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MICROORGANISMS INVOLVED IN ACUTE BACTERIAL MENINGITIS IN CHILDREN AND THE ROLE OF HAEMOPHILUS INFLUENZAE*

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Acute bacterial meningitis (ABM) is an important cause of mortality and neurological damage in children. Documentation of the etiological agents is very important both for the treatment of patients and for prophylactic approaches. H.influenzae, N.meningitidis and S.pneumoniae are the three major pathogens involved in ABM. In Turkey for many years H.influenzae has not been isolated in cerebrospinal fluid (CSF) specimens. In order to show the bacteria involved in ABM in our hospital and to see the role of H.influenzae, we investigated the CSF of 59 patients with bacterial meningitis using Gram and Wayson stains, culture and latex agglutination techniques. The agents were determined in 38 (64.4%) specimens by using culture positivity in 30 (50.8%), and latex or stain positivity in eight (13.6%) specimens. The microorganisms causing ABM included S.pneumoniae (25.6%), gram-negative enteric bacilli (17.9%), N.meningitis (12.8%), alpha hemolytic streptococci (10.3%), H.influenzae (10.3%), nonfermentative gram-negative bacilli (5.1%), candida spp. (5.1%), group B streptococci (2.6%), coagulase negative staphylococci (2.6%), S.aureus (2.6%) and pseudomonas spp. (5.1%). In this study it has been shown that H.influenzae can cause ABM in Turkish children. Multicentric studies from different parts of Turkey will be helpful in showing the real incidence in our country. *Key words: acute bacterial meningitis, bacterial pathogens, Haemophilus influenzae.*

Despite research in diagnosis, prevention and therapy, acute bacterial meningitis (ABM) in children is still an important cause of death and major neurological damage[1]. ABM is caused by a variety of microorganisms. Age and underlying conditions of the patients as well as seasonal and geographic differences influence the types of microorganisms involved in these infections. Together with N.meningitidis and S.pneumoniae, H.influenzae is one of the three most common organisms causing ABM[2]. Infants who have medical devices or underlying illnesses can be infected with less virulent microorganisms[3]. Documentation of

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the etiological agents involved in ABM is very important not only for the treatment of patients but for prophylactic approaches as well. Development of immunization policies depends on a reliable bacteriological analysis showing the prevalence of microorganisms in cerebrospinal fluid (CSF) specimens. In the USA, vaccination against H.influenzae, which is the most common agent in ABM, has decreased the incidence of H.influenzae type b (Hib) meningitis[1]. In Turkey H.influenzae has been isolated very rarely in CSF specimens in recent years[4-7]. It is believed that H.influenzae is not a danger for Turkish children.

This study was performed to investigate the types of bacteria involved in ABM and the role of H.influenzae in childhood meningitis in our hospital.

Materials and Methods

CSF specimens were obtained from children with clinically suspected meningitis in the emergency ward of Hacettepe Children's Hospital. Specimens from hospitalized patients who developed ABM during their hospital stay were also included in the study. Quantitative cell counts were immediately performed in the counting chambers, and CSF specimens lacking polymorphonuclear leukocytes and those with predominant lymphocytes were excluded from the study. Culture, stain and latex agglutination techniques were performed 30 minutes after the lumbar puncture.

Culture Technique: Two to three ml of the CSF specimen was centrifuged for 15 minutes at 2000-3000 rpm. The supernatant was poured in another test tube for other analysis, and the sediment was vortexed for 10 minutes. Using a sterile Pasteur pipette, inoculations on GC-chocolate agar with 10 percent horse blood, five percent sheep blood agar and thioglycollate broth were performed. Agar plates were incubated for 48 hours in a CO_2 incubator at 35 °C by daily examination. Broth was observed for five days in ambient air at the same temperature. Bacterial identification was performed using standard techniques in case of any growth. X, V and XV factor discs (Difco) were used to identify H.influenzae. The colonies of H.influenzae were serotyped using H.influenzae antiserum poly a-f (Difco)[8].

Stain: A few drops of sediment were placed on alcohol-soaked microscopic slides and stained using Gram and Wayson stains[9].

Latex Agglutination: Supernatant fluid was used for this procedure. Latex agglutination was performed using kits by Biomerieux (France) in only half of the specimens according to the instructions.

Results

Over a 16-month period, a total of 59 specimens were obtained from 32 boys and 22 girls between four days and 14 years of age (mean age 2.5 ± 3.3 years).

Thirty (50.8%) of the specimens revealed growth of various microorganisms. In eight (27.6%) of 29 culture-negative specimens, the etiological agent was determined by either direct smear or latex agglutination tests. Using the three methods, causative agents were determined in 38 (64.4%) of the 59 specimens. The types of microorganisms are listed in Table I. Among 29 patients who had no growth, six of them had Gram (+) diplococci on Gram stain and one had latex positivity against group B streptococci. Fifty-nine percent of the culture-negative patients had a history of prior antibiotic usage. Table II shows the age distribution of the patients whose etiological agent was documented by at least one of the three methods.

Table I: Microorganisms Causing ABM

Microorganisms	n	%	Culture (+)	Culture (-) stain and/or latex (+)
N.meningitidis	5	12.8	5	0
S.pneumoniae	10	25.6	4	6
Gram (-) enteric bacilli	7	17.9	7	0
H.influenzae	4	10.3	4	0
Alpha hem. strep.	4	10.3	4	0
Candida spp.	2	5.1	2	0
Nonferm. gram (-) bacilli	2	5.1	2	1
Pseudomonas spp.	2	5.1	2	0
Group B strep.	1	2.6	0	1
Staph. aureus	1	2.6	1	0
Stap. coag. (-)	1	2.6	1	0

Table II: Age Distribution of Patients Whose Microorganisms were Documented

0-1 Month	n	1 Month-3 Years	n	> 3 Years	n
Gr. B strep.	1	N.meningitidis	2	N.meningitidis	3
K.pneumoniae	3	S.pneumoniae	5	S.pneumoniae	4
E.coli	1	H.influenzae	2	Staph coag. (-)	1
S.bovis + proteus spp.**	1	Alpha hemolytic streptococci**	3		
Pseudomonas spp.	1	K.pneumoniae	1		
S.aureus	1	Erwinia spp.	1		
		Candida spp.	1		
		Pseudomonas spp.	1		
		Nonfermentative bacilli	2		

* mixed growth.
** Streptococcus intermedius (2), nontypable streptococcus (1).

Discussion

In surveillance studies from developed countries, H.influenzae has been reported to be the most common agent (45%) causing ABM after the neonatal period[10]. In many other countries, though isolation rates are lower, H.influenzae is one of the three most common isolates[11-13]. In our country there has been no isolation of H.influenzae in CSF specimens in most studies[5-7]. In one study performed in our hospital between 1977 and 1980, H.influenzae represented only one percent of 950 CSF isolates[4]. The isolation of H.influenzae from other specimens such as throat cultures revealed isolation rates of 2.96 and 5.2 percent, which are lower than those in the literature[14, 15]. Given these findings, for years it has been believed that H.influenzae did not cause invasive infections in Turkish children because of antigenic cross-reactivity between H.influenzae and E.coli. The capsular antigen of E.coli K100 is immunochemically related to one of H.influenzae type b. Bacterial antibodies against this microorganism are also believed to protect children from Hib infections[16, 17]. In our study, the isolation rate of 10.3 percent shows that H.influenzae may cause ABM in Turkish children. It is the second most common isolate in the one-month to three-year age-group. Why it has not been isolated for years may be explained by the inoculation techniques and the media used in other studies. H.influenzae requires X and V factors for growth, and special culture media are necessary for isolation. This may be supported either by adding these factors to the culture medium or by using chocolate horse-blood agar[8]. In our study S.pneumoniae was the most common isolate, followed by N.meningitidis. Our isolation rate of N.meningitidis was lower than in previous studies from our hospital[4, 5], possibly because most of our patients had previous antibiotic usage. Premature infants with immature immunity, broad-spectrum antibiotic usage or invasive medical approaches are at increased risk of acquisition of meningitis with more noninvasive microorganisms, such as candida or coagulase (-) staphylococci[18]. Both our candida and coagulase (-) staphylococcus isolates were from patients with ventriculoperitoneal shunts. Alpha haemolytic streptococci rarely cause ABM. They mostly infect children with underlying diseases[19]. In our series, three of the four isolations were from children who had brain abscesses ruptured into the meningeal space. The fourth patient had a ventriculoperitoneal shunt, as mentioned before.

The changing pattern of ABM in the neonatal period is noted in some studies, but gram (-) enteric bacilli, reported to be the most common pathogens of the neonatal period, were isolated more frequently in our study[2, 20]. Bacterial culture is inexpensive and still accepted as the "gold standard" for the diagnosis of bacterial meningitis[9]. With the standardized, routine isolation technique, there is still a 49.2 percent rate of culture negativity in our series. More sensitive and rapid techniques such as PCR are being worked on. PCR gives promising results in the early laboratory diagnosis of bacterial meningitis[21].

In this study it has been shown that H.influenzae can cause ABM in Turkish children. A special culture medium, standardized techniques and good cooperation between microbiologists and clinicians can solve the problem of isolation. Multicentric studies from different parts of Turkey covering more patients will be helpful in documenting the real incidence in Turkey.

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