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RESEARCH ARTICLE

Retrospective evaluation of multiplex PCR panel results from CSF samples in a university hospital

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ABSTRACT

Aim: Given the significant mortality and sequelae due to meningitis, rapid diagnosis and initiation of treatment have a major impact on patient outcomes. In many cases of meningitis/meningoencephalitis, empirical treatment should be initiated immediately. This empirical treatment regimen is initiated based on the cumulative antibiotic susceptibility results in the region. The aim of our study was to retrospectively determine the causative agents in cerebrospinal fluid samples of patients who received a presumptive diagnosis of meningitis, using Multiplex Polymerase Chain Reaction (PCR) tests.

Materials and Methods: The study included 206 cerebrospinal fluid samples from different patients with a preliminary diagnosis of meningitis sent from various clinics. The Biospeedy viral nucleic acid isolation kit (Bioeksen, Türkiye) was used for the isolation of genetic material. Genetic materials (DNA/RNA) related *to Herpes simplex virus 1-2, Humman herpesvirus 6-7-8, Varicella zoster virus, Enterovirus, Cytomegalovirus, Human Parechoviruses, Haemophilus influenzae, Listeria monocytogenes, Streptococcus pneumoniae, Neisseria meningitidis, Streptococcus agalactiae, Escherichia coli K1, Cryptococcus gattii/neoformans* in cerebrospinal fluid samples were investigated using the Meningitis/Encephalitis RT-qPCR MX-17 Panel (RT-qPCR MX-17S Panel, Bio-Speedy®, Bioeksen, Türkiye) multiplex PCR kit.

Results: According to the PCR results, the causative agent was identified in a total of 19 patients. Nine patients were found to have *Streptococcus pneumoniae*, two had *Varicella zoster virus*, and two had *Enterovirus*. Additionally, six patients had separate detections of *Haemophilus influenzae, Cytomegalovirus, Herpes simplex virus 1, Human herpesvirus 6, Human herpesvirus 8, and Parechoviruses*.

Conclusion: Recently, simple and rapid molecular tests such as PCR have contributed to an increase in the early detection of causative agents. Based on the performance of diagnostic tests, we propose an algorithm for the use of both syndromic and specific tests in patients at risk for meningitis/encephalitis.

Keywords: cerebrospinal fluid, meningitis, multiplex PCR

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INTRODUCTION

Infectious meningitis and meningoencephalitis are serious, life-threatening conditions. Challenges in diagnosis continue for clinicians to initiate treatment as soon as possible. Early initiation of target-specific treatment improves the consequences of viral encephalitis or bacterial meningitis (1).

Bacterial meningitis is one of the primary infections of the central nervous system (CNS); it results from inflammation of the protective membranes covering the brain and spinal cord, known as meninges, due to bacterial infection of the cerebrospinal fluid (CSF) (2). *Haemophilus influenzae (H. influenzae), Streptococcus agalactiae (S. agalactiae),* and *Streptococcus pneumoniae (S. pneumoniae)* are among the causes of meningitis worldwide (3). The susceptibility of patients to certain pathogens appears to be age-related. In the newborns, it is usually *Escherichia coli (E. coli), Streptococcus agalactiae (S. agalactiae), Listeria monocytogenes (L. monocytogenes),* and bacteria from the *Enterobacteriaceae* family. In children, *H. influenzae* and *Neisseria meningitidis (N. Meningitidis)*; in adults, *N. meningitidis* and *S. pneumoniae* (4,5). The most common agent of viral meningitis is *Enteroviruses* (6).

Early etiologic diagnosis allows for appropriate and targeted treatment to be initiated as soon as possible. Culture of the causative microorganism is the "gold standard" for diagnosis and antimicrobial susceptibility, and culture is mandatory for community-acquired bacterial meningitis (7). In the clinical microbiology laboratory, Gram staining of CSF helps to identify the microorganism in 50-90% of cases, CSF culture is positive in approximately 80% of CSFs obtained from patients who have not started treatment (8,9). Although Gram staining is rapid and highly specific, its sensitivity is low (sensitivity varies from 10-93% depending on the use of antibiotics before taking CSF). Gram staining is most useful in the diagnosis of *S. pneumoniae* infections (1). In recent times, with the widespread adoption of molecular methods, significant contributions have been made to the identification of causative agents in meningitis (10,11). With the widespread use of molecular tests, it has been shown to be rapid, inexpensive, reliable, and effective in the identification of different agents such as bacteria, viruses, or fungi. Recent studies indicate that molecular diagnostic methods can be sensitive and specific for different microorganisms. They have been reported to be applicable in identifying pathogens in CFS from patients who have started antimicrobial treatment or have negative cultures. In addition, one of the major advantages of Polymerase Chain Reaction (PCR) is that very small amounts of clinical samples are used for molecular testing (6,11,12).

In our study, we aimed to determine infectious agents by multiplex PCR method from CSF samples sent from various clinics with a pre-diagnosis of CNS infection.

MATERIALS AND METHODS

Between January 1 and December 31, 2021, PCR results of CSF samples of 206 patients referred from different clinics of Dicle University Medical Faculty Hospital with a preliminary diagnosis of meningitis were evaluated retrospectively.

The Biospeedy viral nucleic acid isolation kit (Bioeksen, Türkiye) was used for the isolation of genetic material. After nucleic acid isolation, patient samples and PCR mixes were added to the strips in the kit on the cold tube stand in the biosafety cabinet in accordance with the manufacturer's instructions. After nucleic acid isolation, patient samples and PCR mixes were added to the strips provided in the kit, placed on a cold tube rack inside a biosafety cabinet, following the manufacturer's instructions.

Genetic materials (DNA/RNA) related *to Herpes simplex virus 1-2 (HSV 1-2), Human herpesvirus 6-7-8 (HHV 6-7-8), Varicella zoster virus (VZV), Enterovirus, Cytomegalovirus (CMV), Human Parechoviruses (HPeV), H. influenzae, L. monocytogenes, S. pneumoniae, N. meningitidis, S. agalactiae, E. coli K1, Cryptococcus gattii/ neoformans* in CSF samples were investigated using the Meningitis/Encephalitis RT-qPCR MX-17 Panel (RT-qPCR MX-17S Panel, Bio-Speedy®, Bioeksen, Türkiye) multiplex PCR kit.

The descriptive data in the study are presented with numbers and percentages for categorical data.

This study was approved by the Dicle University Medical Faculty Ethics Committee for Noninterventional

Studies (approval date 13.09.2023, number 09.2023.253). We conducted the study following the ethical principles of the Declaration of Helsinki. As this was a retrospective study, informed consent was not required.

RESULTS

CSF samples from 206 patients with a pre-diagnosis of meningitis were analyzed. According to the PCR results in our study, the causative agent was identified in 19 patients. The median age of the patients was 22 years (range: 0–67 years).

S. pneumoniae was detected in nine patients, *VZV* and *Enterovirus* in two patients each, and *H. influenzae, CMV, HHV-6, HHV-8, HSV-1,* and *HPeV* in six patients separately (Table 1). 47.4% of our patients were <18 years old and 52.6% were >18 years old (Table 2).

Table 1. The number of Meningitis Agents in the Multiplex PCR Panel

Infection Agents	Number
S. pneumoniae	9
VZV	2
Enterovirus	2
H. influenzae	1
CMV	1
HHV-6	1
HHV-8	1
HSV-1	1
HPeV	1

DISCUSSION

Infectious meningitis can be caused by many microorganisms. Despite current treatments, many types of infectious meningitis remain associated with mortality and morbidity (6). Meningitis caused by bacteria is particularly important in terms of mortality and morbidity. Despite the availability of many diagnostic methods, establishing the etiology of infectious meningitis is in many cases challenging and laborious. Physicians should consider multiple factors when assessing a meningitis patient (8). Glucose, white blood cell count, and total protein levels from CSF are all useful parameters in the diagnosis of meningitis. In spite of these 'classic' patterns, these non-specific markers are not specific enough for a definitive diagnosis. CSF culture continues to be the basis for the diagnosis of bacterial meningitis (13). In recent times, rapid tests such as the Cryptococcal Lateral Flow Assay (IMMY), FilmArray Multiplex PCR (Biofire), GeneXpert MTB/Rif Ultra (Cepheid) and Real-Time PCR have been used (6). Currently, syndrome-based tests have indicated a new approach to the diagnosis of infectious diseases, suggesting that PCR methods

Table 2. Distribution of the detected agents by age

Detected Agent	Age Range					
	0-5	5-18	18-45	45-65	>65	Total
S. pneumoniae	1	3	4	1	-	9
VZV	-	1	-	1	-	2
Enterovirus	-	2	-	-	-	2
H. influenzae	1	-	-	-	-	1
CMV	-	-	1	-	-	1
HHV-6	1	-	-	-	-	1
HHV-8	-	-	-	-	1	1
HSV-1	-	-	-	-	1	1
HPeV	-	-	1	-	-	1
Total	3	6	6	2	2	19

may be useful in the detection of meningitis. Molecular methods can determine the quantity of bacteria and detect microorganisms in the presence of antimicrobial use (11). However, with the widespread adoption of newly developed nucleic acid detection techniques, they also play an important role in the identification of viral agents. Rapid and reliable detection of viral agents prevents unnecessary antiviral use and circumvents the need for other expensive invasive tests. In a retrospective study conducted in Türkiye to investigate viral etiology, causative agents were isolated in approximately 2.3% of cases using nucleic acid tests (NAT) (14). In our study, one of the advanced diagnostic methods used was multiplex PCR.

The number of bacterial meningitis cases reported to the global surveillance center between 2006 and 2016, while the incidence in developed countries was 0.5-1.5/100,000, incidences of up to 1000 per 100,000 population have been reported, especially in west-central Africa and sub-Saharan regions (15). The symptoms of viral meningitis are generally less severe than bacterial meningitis, but identification of the causative agent is more challenging. The most common causes of viral meningitis are *Enterovirus, VZV*, and *HSV*-2 (16). In this study, the most frequently isolated agents were *S. pneumoniae*, *VZV*, and *EV*. In this study, children with *Enterovirus* were aged 5-15 years. Although enterovirus causes viral meningitis in all age groups, pediatric age is a risk factor that requires special attention (17,18). Although the bacteria that cause meningitis vary in different parts of the world, the most common causative agent worldwide is *S. pneumonia* (19).

Pean et al. retrospectively analyzed the results of 4,100 patients between April 2014 and March 2017 and reported that the most widespread causes of meningitis were *EV* 23.9%, *VZV* 10.2%, and *HSV*-2 4.2% (20). In our study, *EV* and *VZV* were detected in two different patients. Schnuriger et al. analyzed 1,744 CSF samples from 1,344 pediatric and 336 adult patients between May 2017 and November 2019 and detected, viral pathogens in 361 (21%) CSF and bacterial pathogens in 52 (3%) (21). In another study conducted over 34 months, a total of 4,199 patients' CFS were examined, and pathogens were detected in 315 (7.5%) according to Real-Time PCR results. The rate of detected pathogens was 38% for *EV*, 13% for HSV-2, and 19% for *VZV* (22). In this study, *Enterovirus* and *VZV* were the most common viral agents, while *S. pneumoniae* was the most common bacterial agent. *S. pneumoniae* is the most common etiologic agent of community-acquired bacterial meningitis (23). In Türkiye, 470 CFS were examined using Real-Time PCR to investigate the causative agents of meningitis. In the study, a bacterial or viral agent was identified in 21% (98 samples) of the sample. In total, *EV* (25%) was the most commonly detected agent, followed by *Adenovirus* (22%), and *S. pneumoniae* (15%) (24). Recent studies conducted Türkiye have reported that the causative agent of meningitis is *HSVs* (12). The *HSV* agent was detected in patients over 65 years of age, and it has been reported that the frequency of *HSV*-related infections increases at extreme ages (< 1 year and ≥ 65 years) (17). In our study, we observed that the pathogens identified were consistent with those reported in other studies (12,17,18).

Recent studies have included the comparison of the FilmArray Meningitis/Encephalitis (ME, BioFire Diagnostics) panels with other tests in identifying CNS infections. They examined a previously identified set of 291 CFS samples using the FilmArray ME panel. At the end of the study, the concordance rate was 52% (26/50) for viruses, 97.5% (78/80) for bacterial pathogens, and 90.1% (145/161) for Cryptococcus neoformans (25). In recent times, syndrome-based tests indicate a new approach to the diagnosis of infectious diseases. In a multicenter study, when the QIAstat-Dx ME Panel was compared with the BioFire FilmArray ME Panel and it was observed that they were in concordance with each other (26). Clinicians should be aware of the benefits and limitations of each test when evaluating a patient with meningitis/encephalitis, considering the potential for false-positive and false-negative results.

CONCLUSION

Timely identification of the causative agents is of critical importance in CNS infections. Traditionally, microbiological culture of CFS has been time-consuming for the identification of pathogens. Today, with the use of rapid tests such as PCR, the detection of causative pathogens can be achieved in a short period, as quick as one hour. Moreover, the microbiological culture

sensitivity of CFS decreases with the initiation of empirical treatment. Multiplex PCR panels offer many advantages over various methods in the diagnosis of CNS diseases. These panels have the capacity to rapidly detect a potentially large number of agents. This study highlights the value of rapid diagnostic methods with high levels of specificity and susceptibility to reduce the time and cost of testing, shorten the length of hospital stay and reduce antibiotic use.

Ethical approval

This study has been approved by the Dicle University Medical Faculty Ethics Committee for Noninterventional Studies (approval date 13.09.2023, number 09.2023.253). Written informed consent was obtained from the participants.

Author contribution

Concept: HT, FŞ, NÖ; Design: FŞ, NÖ, EÖ, SA, HT; Data Collection or Processing: HT, FŞ, NÖ; Analysis or Interpretation: HT, FŞ; Literature Search: FŞ, NÖ, EÖ, SA, HT; Writing: FŞ, NÖ, EÖ, SA, HT. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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