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Causative agents of bacterial meningitis

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Central nervous system infections include several clinical involvements with a wide clinical distribution and variable course and outcome. One of the most frequently encountered infections is bacterial meningitis which may lead to serious consequences with respect to development of complications. This study has been conducted with the aim to investigate the causative agents of bacterial meningitis. One hundred patients with a clinical prediagnosis of meningitis were enrolled in the study. Their cerebrospinal fluid samples were obtained for biochemical examination, cell count, Gram staining and giemsa staining. Samples were cultured for bacteriological examination. Conventional methods and Vitek2 were used for identification. Molecular evaluation of the samples was carried out with multiplex PCR based on hybridization. For this purpose, Speed-Oligo Bacterial Meningitis kit was used and *Haemophilus influenza*, *Neisseria meningitidis* and *Streptococcus pneumoniae* were explored. A total of 34 samples were shown to have bacterial infection; a bacterial agent was isolated by culture from each of 25 CSF samples of all 100 tested samples. Most frequently isolated microorganisms were *Staphylococcus epidermidis* (4 samples, 16%), *Corynebacterium* spp. (4 samples, 16%) and *S. pneumoniae* (2 samples, 8%). Only 15 samples were positive with PCR, in 10 of the samples (10%). *S. pneumoniae* was detected and *H. influenzae* was detected in 5 of them (5%) while *N. meningitidis* was not found in any of the samples. Only one sample showed yeast infection. Aseptic viral infection was evident based on leukocyte, glucose, and protein vales, but was not demonstrated by specific tests.

Key words: Bacterial meningitis, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*.

INTRODUCTION

Among the central nervous system infections, bacterial or viral meningitis is the one that most commonly occurs. Apart from being frequently encountered, meningitis has a distinct importance due to its widespread mortality and morbidity. Although, clinical findings help establishing a preliminary diagnosis of meningitis, specific diagnosis is crucial for correctly guiding the treatment in order to decrease the rate of complications. Indirect diagnostic methods are widely used in meningitis and include cell counts in cerebrospinal fluid (CSF) samples, CSF protein and simultaneously obtained CSF/blood glucose ratio. Typically, in bacterial meningitis cases, increases are seen in bacterial counts (normally 0 to 5 cell/ml) and protein (>40 mg/dL) content of the CSF with a low CSF glucose level (<40 mg/dL) and decreased CSF-glucose/serum glucose ratio (normally 0.6). Increases in cell count are usually excessive in the bacterial meningitis cases (>500 cell/mm^3, whereas normal level is <10 cell/ mm^3) and polymorphonuclear leucocytes are dominant (Kanra et al., 2003; Özdemir et al., 2010). Despite availability of vaccination programs for bacterial meningitis, the most commonly seen causative agents in our country and worldwide include *Haemophilus influenza*, *Neisseria meningitidis* and *Streptococcus*

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pneumoniae (Silva et al., 2010; Pehlivanoglu et al., 2011). These agents are microorganisms that can be cultured by several laboratories with their available sources. However, factors such as insufficient amount of CSF obtained and further reduction of microorganism counts which are already scarce in the sample due to empirical antimicrobial therapy (Barghouthi and Al Zughayyer, 2012), physical conditions and atmospheric conditions are the major factors that limit the sensitivity of culture.

It has been possible to identify potential agents in a short time and in small amounts of samples owing to molecular methods which allowed development of tests that can be used to investigate multiple agents concurrently. This study has been conducted for epidemiological purposes both in order to find out whether there were any changes in bacterial meningitis agents in parallel with a more widespread use of the vaccination programs, and to consider the role of molecular tests in diagnosing meningitis.

MATERIALS AND METHODS

Collection of samples

CSF samples were used for the study, which have been obtained from patients with a "prediagnosis of meningitis" presenting with complaints, symptoms and physical examination findings and sent to Gaziantep University, School of Medicine, Microbiology Laboratory. In the event that the amount of the sample was <2 ml, the sample was not included in the study. History and demographic characteristics of the patients were obtained from their filed information. Two aliquots (2 to 3 ml) were prepared from the samples collected (samples were processed within 1 to 2 h, frozen at -80°C until used). The first aliquot was used for cell counts, Gram staining and detection of protein and glucose. The second was used for culture and molecular tests. Cell count was carried out by using a thoma camera. Samples were concentrated with centrifugation and then, stained with Gram. Serum samples taken concurrently from the patients were used for detecting serum glucose level.

Culture

CSF samples were inoculated into sheep blood agar, eosin - methylene blue agar and chocolate agar, and incubated at 37°C for 24 to 48 h. Inoculations were carried out in pairs and one of the agar plates was kept in a 5 to 10% CO_2 environment. Bacteria detected were defined by using conventional methods and Vitek2 (Biomerieux).

DNA extraction and amplification

DNA isolation process was carried out by using a High Pure PCR Template Preparation kit (Roche Diagnostics, Germany). Amplification of the extraction samples was carried out with a Speed-Oligo Bacterial Meningitis kit (Vircell Microbiologists, Spain). Based on Multiplex PCR and hybridization, this kit has areas specific to *lyt*A gene to detect *S. pneumoniae*, *bex*A gene to detect *H. influenzae* and *ctr*A gene to detect *N. meningitidis*. These three bacteria were studied by placing separate strips containing complementary probes for the target genes on a single test strip. The study was conducted after obtaining approval from local ethics committee and supported by funding from Gaziantep University Research projects with the research project number TF.10.03.

RESULTS

The study was conducted by using CSF samples from 100 patients who have been referred to our laboratory with a pre-diagnosis of meningitis between August 2009 and June 2010, each sample was used once. Forty-three of the study patients were (43%) female and (57%) male. Age of patients ranged from 0 to 85. Among the CSF cultures obtained from samples of the study patients, growth was detected in 25 (25%); 24 bacteria and one yeast, and no growth was observed in 75 (75%). Microorganisms detected by culture are shown in Table 1. Coagulase-Negative Staphylococcus (CNS) was the most common isolate, it was detected in 11 (45.8%) of the 25 bacterial samples. With PCR, *S. pneumoniae* was detected in 10 of the samples (10%) and *H. influenzae* in (5%) samples, while *N. meningitidis* was not detected in any of the samples. At the molecular level, *S. pneumoniae* was detected in 10 patients; two of these had the same agent detected additionally with the CSF culture and 8 patients did not have them in their CSF. Cultures of 5 patients in which *H. influenzae* was detected did not show growth of this microorganism. All findings related to 38 microorganisms detected in 35 samples with culture and/or PCR are shown in Table 2. Bacteria were observed in 4 samples (4%) with Gram staining method.

Culture positivity was detected in 2 of these samples, while PCR was positive for one sample and the remaining sample showed both culture- and PCR positivity. It was observed that microorganisms detected by Gram staining were consistent with the isolated ones. Additional findings obtained by examination of CSF samples are shown in Table 3. CSF protein content was found to be >40 mg/dL in 65% of the samples. In 29 of these samples (44.6%), a bacterial agent was isolated. One agent (17.1%) was detected in 6 out of 35 samples with normal microbial total protein content (<40 mg/dl). Statistical analysis of all the tests are shown in Table 4.

DISCUSSION

Bacterial meningitis is one of the infections with severe progression. Delays in diagnosis and treatment give rise to increased rates of complications and mortality along with treatment failure (Hacımustafaoglu, 2008). The golden standard for diagnosis is culturing the growth of the causative agent from CSF. Isolation rate of the agents shows a great variation in separate publications and it is reported to be between 5.5 and 52.7% (Taşkesen and Taş, 2007; Sümer et al., 2000; Pişkin et al., 2005; Silva et al., 2010; Zimba et al., 2009; Abdulrab et al., 2010). Differences in the isolation rates may be attributed to several factors such as small number of bacteria found in CSF of meningitis cases, storage of samples in unsuitable conditions until the time of inoculation and require-

Table 1. Bacteria that grown in CSF cultures (N = 100).

Microorganism	Count	Percentage (%)
Staphylococcus epidermidis	4	16
Corynebacterium spp.	4	16
Streptococus pneumoniae	2	8
Staphylococcus warneri	2	8
Staphylococcus hominis	2	8
Enterococcus faecalis	2	8
Staphylococcus capitis	1	4
Staphylococcus haemolyticus	1	4
Staphylococcus aureus	1	4
Staphylococcus lentus	1	4
Acinetobacter lwoffii	1	4
Escherichia coli	1	4
Kocura kristina	1	4
Candida parapsilosis	1	4
Micrococcus luteus	1	4
Total	25	100

ment of special growth conditions for some causative agents such as *H. influenzae* and *N. meningititis* bacteria which are difficult to grow antibiotic adminestration before sample collection. From this perspective, culture is the golden standard but its diagnostic value is limited. In our study, rate of growth in the culture was found to be 25%, and CNS was isolated mostly. CNS was isolated in 11 out of the 25 samples in which growth was detected. Although, isolation rates of CNS as a meningitis agent increased with the extensive use of foreign body implementation as in shunting operations, it needs to be validated with repeated cultures in order to eliminate contamination and contraction of skin bacteria (Tülek and Fişkin, 2008; Güzel et al., 2003; Sarguna and Laakshmi, 2006).

In bacterial meningitis, cytologic and biochemical examinations of CSF samples are tests that provide rapid results with great diagnostic value. Since it takes considerable time to specifically detect the causative agent in diagnosis of meningitis, cytologic and biochemical examination of CSF samples are used frequently in the routine diagnosis as well as clinical pre-diagnosis. Detection of increased cell counts in the cytologic examination of the samples with PMNL being the cells that increase abundantly is used as the first finding that supports the preliminary diagnosis of the patient with meningitis (Tülek and Tanyel, 2008). In our study, one causative agent was detected in 23 samples that showed increased cell counts in CSF samples and in 12 samples with normal cell count. While sensitivity of increased cell count was found to be insignificant, specificity was 63.79%. However, lymphocyte domination was detected in 7 out of 24 samples showing increased cell count with no bacterial agent detected and other meningitis agents were thought to be present in these samples (viral agents in particular). Another finding observed in bacterial meningitis is the increased CSF protein content. In our study, it was found to be normal in 6 of the patients in whom agents were detected, and no growth was detected in 29 samples despite increased protein content. Other systemic conditions should investigate in the patients that explored protein increase and not explored determinant. CSF glucose/serum glucose ratios were found to be well below 0.8 in all samples with established causative agents (this ratio was 0.81 in one sample and it was included in the normal range). No growth was detected in 31 samples with a ratio of <0.5, these may have had bacterial species other than those detectable using cultural, microscopic or multiplex PCR.

Several studies are being conducted to explore the use of PCR for making a diagnosis due to the delays in obtaining culture results in acute bacterial meningitis and lack of adequate sensitivity of Gram staining results. Sensitivity and specificity of these methods have been improved by the use of new technological developments (Tülek and Fişkin, 2008). Apart from benefits derived from use of molecular tests in the diagnosis of meningitis such as faster and more precise detection of the demanding microorganisms compared to the conventional methods, another advantage is that the need for identifying causative agent for epidemiological reasons is satisfied (Tekeli and Ustaçelebi, 2006). Today, owing to multiplex PCR that helps detection of multiple agents (bacterial, viral and fungal) at the same time, it is possible to achieve results faster with greater sensitivity (Yamamoto, 2002; Lopez et al., 2003). Clearly, these two advantages are crucial for clinical manifestations that require urgent diagnosis such as meningitis. Even the high costs incurred by use of PCR may become of secondary importance due to the aforementioned

Table 2. Findings related to samples in which the causative microorganism was detected with culture and /or PCR.

Age/ Gender	Cell counts /mm^3 and type (%)	CSF /Blood glucose (mg/dL)	Protein (mg/dL)	Gram staining	Culture	PCR[2]
15-M	380 79 PMNL[1]	0.32	247	No bacterium was observed	*S. pneumoniae*	*S. pneumoniae*
21-F	350 100 PMNL	0.39	140	No bacterium was observed	*Kocura kristina*	Negative
14-M	Dense PMNL	0.33	219	No bacterium was observed	Corynebacterium spp.	*H. influenzae*
28-M	350 86 PMNL	0.15	712	No bacterium was observed	No growth	*H. influenzae*
10-F	70 57 PMNL	0.51	223	Gr (+) cocci	*Enterococcus faecalis*	Negative
8-M	550 55 PMNL	0.32	241	No bacterium was observed	No growth	*S. pneumoniae*
10-F	180 100 PMNL	0.50	466.62	No bacterium was observed	*E. faecalis*	Negative
46-F	Dense PMNL	0.02	177	Gram (+) diplococci	*S. pneumoniae*	*S. pneumoniae*
8-M	Normal	0.58	59	No bacterium was observed	*S. warneri*	Negative
10-F	250 100 PMNL	0.40	223	Gram (-) cocci	No growth	*H. influenzae*
46-F	800 100 PMNL	0.39	111.11	No bacterium was observed	No growth	*S. pneumoniae*
21-M	620 68 PMNL	0.55	61	No bacterium was observed	Corynebacterium spp.	Negative
10-F	200 80 PMNL	0.45	136	No bacterium was observed	No growth	*H. influenzae*
54-M	510 100 PMNL	0.37	128.75	No bacterium was observed	No growth	*S. pneumoniae*

Table 2. Contd

80-F	Normal	0.11	621.35	No bacterium was observed	*Micrococcus luteus*	Negative
1,5-F	Dense PMNL	0.21	198	No bacterium was observed	*Corynebacterium spp.*	*S. pneumoniae*
22-F	Dense PMNL	0.35	174.74	No bacterium was observed	No growth	*S. pneumoniae*
6-M	Normal	0.57	7.21	No bacterium was observed	*S. epidermidis*	Negative
10-F	Normal	0.53	32.79	No bacterium was observed	*S. capitis*	Negative
3-M	550 100 PMNL	0.36	259.40	No bacterium was observed	No growth	*S. pneumoniae*
1 month-M	25 80 PMNL	0.10	1446.1	Gram (-) bacillus	*E. coli*	Negative
19-F	230 78 PMNL	0.59	99.27	No bacterium was observed	*S. haemolyticus*	Negative
19-M	Dense PMNL	0.47	131.73	No bacterium was observed	*S. warneri*	*S. pneumoniae*
30-M	10.000 100 PMNL	0.11	200.36	No bacterium was observed	No growth	*S. pneumoniae*
15-M	Dense PMNL	0.37	283.82	No bacterium was observed	No growth	*H. influenzae*
5-M	Normal	0.61	63.96	No bacterium was observed	*S. hominis*	Negative
1-M	Normal	0.24	1213.7	No bacterium was observed	*Candida parapsilosis*	Negative
1-M	Normal	0.37	617.88	No bacterium was observed	*S. epidermidis*	Negative
2-M	Normal	0.57	50.23	No bacterium was observed	*S. epidermidis*	Negative
6-F	Normal	0.81	13.71	No bacterium was observed	*Corynebacterium spp.*	Negative
33-F	Normal	0.43	21.49	No bacterium was observed	*Acinetobacter lwoffii*	Negative
33-F	Normal	0.49	20.95	No bacterium was observed	*S. epidermidis*	Negative
33-F	Normal	0.38	42.76	No bacterium was observed	*S. hominis*	Negative
31-M	20 70 PMNL	0.45	81.59	No bacterium was observed	*S. aureus*	Negative
31-M	20 100 PMNL	0.53	37.40	No bacterium was observed	*S. lentus*	Negative

[1]PMNL: Polymorphonuclear leukocytes.
[2]PCR: Polymerase chain reaction.

Table 3. Cytologic and biochemical results of CSF samples (N = 100).

Microorganism	Cell count		Protein content		CSF glucose/serum glucose	
	Normal	High	Normal	High	Normal	Low
Yes	12	23	6	29	2	33
No	41	24[1]	29	36	34	31
Total	53	47	35	65	36	64

[1] Lymphocytes were dominant in 7 samples.

Table 4. Statistical analysis of the findings.

Test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Cell count	48.94 (34.08 to 63.94)	77.36 (63.79 to 87.72)	65.71 (47.79 to 80.87)	63.08 (50.20 to 74.72)
Protein value	44.62 (32.27 to 57.47)	82.86 (66.35 to 93.44)	82.86 (66.35 to 93.44)	44.62 (32.27 to 57.47)
CSF glucose /serum glucose	51.56 (38.73 to 64.25)	94.44 (81.34 to 99.32)	94.29 (80.84 to 99.30)	52.31 (39.54 to 64.85)

The most significant diagnostic finding is that the specificity for CSF glucose/serum glucose was 94.44%.

advantages. However, there are situations in which PCR investigation of only the sought agents are performed and when those agents are not found, CSF sample cannot be ascertained to be sterile, considering new or unknown bacterial agents could be present. Therefore, commercial kits targeting the most commonly encountered agents are being developed.

A commercially available kit based on hybridization was used in this study which investigated DNA of the three agents most commonly detected during the study (*H. influenzae*, *N. meningitidis* and *S. pneumoniae*). These three agents studied are reported to be the three most frequently encountered meningitis agents (Booy and Kroll, 1994; Silva et al., 2010). *S. pneumoniae* and *H. influenzae* were detected in 10 of the samples examined. It is believed that adding multiplex PCR tests to routine diagnostic algorithm would be beneficial when investigating the presence of an agent in sterile body fluids such as CSF.

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