Evaluation of Viral and Bacterial Pathogens in the Central Nervous System Infections with Multiplex PCR

Sedef Zeliha Oner, Ilknur Kaleli, Melek Demir, Ergun Mete and Ahmet Caliskan

Department of Medical Microbiology, Faculty of Medicine, Pamukkale University, Denizli, Turkey

ABSTRACT

Objective: To evaluate the bacterial and viral causes of central nervous system (CNS) infection by multiplex PCR.

Study Design: Descriptive study.

Place and Duration of Study: Department of Medical Microbiology, Pamukkale University Faculty of Medicine, Turkey, from March 2016 to December 2021.

Methodology: Cerebrospinal fluid (CSF) samples of patients prediagnosed with CNS infection were included in the study. Viral pathogens were detected with the Multiplex real-time PCR panel (FTD Neuro9, Fast Track Diagnostics, Luxembourg) and bacterial pathogens with the multiplex real-time PCR panel (FTD Bacterial Meningitis, Fast Track Diagnostics, Luxembourg). The identification of bacteria growing in samples was done by conventional methods and with the Phoenix™ (Becton Dickinson Diagnostics, USA) automated system.

Results: CSF samples of 440 patients were evaluated using multiplex PCR panel. The viral factors included adenovirus (14.2%), human herpes virus 7 (1.5%), varicella zoster virus (1.3%), herpes simplex virus 1 (1.3%), cytomegalovirus (1.3%), Epstein-Barr virus (0.8%), human herpes virus (0.8%), herpes simplex virus 2 (0.3%), varicella zoster virus (0.3%), and parvovirus B19 (0.3%); and bacterial factors included *Streptococcus pneumoniae* (7.0%) and *Neisseria meningitidis* (0.9%). The bacterial growth was detected in the CSF culture was 4.9%. Among the growing bacteria, there were six different types that were not found on the multiplex PCR panel.

Conclusion: The use of a comprehensive bacterial multiplex PCR panel containing common pathogens will be more effective in pathogen detection. Care should be taken, especially when interpreting the viral Multiplex PCR.

Key Words: *Viral multiplex PCR, Bacterial multiplex PCR, Bacteria culture.*

How to cite this article: Oner SZ, Kaleli I, Demir M, Mete E, Caliskan A. Evaluation of Viral and Bacterial Pathogens in the Central Nervous System Infections with Multiplex PCR. *J Coll Physicians Surg Pak* 2022; **32(12)**:1605-1608.

INTRODUCTION

Nervous system infections have the potential to threaten life. Pathogens such as bacteria, viruses and fungus cause these infections. Central nervous system (CNS) infections include meningitis, encephalitis, and cerebral abscess. Bacterial meningitis is a disease that can be seen in any age group. Common bacteria include *Group B Streptococcus, Listeria monocytogenes, Escherichia coli* in newborns and infants, *Streptococcus pneumonia (S. pneumonia), Neisseria meningitis (N. mengitis), Haemophilus influenzae* in children aged 2-18 years, and *S. pneumoniae, N. meningitidis and L monocytogenes* in adults.[1]

Correspondence to: Dr. Sedef Zeliha Oner, Department of Medical Microbiology, Faculty of Medicine, Pamukkale University, Denizli, Turkey
E-mail: soner@pau.edu.tr

Received: March 31, 2022; Revised: May 09, 2022; Accepted: May 12, 2022
DOI: https://doi.org/10.29271/jcpsp.2022.12.1605

Viral infections in the CNS cause meningitis and encephalitis. Inflammation isolated in the meninges leads to meningitis, whereas the involvement of the cerebral parenchyma causes encephalitis. Meningitis caused by viruses is a disease that is often benign and self-limited, and it heals without sequela in patients with sufficient immunity. Common causes include human enteroviruses, mumps, lymphocytic choriomeningitis virus and herpes viruses. Viral aetiology varies by age and country. Unlike viral meningitis, encephalitis caused by viruses can be life-threatening and can cause permanent neurological damage, although it often limits itself.[2,3] Viruses that cause encephalitis include Herpes viruses, Arboviruses, Enteroviruses, Par Echo viruses, Mumps, Measles, Rabies, Ebola, Lymphocytic choriomeningitis viruses and Lentiviruses.[4]

As nervous system infections present high morbidity and mortality, their rapid identification and treatment are crucial for the patient.[1] The aim of this study was to evaluate retrospectively the bacterial and viral causes of CNS infection over a five-year period, through the multiplex PCR method.

METHODOLOGY

Cerebrospinal fluid (CSF) samples from patients prediagnosed with CNS infection, sent to the Medical Microbiology Molecular

Department of the Hospital between March 2016 and December 2021, were included in the study. The bacterial/viral PCR panel and the bacterial culture results were simultaneously evaluated. Repeated samples of the same patient were excluded. The patients' demographic data were obtained from the hospital information system.

In CSF samples, cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV) 1/2, varicella zoster virus (VZV), adenovirus (ADV), enterovirus (EV), parvovirus B19, par echovirus (PV), and human herpes virus (HHV) were detected with 6/7 multiplex real-time PCR panel (FTD Neuro9, Fast Track Diagnostics, Luxembourg); and *S.pneumoniae, N.meningitidis, Haemophilus influenzae* were detected with the multiplex real--time PCR panel (FTD Bacterial Meningitis, Fast Track Diagnostics, Luxembourg).

CSF was inoculated on blood agar, Eosin Methylene Blue agar and chocolate agar incubated for 24-48 hours at 37°C. The identification of bacteria growing in culture samples was done by conventional methods and with the Phoenix™ (Becton Dickinson Diagnostics, USA) automated system. The Ethical Committee approved the study.

The statistical analysis of data was performed with SPSS Ver. 25 [(IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)] package program. Categorical variables were expressed in numbers and percentages.

RESULTS

CSF samples of the 440 patients considered to present CNS infection were evaluated by the multiplex PCR panel (bacterial and viral). In total, 45 (10.2%) patients were evaluated with bacterial multiplex PCR panel, 111 (25.2%) patients with viral multiplex PCR panel, and 284 (64.6%) patients with bacterial and viral multiplex PCR. Samples of the same patient were not included in the study. There were 45.2% female and 54.8% male patients, and 60.22% of patients were >18 years old. The highest number of CSF samples was evaluated in the summer (Table I).

In the multiplex PCR panel, viral factor was detected in 83 (21.01%) patients and bacterial factor in 26 (7.9%) patients. In the order of frequency, viral factors were detected as adenovirus (14.2%), HHV7 (1.5%), VZV, HSV1, and CMV (1.3%), EBV and HHV6 (0.8%), VZV (1.3%), HSV2 and parvovirus B19 (0.3%) (Table II).

Bacterial factors were S. pneumonia (n= 23, 7.0%) and; N. meningitides (n=3, 0.9% occurring with viruses (Table II).

There was a virus-virus combination in three samples (CMV+HHV6, EBV+HHV7, HHV6+parvovirus B19) and bacteria-virus combination in one sample (*S. Pneumoniae*+ EBV).

In the bacterial multiplex PCR panel, bacterial factors in 26 patients were 23 (7.0%) *S. pneumoniae* and 3 (0.9%) N. meningitis in the order of frequency. The CSF culture results of 329 patients were evaluated simultaneously with bacterial multiplex PCR panel results. Ten patients were excluded from the study due to the absence of consent for the CSF culture. Sixteen (4.9%) instances of bacterial growth were detected in 319 CSF cultures. Six patients presented bacterial growth outside the multiplex PCR panel (*Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa*, coagulase-negative staphylococcus, *Enterobacter cloacae, Streptococcus mitis*).

DISCUSSION

Amplification tests based on nucleic acid sequencing are considered to be the gold standard in the diagnosis of viral pathogens.[3] Multiple viruses are evaluated simultaneously with the Multiplex PCR test, which is promising for improved diagnosis.[2] Ten different viral pathogens were evaluated in this study.

The prevalence of factors varies according to geographic differences. A study that evaluated viral factors detected in CNS infections from 2010 to 2020 reported EV 2.64%, HSV 2.58%, HHV 6 and 7 1.90 % and 0.41%, ADV 1.71%, EBV 1.57%, CMV 1.24%, VZV 0.83%, PV 0.47% and parvovirus B19 0.05%.[5] In a study that examined the epidemiology of viral meningitis infections in Qatar from 2015 to 2018, the results were reported for positive samples as EV (68.7%), EBV (7.5%), ADV (6.8%), VZV (4.8%), CMV (4.5%), PV (4.1%), HSV1 (1.6%), and HSV2 (1.9%).[6] In this study, viral factors were found in the order of frequency as adenovirus (14.2%), HHV7 (1.5%); VZV, HSV1 and CMV as (1.3%) each; EBV and HHV6 (0.8%) each; and HSV2, enterovirus and Parvovirus B19 (0.3%) each.

The most frequent viral factor detected in this study was ADV. An evaluation of the studies conducted with the same kit showed that one study reported ADV (5%) to follow EV (6%) in the order of frequency, and another study reported ADV (22%) to be the second most frequently detected factor after EV (25%) among the positive viruses.[7,8] To eliminate false-positive results that may result from sample or laboratory contamination, ADV--positive samples were consulted with clinical physicians. An evaluation with the clinic will clarify whether the virus is a factor for disease or a bystander. The detection of a virus on PCR does not always mean that it is clinically significant.[9]

The three most common factors of bacterial meningitis in the world include *S. pneumoniae, H. influenza,* and *N. meningitidis.* The conjugated vaccines have led to a decreased incidence of the disease. However, the type of meningitis that occurs with pneumococcus serotypes not covered by vaccine is still a major health problem.[10] In a study of bacterial meningitis cases in Brazil between 2009 and 2018, the most common aetiological agent was *S. pneumoniae*, followed by *N. meningitides.*[11] A literature review that investigated the prevalence and aetiology of meningitis in India between 1990 and 2020 reported *S. pneumoniae* to be the predominant pathogen that caused meningitis.[12] In this study, *S. pneumoniae* and *N. meningitis* were the most common bacterial pathogen, respectively.

To establish a definitive microbiological diagnosis for CNS infections, multiple patient-specific diagnostic tests are selected according to the patient's clinic, which are usually performed simultaneously.[9]

Table I: Demographic data of patients.

Multiplex PCR panel		Bacterial (N=45) n (%)	Viral (N=111) n (%)	Bacterial+ Viral (N=284) n (%)	Total (N=440) n (%)
Gender	Female	14 (31.1)	63 (56.8)	122 (43.0)	199 (45.2)
	Male	31 (68.9)	48 (43.2)	162 (57.0)	241 (54.8)
Age (years)	≤18	28 (62.2)	73 (65.8)	74 (26.0)	175 (39.8)
	>18	17 (37.8)	38 (34.2)	210 (74.0)	265 (60.2)
Seasons	Winter	6 (13.3)	33 (29.7)	71 (25.0)	110 (25.0)
	Spring	6 (13.3)	26 (23.5)	67 (23.6)	99 (22.5)
	Summer	24 (53.4)	19 (17.1)	82 (28.9)	125 (28.4)
	Autumn	9 (20.0)	33 (29.7)	64 (22.5)	106 (24.1)

Table II: Distribution of agents in the viral and bacterial multiplex PCR panel (FTD Neuro9®).

Multiplex PCR panel	Viral (N=111) n (%)	Bacterial+ Viral (N= 284) n (%)	Total (N=395) n (%)
CMV	2 (1.8)	3 (1.0)	5 (1.3)
EBV	-	3 (1.0)	3 (0.8)
HSV1	-	5 (1.8)	5 (1.3)
HSV2	-	1 (0.4)	1 (0.3)
Adenovirus	14 (12.6)	42 (14.8)	56 (14.2)
Enterovirus	-	1 (0.4)	1 (0.3)
HHV6	1 (0.9)	2 (0.7)	3 (0.8)
HHV7	1 (0.9)	5 (1.8)	6 (1.5)
VZV	2 (1.8)	3 (1.0)	5 (1.3)
Parvovirus B19		1 (0.4)	1 (0.3)
Par echovirus	-	-	0 (0.0)
Multiplex PCR panel	**Bacterial (N=45) n (%)**	**Bacterial +Viral (N=284) n (%)**	**Total (N=329) n (%)**
S. pneumoniae	5 (11.1)	18 (6.3)	23 (7.0)
N. meningitidis	-	3 (1.0)	3 (0.9)

CMV = cytomegalovirus, EBV = Epstein-Barr virus, HSV ½ = Herpes Simplex virus 1/2, VZV = Varicella zoster virus, ADV = Adenovirus, EV = Enterovirus, PV = Parechovirus, HHV 6/7 = Human Herpes virus 6/7. S. pneumonia = Streptococcus pneumonia; N. meningitides = Neisseria meningitides.

In a study where community-acquired CNS infections are evaluated, factors were demonstrated in 21.7% of CSF samples and 58.6% of cases in multiplex PCR.[13] The present study, the simultaneous CSF culture and bacterial multiplex PCR presented 16 (4.9%) and 26 (7.9%) factors, respectively. As with many studies, the rate of diagnosis by the molecular method was higher than by conventional methods.

One of the advantages of using multiplex PCR tests is that multiple pathogens can be targeted in a single test.[14] Three different bacterial meningitis pathogens were evaluated with the kit used as part of this study. A study that evaluated patients with laboratory-verified bacterial meningitis from 2007 to 2016 reported more than ten different instances of bacterial growth.[15] However, in this study, six different instances of bacterial growth that was not on the multiplex PCR panel were detected by the culture method. It should be taken into account that many aetiological agents cause bacterial meningitis.

CONCLUSION

The PCR method is more sensitive than culture in the detection of bacterial pathogens. However, the use of a comprehensive bacterial multiplex PCR panel containing common pathogens will be more effective in pathogen detection.

ETHICAL APPROVAL:

Ethical approval of this study was obtained from Pamukkale University Ethics Committee prior to initiation of the research work.

PATIENTS' CONSENT:

Informed consent forms were obtained from patients to publish the data concerning this study.

COMPETING INTEREST:

The authors declared no competing interest.

AUTHORS' CONTRIBUTION:

SZO, IK, MD, EM, AC: Conception of work, analysis and interpretation of data, revision of work, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Giovane RA, Lavender PD. Central nervous system infections. *Prim Care* 2018; **45(3)**:505-18. doi:10.1016/j.pop.2018.05.007.
2. Lyons JL. Viral meningitis and encephalitis. *Continuum (Minneap Minn)* 2018; **24(5)**:1284-97. doi:10.1212/CON.0000000000000650.
3. Wright WF, Pinto CN, Palisoc K, Baghli S. Viral (aseptic) meningitis: A review. *J Neurol Sci* 2019; **398**:176-83. doi:10.1016/j.jns.2019.01.050.
4. Venkatesan A, Murphy OC. Viral encephalitis. *Neurol Clin* 2018; **36(4)**:705-24. doi. doi.org/10.1016/j.ncl.2018.07.001.
5. Gökkılıç B, Çiçek C, Zeytinoğlu A, Kartal E. Evaluation and a bibliometric analysis of viral factors in central nervous system ınfections detected in the last ten years in Turkey. *Turk Mikrobiyol Cemiy Derg* 2021; **51(1)**:70-85. doi:10.5222/TMCD.2021.74508.
6. Mathew S, Al Khatib HA, Al Ansari K, Nader J, Nasrallah GK, Younes NN, *et al.* Epidemiology profile of viral meningitis ınfections among patients in Qatar (2015–2018). *Front Med* 2021; **8**:809. doi.org/10.3389/fmed.2021.663694.
7. Ntagwabira E, Mureithi MW, Mazarati JB, Jaoko W, Anzala O. Utility of molecular diagnostic method compared with

conventional methods in detection of etiologic agents of central nervous system infections in Rwanda. *African Journal of Microbiology Research* 2019; **13(9)**:176-87. doi.org/10.5897/AJMR2018.9029.

8. Akkaya O, Guvenc HI, Yuksekkaya S, Opus A, Guzelant A, Kaya M, *et al*. Real-time PCR detection of the most common bacteria and viruses causing meningitis. *Clinical laboratory* 2017; **63(4)**:827-32. doi 10.7754/clin.lab.2016.160912.
9. Vetter P, Schibler M, Herrmann JL, Boutolleau D. Diagnostic challenges of central nervous system infection: Extensive multiplex panels versus stepwise guided approach. *Clinical Microbiology and Infection* 2020; **26(6)**:706-12. doi.org/10.1016/j.cmi.2019.12.013.
10. Van de Beek D, Brouwer MC, Koedel U, Wall EC. Community-acquired bacterial meningitis. *Lancet* 2021; **398(10306)**:1171-83. doi.org/10.1016/S0140-6736 (21)00883-7.
11. Silva AFT da, Valente F de S, Sousa LD de, Cardoso PNM, Silva MA da, Santos DR dos. Epidemiological study of bacterial meningitis cases in Brazil between 2009 and 2018. *Revista De Medicina* 2021; **100(3)**:220-8. doi.org/10.11606/issn.1679-9836.v100i3p220-228.
12. Ghia CJ, Rambhad GS. A systematic literature review on the prevalence and etiology of meningitis among critically ill and hospitalised patients in India. *Ther Adv Infect Dis* 2021; **8**. doi.org/10.1177/20499361211046453.
13. Kahraman H, Tünger A, Şenol Ş, Gazi H, Avcı M, Ormen B, *et al*. Investigation of bacterial and viral etiology in community acquired central nervous system ınfections with molecular methods. *Mikrobiyol Bul* 2017; **51(3)**:277-85. doi: 10.5578/mb.57358.
14. Diallo K, Feteh VF, Ibe L, Antonio M, Caugant DA, Du Plessis M, *et al*. Molecular diagnostic assays for the detection of common bacterial meningitis pathogens: A narrative review. *EBio Medicine* 2021; **65**:103274. doi.org/10.1016/j.ebiom.2021.103274.
15. Sunwoo JS, Shin HR, Lee HS, Moon J, Lee ST, Jung KH, *et al*. A hospital-based study on etiology and prognosis of bacterial meningitis in adults. *Sci. Rep* 2021; **11**:1-8. doi.org/10.1038/s41598-021-85382-4.