suggested cerebrospinal fluid (CSF) analysis and clinical assessments to differentiate between the various types of meningitis [3,4,6–10]. However, now researchers pay less attention to that in favor of distinguishing suspected cases of meningitis from other infections (e.g.

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sinusitis, encephalitis, septicaemia) and neurologic disorders that may present similarly (e.g. hydrocephalus, seizure). Thus, well-designed studies are essential to accurately and rapidly distinguish suspected cases of meningitis by using clinical and laboratory findings that add potential value to standard laboratory tests [3,11].

A systematic search performed in our recent meta-analysis indicated that the distribution of common bacterial meningitis agent genotypes or capsular types is unclear in Iran—information essential for vaccine and prevention strategies [12]. Furthermore, some additional factors such as having poor laboratory facilities, as well as in a region of the world with high rates of advanced HIV- or tuberculosis-infected patients and drug-resistant pathogens make the diagnosis and prevention of meningitis complicated in Iran [13]. These issues create an exceptional challenge for clinicians in terms of the management of meningitis [13,14]. The 7-valent pneumococcal conjugate vaccine has become commercially available; however, it is not a mandatorily prescribed vaccine in Iran, although the conjugate polysaccharide vaccine against *Haemophilus influenzae* type b (Hib) is [12].

In this study, we focused on characteristics of clinical features and CSF findings solely and in combination with each other in children and neonates with suspected meningitis to create a multivariable diagnosis model for the diagnosis of meningitis. In addition, we aimed to determine the distribution of genotype/capsular types of common bacterial meningitis agents in children in Iran for vaccine and prevention strategies.

Materials and methods

Ethics statement

The ethics committee of Shahid Beheshti University of Medical Sciences approved this study (IR.SBMU.RETECH.REC.1395.212).

Study population and study design

We included all consecutive children (defined as patients <16 years of age) who were suspected of having meningitis in Baqiyatallah and Mofid paediatric hospitals (territorial hospitals in Tehran) in a retrospective cross-sectional study from March 2015 to March 2017. In a masked study, all of the clinical diagnoses, molecular assays, and CSF and serum analyses were performed by separate personnel unknown to each other. All included patients were completely vaccinated according to Iran's vaccine schedule.

Patients with suspected meningitis had any of the following criteria: (a) fever with signs of meningeal irritation, bulging fontanels, unexplained irritability, persistent nausea and vomiting, petechial rash or purpura; (b) unexplained alteration of consciousness with or without fever; (c) fever with no localizing signs in an ill or toxic child; and (d) complex febrile seizure with or without fever. Inclusion criteria were age <16 years old and suspected diagnosis of meningitis. Exclusion criteria were neurosurgical procedure performed before the onset of meningitis, presence of a shunt within the central nervous system or presence of a known immunodeficiency (Fig. 1). Duplicate samples from the same patient were excluded.

Conventional bacteriologic diagnostic tests such as smear staining and culture tests were performed for CSF and blood samples according to the laboratory methods for the diagnosis of meningitis reported by the World Health Organization [15] and the US Centers for Disease Control and Prevention guidelines (https://www.cdc.gov/meningitis/lab-manual/index.html) (Fig. 2).

On the basis of clinical and laboratory combination tests, confirmed patients were categorized into three groups: bacterial meningitis, aseptic meningitis and NMC. Clinical status was evaluated with the Glasgow Outcome Scale, which ranges from 1 to 5, as follows: 1 = death; 2 = persistent vegetative state (the patient is unable to interact with the environment); 3 = severe disability; 4 = moderate disability; 5 = good recovery [4]. Severe or moderate disability was defined as any of the following conditions: muscle weakness and immobility in one or more limbs; hearing loss; microcephaly; spasticity hydrocephalus; and seizure disorder. For the purpose of this analysis, a good outcome was defined as a score of 5, and a poor outcome was a score of 1 to 4. All demographic characteristics, serum and CSF findings were recorded for each patient.

DNA extraction

Upon arrival at the laboratory, 200 to 400 μL of each CSF and serum sample was extracted according to the Roche High Pure template DNA kit manual (Roche, Basel, Switzerland).

PCR survey

Conventional PCR for species identification was performed with primers specific for amplifying the genes *CtrA* (*Neisseria meningitidis*), *lytA* (*Streptococcus pneumoniae*) and *hpd* (*H. influenzae*) for all samples from patients suspected to have meningitis. PCR was performed as described previously [16]. Standard strains were obtained from the Persian Type Culture Collection (PTCC) and Microbial Collection of Pasteur Institute of Iran, *H. influenzae* Type Cultures (ATCC 35056), *N. meningitidis* (PTCC 1760) and *S. pneumoniae* (PTCC 1800 and PTCC 1240). Specificity of the three primer sets was determined by using genomic DNA from bacteria and viruses which are most likely to be present in CSF samples, such as

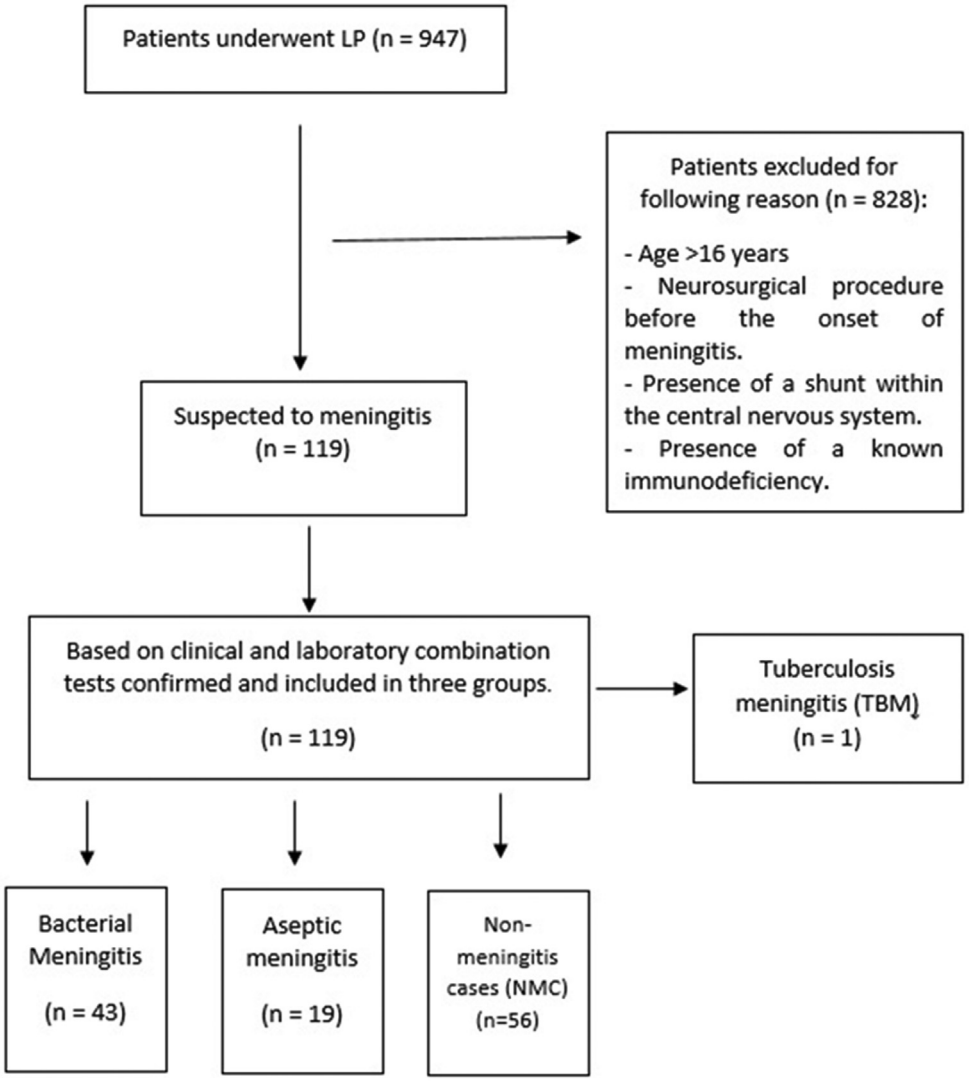

FIG. 1. Flow diagram of patient selection.

group B *Streptococcus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter, Staphylococcus aureus,* herpes simplex virus, Epstein-Barr virus, cytomegalovirus and varicella-zoster virus. Also, we prepared human genomic DNA from the microbiology department of Shahid Beheshti University. We used Taq DNA Polymerase 2× Master Mix Red (Ampliqon, Odense, Denmark) and optimized it in a gradient cycler (Mastercycler Gradient; Eppendorf, Hamburg, Germany). Primers, PCR assembly and cycling conditions are described in Supplementary Table S1.

Molecular genotyping by multiplex PCR

First, all of the confirmed bacterial meningitis CSF samples were examined with minor modifications designed to cover most of the *N. meningitidis*, *S. pneumoniae* and *H. influenzae* predominant serotypes reported in Asia and Africa [17,18]. Accordingly, each reaction was designed as uniplex or multiplex for targeting the serotype-specific genes of individual serotypes; it included an internal positive control (Supplementary Table S1).

Statistical analysis

All statistical analyses were performed by SPSS 16.0 software (IBM, Armonk, NY, USA) and MedCalc 12.1.4 (MedCalc Software, Ostend, Belgium). The chi-square test, Fisher exact test, Student *t* test or Mann-Whitney *U* test were used for comparing categorical and continuous variables and for comparisons between groups in nonnormally distributed variables. We determined a cutoff point for each scale variable (biomarkers) to differentiate the bacterial and aseptic meningitis from other similar disorders by using the receiver operating characteristic curve. Likewise, for each biomarker, sensitive specific values with 95% confidence interval were calculated on

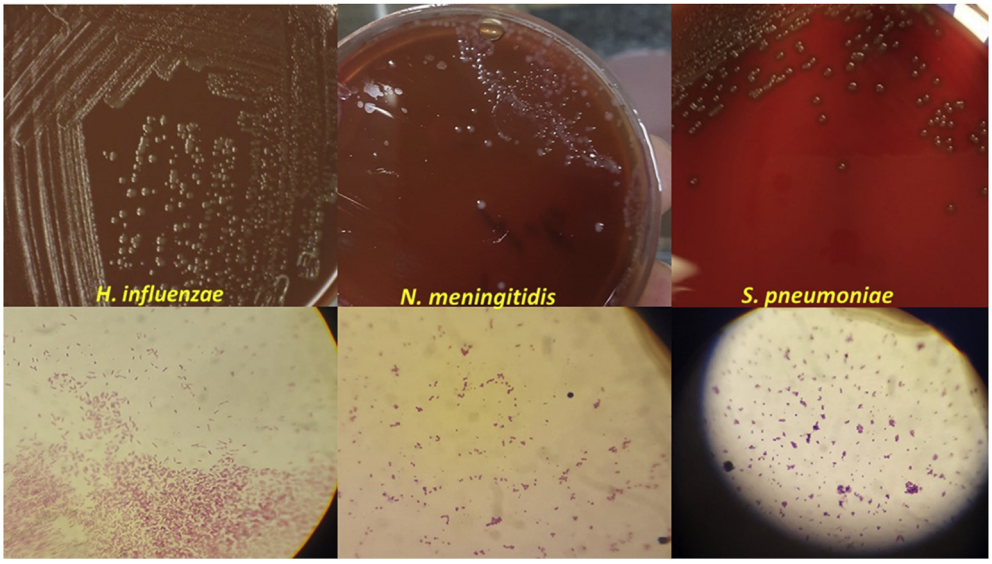

FIG. 2. *Haemophilus influenzae, Neisseria meningitidis* and *Streptococcus pneumoniae* colonies in upper images, and Gram staining in lower pictures.

the basis of the chosen cutoff point. Results are presented as mean ± standard deviation, or number and percentage. We used logistic regression to examine the accuracy of association of various biomarkers in distinguishing bacterial and aseptic meningitis from the other three groups. All statistical tests were two tailed with p <0.05.

Results

Among 119 patients suspected to have meningitis, 43 were confirmed as bacterial, 19 as aseptic and one as tuberculous, as well as 56 NMC, by using a combination of clinical and

TABLE 1. Clinical features and laboratory findings of study patients

Characteristic	Bacterial meningitis (*n* = 43)	Aseptic meningitis (*n* = 19)	NMC (*n* = 56)	Total (*n* = 118)	p
Male sex	25 (58)	10/17 (52.6)	32 (57.7)	67/116 (56.3)	0.91
Age group					0.17
<1 month	9 (21)	0	4/55 (5.3)	13/115 (11.3)	
1 month to 1 year	20 (46.5)	11/17 (64.7)	25/55 (44.7)	56/ 115 (48.7)	
1–7 years	9 (21)	4/17 (23.5)	19/55 (36.8)	32/ 115 (27.8)	
7–16 years	5 (11.6)	2/17 (11.8)	7/55 (13.2)	14/115 (12.2)	
Clinical features					
Fever	41 (95.3)	18 (95)	30 (53.6)	89 (75.5)	>0.005
Seizure	12 (28)	6 (31.5)	7/55 (12.7)	25/117 (21.4)	0.09
Sleepy	11/18 (61)	5/8 (62.5)	9/12 (75)	25/38 (65.8)	0.71
Hydrocephalus	3 (7)	1/17 (6)	0	4/115 (3.5)	0.14
Anorexia	4/35 (11.5)	4/13 (31)	6/36 (16.7)	14/84 (16.7)	0.28
Nausea, vomiting	19 (44.2)	12/18 (66.7)	18/56 (32)	49/117 (42)	0.03
Cough	6 (14)	5/18 (28)	15/55 (27.3)	26/116 (22.4)	0.24
Diarrhea	5 (11.5)	3/18 (16.7)	7/55 (12.7)	15/116 (13)	0.86
Poor feeding	5 (11.5)	0	9/55 (16.4)	14/115 (12.2)	0.19
Headache	7 (16.3)	4/17 (23.5)	8 (14.3)	19/116 (16.5)	0.66
Coryza	1/29 (3.4)	4/12 (33.3)	2/22 (9)	7/63 (11)	0.02
Neck stiffness	1/25 (4)	0	1/14 (7)	2/50 (4)	0.66
Rash	5 (11.5)	1/17 (6)	1/56 (1.8)	7/116 (6)	0.12
Daze	3 (7)	1 (5.3)	3 (5.4)	7/118 (6)	0.93
Double vision	2/7 (28.6)	0	3/6 (50)	5/14 (35.7)	0.53
Birth, term	7/9 (77.8)	6/6 (100)	11/11 (100)	24/26 (92.3)	0.13
Parent, relatives	20/30 (66.7)	7/10 (70)	24/34 (70.6)	51/74 (69)	0.94
Surgery history	1 (2.3)	0	2 (3.6)	3 (2.5)	0.7
Outcome, good	27/33 (82)	10/12 (83.3)	38/42 (90.5)	75/87 (86)	0.53
Lab findings					
Blood culture	3 (7)	0	3/55 (5.5)	6/115 (5.2)	0.02
CSF culture	3 (7)	0	0	3/115 (2.6)	0.1
CSF smear	2 (4.7)	0	0	2/118 (1.7)	0.31
Blood smear	1 (2.3)	0	0	1/116 (1)	0.63
Urine culture	3 (7)	0	1/55 (1.8)	4/113 (3.5)	0.26

Data are presented as *n* (%) or *n*/*N* (%).
CSF, cerebrospinal fluid; NMC, nonmeningitis cases.

TABLE 2. Serum findings in study patients

Characteristic	Bacterial meningitis (n = 43)			Aseptic meningitis (n = 19)			NMC (n = 56)			p
	n	Mean ± SD	Range	n	Mean ± SD	Range	n	Mean ± SD	Range	
CSF RBC	29	3178 ± 15753	0–85000	8	587 ± 1386	1–4000	38	108 ± 304	0–1500	0.44
ESR (mm/h)	25	33.8 ± 37.3	2–85	12	23 ± 20	5–65	29	32.5 ± 27.27	2–95	0.45
Blood WBC	36	16285 ± 15285	3300–88000	18	12365 ± 4880	4400–22000	52	6415 ± 3901	1100–18000	>0.001[a]
Blood Poly	28	57.25 ± 19.87	23–91	16	53.68 ± 15.3	30–85	33	53.7 ± 22.3	20–95	0.76
Blood lymphocytes	28	39.6 ± 19.3	6–75	16	41.56 ± 15.3	14–70	32	41.5 ± 21	5–80	0.91
Blood Na	34	134.2 ± 2.7	129–139	12	134.2 ± 2.8	130–139	33	135.8 ± 7	125–165	0.37
Blood K	34	4.6 ± 0.76	3–7	12	4.5 ± 0.37	4–5	32	4.1 ± 1.2	0–8	0.12
CRP	28	64 ± 64.3	1–149	15	16.9 ± 12.7	1–45	28	14.64 ± 25.15	1–84	>0.001[a]
Body temperature	43	38.3 ± 0.75	37–40	19	38.4 ± 0.84	37–40	56	37.7 ± 1	36–40	0.002

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NMC, nonmeningitis cases; Poly, polymorphonuclear leukocytes; RBC, red blood cell; WBC, white blood cell count.
[a]Leven test was statistically significant; we used Welch analysis of variance.

laboratory findings (Fig. 1). Bacterial meningitis was confirmed by the following positive tests or combination of tests: 28 PCR (seven *S. pneumoniae,* 11 *N. meningitidis*, ten *H. influenzae*), three CSF culture and PCR (two *S. pneumoniae* and one *Acinetobacter baumannii*), one blood culture and PCR (*S. pneumoniae*), one CSF microscopy examination (Gram-positive coccobacillus), one CSF microscopy examination and blood smear (Gram-negative diplococci) and one blood smear and BACTEC (Gram-negative coccobacillus). Seven meningitis cases were confirmed by a combination of clinical and CSF findings. Moreover, aseptic meningitis was confirmed using the combination of clinical and CSF findings. In addition, 67 patients (56%) were boys and 52 (44%) were girls, with a minimum age of 4 days and a maximum age of 15 years. Fever was the most frequent feature in patients with bacterial and aseptic meningitis, with 41 (95.3%) of 43 and 18 (95%) of 19 respectively (Table 1).

Clinical features

In terms of the clinical features, body temperature, nausea/vomiting and coryza/snivel were statistically significant for differentiation from each other of bacterial meningitis, aseptic meningitis and NMC (Table 1). Furthermore, *post hoc* analysis showed that body temperature was significant for differentiation of bacterial and aseptic meningitis from NMC (Supplementary Table S2).

Serum findings

Only serum C-reactive protein (CRP) and blood white blood cell count (WBC) were significant for differentiating between

TABLE 3. CSF findings in study patients

Characteristic	Bacterial meningitis (n = 43)			Aseptic meningitis (n = 19)			p
	n	Mean ± SD	Range	n	Mean ± SD	Range	
CSF RBC	29	3178 ± 15 753	0–85 000	8	587 ± 1386	1–4000	0.65
CSF WBC (cell/mm^3)	43	1809 ± 4430	230–21 200	19	324 ± 458	20–2050	0.03[a]
CSF poly (%)	37	64.3 ± 26.7	3–95	18	29.6 ± 21.7	1–85	>0.001
CSF lymph (%)	37	33 ± 25	2–90	18	70.3 ± 22	15–99	>0.001
CSF Pro	41	144.7 ± 131	35–670	14	54.3 ± 64	10–260	0.01
CSF Glu	41	42.6 ± 21.7	10–116	14	61.6 ± 20	39–112	0.01
CSF:blood glucose ratio	15	0.53 ± 0.34	0–1.22	5	0.37 ± 0.28	0–0.66	0.28

CSF, cerebrospinal fluid; Glu, glucose; Poly, polymorphonuclear leukocytes; Pro, protein; RBC, red blood cell; WBC, white blood cell count.
[a]Leven test was statistically significant; we used Welch analysis of variance.

TABLE 4. Accuracy of CSF and serum findings for differentiation of bacterial meningitis from aseptic meningitis

Biomarker	AUC	Cutoff values	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CSF WBC	0.881	420	81.4	84.2	92	66
CSF Pro	0.880	79	80.5	78.6	91	57
CSF poly	0.789	50	78.4	88.9	93	66
CSF Glu	0.784	51.5	71.4	73.2	47	88
Serum CRP	0.894	32	78.3	80	88	66.4
Blood WBC	0.476	10 250	61	49	70	38
CSF WBC; Pro, Poly and serum CRP	0.977	—	100	86.4	80	100
CSF WBC and Pro, Glu, Poly and serum CRP	1	—	100	100	100	100

AUC, area under the plasma concentration vs. time curve; CRP, C-reactive protein; Glu, glucose; NPV, negative predictive value; Poly, polymorphonuclear leukocytes; PPV, positive predictive value; Pro, protein.

bacterial meningitis, aseptic meningitis and NMC (Table 2). Moreover, *post hoc* analysis indicated that serum CRP and blood WBC were significant for differentiation of bacterial and aseptic meningitis from NMC (Supplementary Tables S3 and S4).

CSF findings

As shown in Table 3, amounts of CSF WBC, and percentage of polymorphonuclear leukocytes (Poly), lymphocytes, protein (Pro) and glucose (Glu) were significant for differentiation of bacterial and aseptic meningitis from each other.

Accuracy of CSF and serum findings

Table 4 shows the biomarkers with the highest sensitivity, specificity, area under the plasma concentration vs. time curve (AUC) and cutoff values for differentiation of bacterial meningitis from aseptic meningitis. Except serum CRP, with 78.3% sensitivity and 80% specificity with a cutoff value of 32, most of the serum findings had low sensitivity and specificity for distinguishing bacterial and aseptic meningitis from each other. However, CSF findings such as WBC, Pro, Poly and Glu had high accuracy, with 0.881, 0.880, 0.789 and 0.784 AUC respectively for differentiation of bacterial meningitis from aseptic meningitis. Logistic regression analysis showed that the presentation of four features at the same time (CSF WBC, Pro, Poly and serum CRP) revealed 100% sensitivity and 86.4% specificity (Fig. 3), and association of five features (CSF WBC, Pro, Poly, Glu and serum CRP) showed 100% sensitivity and 100% specificity for differentiation of bacterial and aseptic meningitis from each other (Fig. 4 and Table 4).

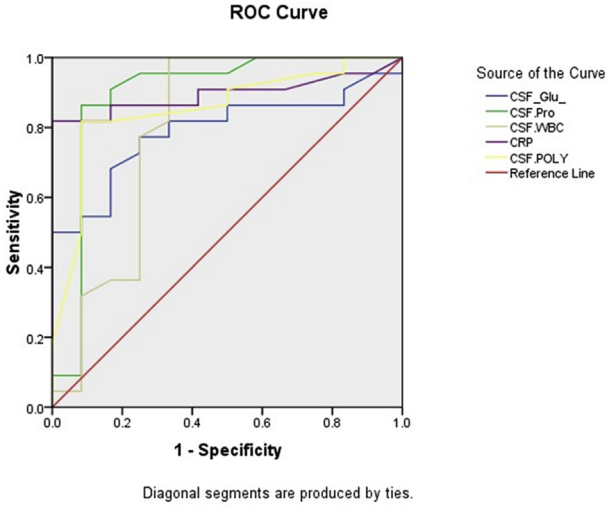

FIG. 3. ROC plot, accuracy of best biomarkers for differentiation of bacterial meningitis from aseptic meningitis. Left upper corner shows accurate biomarker with high sensitivity and specificity. ROC, receiver operating characteristic.

Molecular genotyping

Of 28 PCR-positive isolates, ten (35.7%) were *H. influenzae,* seven (25%) *S. pneumoniae* and 11 (40%) *N. meningitidis*. Table 5 shows the distribution of *H. influenzae, S. pneumoniae* and *N. meningitidis* serotypes on the basis of patient age group. Hib (60%), *S. pneumoniae* serotype 3 (28.5%) and *Neisseria meningitidis* B (NmB) (63.5%) were the most prevalent serotypes in *H. influenzae, S. pneumoniae* and *N. meningitidis* respectively.

Discussion

Meningitis is an emergency disease with a high rate of mortality and morbidity in Iran, especially in children. The molecular epidemiology of meningitis in children is unclear in Iran and is rarely performed. We aimed to characterize clinical and paraclinical features as well as the distribution of genotype/capsular types of common bacterial meningitis agents in children in Iran.

Early diagnosis of meningitis is essential to improve the prognosis of the disease. Physicians find it difficult to diagnose the various forms of meningitis and separate meningitis from other disorders with similar presentations [13,19]. PCR has a wide range of specificity (57–94%) and sensitivity (30–95%) in diagnosing the cause of meningitis [20–24]. In this study, 72% of the cases of bacterial meningitis were PCR positive, which

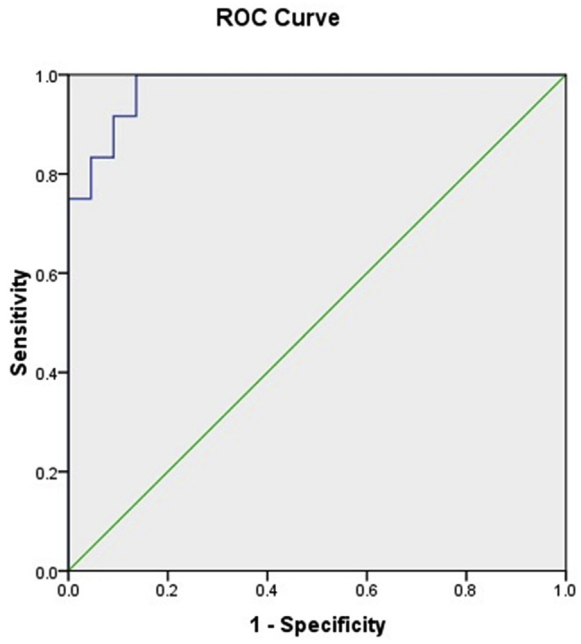

FIG. 4. ROC plot, accuracy of four features (CSF WBC, Pro, Poly and serum CRP) association at same time for differentiation of bacterial meningitis from aseptic meningitis. Left upper corner shows accurate biomarker with high sensitivity and specificity. CRP, C-reactive protein; CSF, cerebrospinal fluid; ROC, receiver operating characteristic; WBC, white blood cell count.

could be due to the low copy numbers of the pathogen's nucleic acid in CSF. However, nucleic acid amplification methods have some limitations because of their high cost and the need for special laboratory facilities, which makes it particularly difficult to use in developing countries [6,13,25]. Moreover, although CSF culture is the reference standard for the diagnosis of bacterial meningitis, only 4% to 70% of meningitis cases are positive by CSF culture [10,13,21,24–27]. Our results showed that only 7% of the bacterial meningitis cases were culture positive. Because the Mofid paediatric hospital is a referral hospital, most meningitis cases are referred to it from other hospitals. Patients routinely receive antibiotic therapy before a lumbar puncture is performed; this could be a reason for the low meningitis culture-positive cases in our study. Studies have indicated that standard microbiology diagnostics have far different sensitivities for meningitis [6,13,24], which we also found in our study. A combination of clinical and laboratory features is therefore essential to diagnose patients suspected to have meningitis [28]. Previously, many studies suggested CSF analysis and clinical assessments to differentiate between the various types of meningitis [3,4,6–10,29]. However, when other similar disorders are considered, assessment of clinical signs and symptoms should be interpreted carefully because of overlapping clinical features.

Our results showed that only fever was a significant clinical feature in distinguishing bacterial and aseptic meningitis from each other. In regard to serum findings, previous studies showed that increasing the CRP level in serum could be helpful in the

TABLE 5. Distribution of *Haemophilus influenzae, Streptococcus pneumoniae* and *Neisseria meningitidis* genotype/capsular types based on patient age group

		Age group				
Organism	**Genotype/capsular type**	**< 1 month (n = 13)**	**1 month to 1 year (n = 56)**	**1–7 years (n = 32)**	**7–16 years (n = 14)**	**Total (n = 115)**
H. influenzae	Hia	0	0	0	0	0
	Hib	1	2	2	1	6 (60%)
	Hic	0	0	1	1	2 (20%)
	Hid	0	0	0	0	0
	Hie	0	0	0	0	0
	Hif	0	0	0	0	0
	Nontypeable[a]	0	2	0	0	2 (20%)
	Total	1 (10%)	4 (40%)	3 (30%)	2 (20%)	10 (100%)
S. pneumoniae	3	0	1	1	0	2 (28.5%)
	23F	0	1	0	0	1 (14.3%)
	19F	0	1	0	0	1 (14.3%)
	19A	1	0	0	0	1 (14.3%)
	11	0	1	0	0	1 (14.3%)
	14	0	0	0	1	1 (14.3%)
	Total	1 (14.3%)	4 (57%)	1 (14.3%)	1 (14.3%)	7
N. meningitidis	NmB	2	3	1	1	7 (63.5%)
	NmC	0	1	1	1	3 (27%)
	NmA	0	0	0	0	0
	NmW135	0	0	0	0	0
	NmY	0	0	0	0	0
	NmX	0	0	0	0	0
	Nontypeable[a]	1	0	0	0	1 (9%)
	Total	3 (27%)	4 (36.5%)	2 (18%)	2 (18%)	11 (100%)

[a]Nontypeable refers to isolates that did not react with study primers.

differentiation of bacterial meningitis from aseptic meningitis, with 70% to 100% sensitivity and 90% to 100% specificity [6,30]. We found that a serum CRP with a cutoff value of 32 mg/ml had 78.3% sensitivity and 80% specificity for differentiation of bacterial meningitis from aseptic meningitis; however, in a similar study setting, Dashti et al. [6] reported that CRP with cutoff value of 57 mg/mL had 91% sensitivity and 10% specificity in differentiating bacterial meningitis from viral meningitis. Moreover, logistic regression analysis demonstrated that assessment of five biomarkers (CSF WBC, Pro, Poly, Glu and serum CRP) could have 1 AUC for differentiation of bacterial meningitis from viral meningitis; however, Dashti et al. reported 0.994 AUC for the association of three biomarkers (CRP and erythrocyte sedimentation rate; CSF absolute neutrophil count; and CSF lactate) in differentiating bacterial meningitis from viral meningitis [6].

The range of CSF WBC count reported in bacterial meningitis (mean, 1500–3143 cells/mL) was significantly higher than that in viral meningitis (mean, 159–539 cell/mL) [6,12,31,32]. Our results also demonstrated that the mean CSF count was 1809 cells/mL for bacterial meningitis and 324 cells/mL for aseptic meningitis. It should be noted that the marked difference in CSF WBC counts and lower cell counts may be because our study sample included immunocompromised children as well as immunocompetent children with pneumococcus and meningococcus meningitis [6,31]. The left upper corner of the receiver operating characteristic plot shows a higher sensitivity and specificity of biomarkers in distinguishing between bacterial and aseptic meningitis (Figs. 3 and 4). Consistent with previous reports, serum CRP, CSF Poly, CSF WBC and CSF Pro, in order, have the highest accuracy for bacterial meningitis (Figs. 3 and 4) [6,14,32,33].

@@Our study showed that Hib (60%), serotype 3 (28.5%) and NmB (63.5%) were the most prevalent serotypes in *H. influenzae, S. pneumoniae* and *N. meningitidis* respectively. Previously, several studies reported that serotypes 11, 14, 23F, 15B/15C, 18C, 19F and 19A for *S. pneumoniae*, serogroups NmB, NmC *N. meningitides,* and type b and c *H. influenzae* represent the causative agent in approximately 70% to 85% of children with bacterial meningitis, especially in nonvaccine regions [34–38]. Therefore, the distribution of genotype/capsular types of common agents causing bacterial meningitis in children in Iran are not different from other regions of the world.

The main limitation of the present study was that the incidence of meningitis in children in Iran is less considered in this study. For future direction, additional and further well-designed studies are necessary to accurately apply new biomarkers to assess suspected cases of meningitis which will add potential value to standard laboratory tests. It is also necessary to perform comprehensive molecular epidemiology studies from different regions of Iran.

In conclusion, we demonstrated that a well-designed combination of clinical and paraclinical features could be useful to diagnose bacterial meningitis, but these features are not good enough to be relied on as stand-alone, exclusionary tests. In addition, public immunization of infants with the most prevalent bacterial meningitis serotypes is recommended.

Conflict of Interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2019.100594.

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