Study of Bacterial Agents of Meningitis in Children and Detection of Their Antibiotic resistance Patterns in Hamadan, Western Iran.

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Abstract: Bacterial meningitis is still one of the most dangerous infection diseases and causes serious complications in children.The aim of present study was to identify the most common of bacteria causing meningitis in children under 10 years of age and detection of their resistance to antibiotics in patients who referred to hospitals of Hamadan. Overall 582 children suspected to meningitis were investigated for CSF cultures, elevation of CSF leukocyte count, hypoglucorrhachia and clinical manifestations, from January 2009 to December 2013 in two hospitals. The required data of patients were gathered through a questionnaire and analyzed using Epi6 system. The species were identified by biochemical and latex-agglutination tests. Antibiogram tests were also performed by gel-diffusion method of Kirby-Bauer. Out of 582 children suspected to meningitis, 146 children (%25.1) had positive bacterial culture that %58.9 of isolates was Gram-positive cocci and %41.1 was also Gram-negative bacilli. The most common isolates were *Streptococcus* (*S.*) *pneumonia* (%23.9), *Staphylococcus* (*S.*) *aureus* (%13.1), *Escherichia* (*E.*) *coli* (%10.9), *Neisseria* (N.) *meningitides* (%6.2) and *Haemophilus* (*H.*) *influenzae* type b (%4.1). Of 146 patients with bacterial meningitis, 79 cases (54.2%) were males and 67 cases (45.8%) were females. Fever (88.3%), Leukocytosis (74.6%), nausea and vomiting (67.1%), sleepness (66.4%) and headache (46.6%) were most common clinical manifestations of patients with bacterial meningitis. The most effective antibiotics against isolates were ceftizoxime, kanamycin, and gentamicin while most of isolates showed high resistance against ampicillin, sulfamthoxazole-trimethoprim, chloramphenicol and amoxicillin. The present study showed that gram positive cocci in particular, *S. pneumoniae* and *S. aureus* are predominant causes of bacterial meningitis in children under 10 years of age in this region. Most species showed high resistance against routine antibiotics such as amoxicillin, ampicillin and chloramphenicol. The antimicrobial susceptibility pattern for bacteria causes meningitis may provide a guideline for the selection of appropriate drug treatment.

1. Introduction

Bacterial meningitis is a serious infection of the fluid in the spinal cord and the fluid that surrounds the brain. The bacteria often live harmlessly in a person's mouth and throat. In rare instances, however, they can break through the body's immune defenses and travel to the fluid surrounding the brain and spinal cord. There they begin to multiply quickly. Soon, the thin membrane that covers the brain and spinal cord (meninges) becomes swollen and inflamed, leading to the classic symptoms of meningitis [1,2].

Bacterial meningitis is one of the most potentially serious infections in infants and older children because it is associated with a high rate of acute complications, risk of chronic morbidity and death. The complications resulting from this disease are quite common and include blindness, hearing loss, nervous disorders, seizures, decreasing of conscious and paralysis [1,2,3,4]. Before routine use of pneumococcal conjugate vaccine, bacterial meningitis affected almost 6000 people every year in the United States, and about half of all cases occurred in children 18 years old or younger [2,5]. Prompt and accurate diagnosis and adequate treatment of bacterial meningitis in children remains a major challenge, as reflected by the continued high morbidity and case-fatality rates of the disease worldwide [1,6,7].

It is important to know which type of bacteria is causing the bacterial meningitis because antibiotics can prevent some types from spreading and infecting other people. Bacterial meningitis is most commonly caused by one of three types of bacteria: *Haemophilus* (*H.*) *influenzae* type b, *Neisseria* (*N.*) *meningitis*, and *Streptococcus* (*S.*) *pneumoniae*. However, other bacteria such as *Staphylococcus* (*S.*)

aureus, *Streptococcus* (*S.*) *agalactiae*, *Escherichia* (*E.*) *coli*, *Proteus species*, *Pseudomonas* (*P.*) *aeruginosa*, *Klebsiella* (*K.*) *pneumonia* and *Enterobacter species* could be rarely caused of meningitis [3,8,9,10,11].

Before the 1990, *H. influenzae* type b was the leading cause of bacterial meningitis, but new vaccines being given to children as part of their routine immunizations have reduced the occurrence of serious *H. influenzae* type b disease [7,12,13]. Today, *N. meningitis* and *S. pneumoniae* are the leading causes of bacterial meningitis [14,15].

Bacterial meningitis can be treated with a number of effective antibiotics. It is important, however, that treatment be started early in the course of the disease. Appropriate antibiotic treatment of most common types of bacterial meningitis should reduce the risk of dying from meningitis to below 15%, although the risk is higher among the elderly [2,16]. Because of the major increasing of antibiotic resistance in bacteria causing bacterial meningitis in the last few years, attempts have been made to organize an appropriate antibiotic treatment regime with combination of synergistic antibiotics [16,17,18,19].

The purpose of this study was to describe the frequency of etiologic agents of bacterial meningitis among children under 10 years of age in a sample of patients in Hamadan (Western Iran), with emphasis on the frequency of bacterial meningitis of unknown etiology and detection of the drug resistance of isolated bacteria from patients with bacterial meningitis.

2. Material and Methods

A cross-sectional and prospective descriptive study was conducted on children under 10 years of age who were suspected to bacterial meningitis and admitted in the pediatric department of two hospitals (Farshchian and Be'sat) in Hamadan, the west of Iran between January 2009 to December 2013. Active surveillance for cases of bacterial meningitis among the study population, which comprised 582 children, was implemented. The required data of the patients including clinical manifestations, patient demographics (age, sex and season) and types of microorganisms isolated from cerebrospinal fluid (CSF) were recorded in a special questionnaire and then analyzed using EP1 INFO 6 software package.

After the establishing of indication, lumbar puncture was performed. CSF was sent to cytological, biochemical and bacteriological analyses. Diagnosis of meningitis was established when at least three criteria of four were fulfilled: 1. isolation of the cause by CSF culture and identification of bacteria by the Gram stain of CSF sediment; 2. elevation of CSF leukocyte count more than 20 with predominance of polymorphonuclear leukocytes; 3. hypoglucorrhachia (glycaemia less than 40 mg percent or less than 50 percent of glycaemia); 4. clinical manifestations including fever, consciousness, neck stiffness and sign of mental irritation. The sampling of CSF of each patient was done to culture at two times to make sure of growing any pathogenic microorganisms. The etiologic diagnosis was based on either stained-smears (Gram), culture and latex-agglutination test.

Cultivation and isolation of organisms were performed according to the methods outlined in the Bailey & Scott's Diagnostic Microbiology [20]. The sediment of spinal fluid was prepared for providing the smear and cultures. Specimens were then cultured on appropriated culture media including Thayer-Martine agar, Eeosin Methylen Blue (EMB) agar and Blood agar (Merck, Germany). The cultures were incubated in 37^{o}C for 24-48 hours and then the colonies were removed for further studying. Biochemical and antigenic properties of isolates were verified for identification purposes. In essential cases, specific antiserum against bacteria was used for precise identification of bacteria type [21].

In order to detection of the susceptibility of isolates to routine antimicrobial drugs, the all isolates were tested by disc diffusion (Kirby-Bauer) method [22]. Eight antibiotics including amoxicillin (AMX), ampicillin (AM), chloramphenicol (CH), cephalexin (CF), ceftizoxime (CT), gentamicin (GM), kanamycin (KA) and sulfamthoxazole trimethoprim (SXT) were applied. Results were then collected and analyzed.

3. Results

Out of 582 children less than 10 years suspected to meningitis, only 146 children (25.1%) had positive bacterial culture that 58.9% of isolates was Gram-positive cocci and 41.1% was also Gram-negative bacilli. The most common isolates of Gram-positive cocci were as follow: *S. pneumonia* (23.9%), *S. aureus* (13.1%), *S. agalactiae* and *Staphylococcus* (*S.*) *epidermidis* each 13 cases (8.9%). The most common isolates of Gram-negative bacilli were: *E. coli* (10.9%), *N meningitis and p. aeruginosa each 9 cases (6.2%) and H. influenzae type b (4.1%).*

Frequencies distributions of the all isolated

Fever (88.3%), Leukocytosis (74.6%), nausea and vomiting (67.1%), sleepness (66.4%) and headache (46.6%) were most common clinical manifestations of meningitis in children under 10 years of age with bacterial meningitis. Frequencies of clinical manifestations of patients are shown in table 2

Of 146 patients with bacterial meningitis, 79 cases (54.2%) were males and 67 cases (45.8%) were females. The frequencies of age groups of patients

with bacterial meningitis are shown intable 3, the most frequent patients were belonged to the 0-2 years old (28.3%) and the least frequent patients were belonged to the 8-10 years old (13.1%). Our results showed that the most frequent bacterial meningitis (43.7%) was occurred during fall and then winter with frequency of 27.6%, and summer 14.8%. The lowest frequency (13.9%) was belonged to winter.

Table 1. Frequencies of isolated microorganisms from patients with bacterial meningitis

Isolate	Frequency of isolates	Percentage
Streptococcus pneumonia	35	23.9
Staphylococcus aureus	19	13.7
Escherichia coli	16	10.9
Streptococcus agalactiae	13	8.9
Staphylococcus epidermidis	13	8.9
Neisseria meningitides	9	6.2
Pseudomonas aeruginosa	9	6.2
Citrobacter freundii	7	4.8
Klebsiella pneumonia	7	4.8
Haemophilus influenzae	6	4.1
Enterococci spp	3	2.1
Proteus spp.	3	2.1
Unknown	6	4.1
Total	146	100

Table 2.The frequencies of clinical manifestations of the patients with bacterial meningitis

Symptoms	Frequency	Percentage
Fever	129	88.3
Leukocytosis	109	74.6
Nausea and vomiting	98	67.1
Sleepiness	97	66.4
Headache	68	46.6
Hydrocephalus	59	40.4
Neck stiffness	55	37.6
Irritability	52	35.7
Anorexia	51	34.9
Confusion	48	32.9
Feeding poorly	46	31.8
Sensitivity to light	34	23.3
Dyspnea	11	16.4
Seizures	10	6.8

Antibiotic resistance patterns of isolated bacteria from patients with bacterial meningitis are shown in table 4. As it is observed, the most effective antibiotics against both Gram-negative bacilli and gram-positive cocci were ceftizoxime, kanamycin, and gentamicin while most of isolates showed high resistance against ampicillin, sulfamthoxazole-trimethoprim, chloramphenicol and amoxicillin. P. aeruginosa, S. aureus, S. epidermidis and K. pneumonia showed the highest resistance to many of antibiotics applied in this study. P. aeruginosa showed hundred percent resistance to four antibiotics including ampicillin, sulfamthoxazole-trimethoprim, cephalexin and amoxicillin. S. aureus, S. epidermidis and K.
pneumoniae were also resistant (100%) to ampicillin. However, *S. pneumonia, S. agalactiae, N. meningitis* and *H. influenzae* type b, showed low or moderate resistance against antibiotics used in this study. *S. pneumonia, N. meningitis* and *H. influenzae* type b showed no resistance (0%) to ceftizoxime and gentamicin, they were sensitive to antibiotics of aminoglycosides groups including kanamycin and gentamicin, and group of cephalosporines including cephalexin and ceftizoxime

Table 3. The frequencies of the age groups of patients with bacterial meningitis

Age groups	Frequency	Percentage
0-2 years old	41	28.3
2-4 years old	35	23.9
4-6 years old	22	15.2
6-8 years old	29	19.6
8-10 years old	19	13.1
Total	146	100

Table 4. Antibiotic resistance patterns of isolated bacteria from patients with bacterial meningitis

Isolate	Frequencies of resistance to antibiotics (%)							
	SXT*	KA	GM	CT	CF	CH	AM	AMX
Streptococcus pneumonia	27	9	0	0	18	36	63	54
Staphylococcus aureus	68	48	16	33	48	84	100	84
Escherichia coli	44	25	13	0	38	38	57	19
Streptococcus agalactiae	47	0	23	23	0	70	23	0
Staphylococcus epidermidis	70	23	31	39	54	77	100	85
Neisseria meningitides	0	0	0	0	33	33	55	33
Pseudomonas aeruginosa	100	33	33	66	100	66	100	100
Citrobacter freundii	72	58	43	58	43	72	85	100
Klebsiella pneumonia	43	29	29	15	29	85	100	58
Haemophilus influenzae	50	0	0	0	0	0	50	50

* :SXT= Sulfamthoxazole-trimethoprim, KA= Kanamycin, GM= Gentamicin, CT= Ceftizoxime, CF=Cephalexin, CH= Chloramphenicol, AM=Ampicillin, AMX=Amoxicillin

4. Discussions

Knowing whether a virus causes meningitis or bacterium is important because the severity of illness and the treatment differ. Viral meningitis is generally less severe and resolves without specific treatment, while bacterial meningitis can be quite severe and may result in brain damage and learning disability

[1,2]. Etiologic agents of bacterial meningitis are variable and usually depend to time, geographical location, and age of patients. Most researchers have demonstrated that *H. influenzae* type b, *S. pneumonia* and *N. meningitis* are as main agents of bacterial meningitis especially in children [2,3,4,23]. In a study [24] from the United State revealed the most common isolated bacteria from newborns was *H. influenzae* type b (45%), *S. pneumoniae* (18%) and *N. meningitides* (14%), respectively. In a same study [25], other researcher was indicated that the bacterial agents of meningitis in 70% of children between 1 to 5 years of age were *H. influenzae* type b, *N. meningitis* and *S. pneumoniae*, respectively. R. Laxer et al., in a study [26] that carried out in some third world countries including Senegal and Dakar indicated that *S. pneumoniae* was the most common meningitis agent in children. Another study [27] from Central Iran (hospital of Valieasr in Brojen), the most common causes of meningitis in children were *H. influenzae* type b (36.8%), *S. pneumonia* (26.3%), *N. meningitis* (10.5%) and *Salmonlla paratiphy A* (10.5%), respectively. In our study, *S. pneumoniae* (23.9%), *S. aureus* (13.1%) and *E. coli* (10.9%) have been the most important causes of bacterial meningitis in involved children. These findings are very similar to a research that was performed by Laxer et al., in third world countries [26].

As observed, in most studies done in all over the world, *H. influenzae* type b and *N. meningitis* are known as main causes of meningitis in infants and children, whereas in present study only 4.4% of patients had *H. influenzae* type b and 6.5% had *N. meningitides.* This event may be because of widespread of vaccination against these strains. With the decline in *H. influenzae* type b disease, cases of bacterial meningitis have decreased since 1986 [1,13]. Anyone can get bacterial meningitis, but it is most common in infants and children. People who have had close or prolonged contact with a patient with meningitis caused by *N. meningitis* or *H. influenzae* type b can also be at increased risk. Our study revealed a decrease in the proportion of *H. influenzae* type b and *N. meningitides* in children. Meningococcal meningitis is a continuing threat in day-care centers and schools. Healthy children and young adults are susceptible, and death can occur within a few hours of onset. In our study, *N. meningitis* was not a common isolate.

In our study, Gram-negative bacilli such as *E.coli* (10.9%), *P. aeruginosa* (6.2%), Citrobacter (C.) freundii (4.8%) and *K. pneumonia* (4.8%) had important contribution in creating bacterial meningitis. *S. aureus* (13.1%) was also the second predominant cause of bacterial meningitis in patients involved. *P. aeruginosa* and *S. aureus* are versatile human pathogen that continues to be an important cause of nosocomial infections especially in pediatric units [28,29,30]. *P. aeruginosa* and *S. aureus* meningitis in children and neonates were presented to withdraw attention to this clinical entity.

The diagnosis of bacterial meningitis is rarely a difficult diagnostic dilemma when a patient presents with fever, headache, neck stiffness, and altered mental status. Recording of symptoms and signs of infant patients in chart, was a major problem in this study. The most frequent patients were belonged to the 0-2 years of age (28.3%). Unfortunately, for the practicing clinician, patients are rarely that straightforward. Patients who are elderly, very young, or immuno-compromised often present with subtle findings, making the correct diagnosis a challenge. In addition, patients being treated with antibiotics may be misleading in their clinical presentation, leading to a missed diagnosis of meningitis [2,4,16]. Only when one considers the diagnosis or obtains a sample of cerebrospinal fluid is the correct diagnosis made. Although the clinical scenario may suggest meningitis, it is the cerebrospinal fluid white blood cell count that establishes the definitive diagnosis. In our study, Leukocytosis of patients involved was the second predominant of clinical manifestation (74.6%).

Out of 8 tested antibiotics in this study, gentamicin, kanamycin and ceftizoxime were the most effective antibiotics in both Gram-positive cocci and Gram-negative bacilli. Regarding some reports [6,16,18,31,32,33], antibiotics of beta-lactam (ampicillin and amoxicllin), cephalosporin and also chloramphenicol have been recommended as effective drugs for those affected by bacterial meningitis, whereas most of the isolated bacteria in this study, showed high drugs resistance (between 20% to 100%) to many of these mentioned antibiotics (table 4). *S. pneumoniae*, a common pathogen in pediatric infections, has become resistant to penicillin and makes these infections difficult to treat [2,14,17,18,33]. The National Reference Center in France [34] for Pneumococci determined the susceptibility to antibiotics of 2,837 *S. pneumoniae* isolated in 1997. The incidence of *S. pneumoniae* with reduced susceptibility to penicillin G increased from 3.8% in 1987 to 48% in 1997 [34]. In our study, *S. pneumoniae* was the most common causes of meningitis and showed high resistance to amoxicllin, ampicillin and chloramphenicol. Rifampin and chloramphenicol have been recommended as alternative therapies, since they are less costly and more accessible to communities with limited resources. However, the differing levels of resistance found in target populations may restrict their use. In Iran, the increasing cost of treating resistant

infections supports economic arguments for prevention through conjugate *S. pneumoniae* immunization.

To monitor the trends in *H. influenzae* meningitis and the prevalence of resistance, the National Reference Center conducted a survey of approximately 80 hospitals [35]. The percentage of beta-lactamase-producing *H. influenzae* increased progressively from 22% in 1992 to 35% in 1997, with a similar evolution for kanamycin resistance. Tetracycline and chloramphenicol resistance remained stable in 1997-less than 10% and 2%, respectively [35,36]. In our study, *H. influenzae* type b, showed good susceptibility to kanamycin, gentamicin, chloramphenicol and cephalosporin.

Meningococcal resistance to antibiotics is emerging in some part of world. The incidence of *N. meningitis* with reduced susceptibility to penicillin G (MICs from 0.125 mg/L to 1 mg/L) increased from less than 1% in 1991 to 18% in 1996 [37-41]. The strains belonged to various serogroups; most belonged to serogroup B, none produced a beta-lactamase, and all were susceptible to cefotaxime and ceftriaxone. In our study, *N. meningitides* showed a moderate resistance to cephalexin, amoxicllin, ampicillin and chloramphenicol.

In our study, *S. aureus and* Gram-negative bacilli such as *E. coli*, *P. aeruginosa*, and *K. pneumonia* showed high resistance aganst many of tested antibiotics (table 4). The emergence and spread multidrug-resistance among species of *P. aeruginosa* and *S. aureus* has become a major concern worldwide and is seriously challenging current treatment strategies [8,11,16,25]. The emergence of third-generation cephalosporin non-susceptible *E. coli* strains in infant bacterial meningitis, as shown in this study, has caused a therapeutic challenge in choosing initial empirical antibiotics for treating infant patients with post-neurosurgical meningitis.

5. Conclusion

Our results emphasize that the timely use of appropriate antibiotics is essential for the management of this potentially fatal central nervous system infection. However, it should be noted that the number of cases examined in this study is too small to reach a therapeutic conclusion regarding infant bacterial meningitis, and further large-scale studies will be needed for this purpose.

We may come to this conclusion that Gram–positive cocci are major agents in causing bacterial meningitis in children investigated in this region that showed quite high resistance against routine antibiotics such as amoxicillin, ampicillin, sulfamethoxazole-trimethoprim and chloramphenicol. Through this experience, it became apparent that for the initial treatment of bacterial meningitis in infants, it is necessary to apply a combination of two antibiotics, instead of a single agent, and new antibiotics should be considered for such combinations rather than persisting on conventional of cephalosporin and beta-lactam antibiotics. Better understanding of pathophysiologic mechanisms likely would result in more effective therapies in the future. The antimicrobial susceptibility pattern for bacteria causes meningitis may provide a guideline for the selection of appropriate drug treatment.

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