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Evaluation of Pediatric Central Nervous System Infections with Meningitis Encephalitis Panel

Pediyatrik Santral Sinir Sistemi Enfeksiyonlarının Menenjit Ensefalit Paneliyle Değerlendirilmesi

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Abstract

Objective: Central nervous system (CNS) infections require establishment of urgent diagnosis due to rapid progression leading to mortality or severe permanent sequelae. This study aimed to investigate the clinical and epidemiological characteristics of pediatric patients evaluated with FilmArray®, Meningitis Encephalitis Panel (MEP) applied due to suspicion of CNS infection.

Material and Methods: A total of 230 pediatric patients who were followed up for suspected CNS infection at the Eskişehir Osmangazi University Medical Faculty and whose cerebrospinal fluid (CSF) samples were analyzed using BioFire Diagnostics®, FilmArray®,MEP (bioMérieux, Utah, USA) between January 2018 and December 2021 were included in the study. Epidemiological, clinical, and laboratory findings of the patients were retrospectively evaluated.

Results: The median age of 115 pediatric patients with CNS infection was 42 (range 1-192) months and 65 (56%) were male. MEP revealed viral agents human herpes virus-6 (HHV-6), enterovirus, varicella zoster virus, cytomegalovirus in 23 and bacterial agents *Streptococcus pneumoniae, Haemophilus influenzae, Streptococcus agalactiae* in six cases. Pathogenic agents was identified in the conventional CSF culture of five cases in which no causative agent was detected by MEP (five of them were non-panel agents). In two cases, the same pathogenic agents (*S. pneumoniae* and *S. agalactiae*) were detected with both MEP and CSF culture.

Öz

Giriş: Santral sinir sistemi (SSS) enfeksiyonları hızlı ilerlemesi ve mortaliteye ya da ciddi kalıcı sekellere yol açması nedeniyle hızlı tanı gerektirir. Bu çalışmada SSS enfeksiyonu şüphesi nedeniyle FilmArray Menenjit Ensefalit Paneli (MEP) ile değerlendirilen pediyatrik hastaların klinik ve epidemiyolojik özelliklerinin araştırılması amaçlanmaktadır.

Gereç ve Yöntemler: Çalışmaya Ocak 2018 ile Aralık 2021 tarihleri arasında Eskişehir Osmangazi Üniversitesi Tıp Fakültesinde SSS enfeksiyonu şüphesiyle takip edilen ve BioFire®, FilmArray®, MEP (bioMérieux, Utah, ABD) ile beyin omurilik sıvısı (BOS) analizi yapılan 230 çocuk hasta dahil edildi. Hastaların epidemiyolojik, klinik ve laboratuvar bulguları retrospektif olarak değerlendirildi.

Bulgular: SSS enfeksiyonu tanısı ile takip edilen 115 pediyatrik olgunun yaş ortancası 42 ay (1-192) olup, 65 (%56)'i erkekti. MEP ile, 23 vakada viral ajanlar insan herpes virüs-6 (HHV-6), enterovirüs, varisella zoster virüs, sitomegalovirüs ve altı vakada bakteriyel ajanlar *Streptococcus pneumoniae, Haemophilus influenzae, Streptococcus agalactiae* saptandı. MEP ile etken saptanamayan beş olguda (beşi panel dışı ajan) BOS kültüründe üreme tespit edildi. İki olguda hem MEP hem de BOS kültüründe aynı etkenler (*S. pneumoniae* ve *S. agalactiae*) tespit edildi.

Sonuç: Çocuklarda SSS enfeksiyonlarında en sık görülen etiyolojik ajanlar, başta HHV-6 ve enterovirüsler olmak üzere viral ajanlar olup, bunu *S. pneumoniae* ve *H. influenzae* gibi bakteriyel ajanlar takip etmektedir. Menenjit ensefalit panelleri gibi moleküler testler, birçok faktörün aynı

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Conclusion: The most common etiological agents in CNS infections in children are viral agents, primarily HHV-6 and enteroviruses, followed by bacterial agents such as *S. pneumoniae* and *H. influenzae*. Molecular tests such as MEP are important for the establishment of the diagnosis of central nervous system infections and they can be used together with culture and microscopic tests due to their advantages such as screening many factors at once, identifying both viral and bacterial agents, and yielding rapid results.

Keywords: Children, molecular diagnostics, central nervous system infections

anda taranması, viral ve bakteriyel etkenlerin bir arada değerlendirilmesi, hızlı tanı verilmesi gibi avantajlara sahiptir. Santral sinir sistemi enfeksiyonunun tanısında kültür ve mikroskobik testler gibi konvansiyonel yöntemler, menenjit ensefalit paneli gibi moleküler testlerle birlikte kullanılmalıdır.

Anahtar Kelimeler: Çocuklar, moleküler tanı, santral sinir sistemi enfeksiyonu

Introduction

Central nervous system (CNS) infections such as meningitis, encephalitis, and meningoencephalitis are among the leading infectious diseases with high mortality and morbidity in childhood (1). Viruses, bacteria, and rarely parasites and fungi play a role in its etiology. The most common bacterial agents are *Streptococcus pneumoniae, Neisseria meningitidis, and Haemophilus influenza* type b, while common viral agents include enteroviruses, herpes simplex viruses, varicella zoster virus and mumps virus (2,3). Early diagnosis and treatment are of vital importance due to the potential of the infection for rapid progression, permanent neurological damage, and the risk of serious complications. Microscopic examination of the cerebrospinal fluid (CSF), biochemical methods and specific microbiological diagnostic tests are used in the diagnosis of CNS infections (4). Specific microbiological tests include CSF culture, serological investigation, nucleic acid amplification test incluiding multiplex polymerase chain reaction (PCR) tests, and antibody and antigen detection tests. Recently, nucleic acid amplification tests such as multiplex PCR and meningitis/encephalitis panel (MEP) in CSF have been used in increasing frequency due to their advantages such as getting rapid results, screening for bacteria, viruses, fungi and parasites with a single sample, and being less affected by antimicrobial therapy (5,6). This study aimed to investigate the clinical and epidemiological characteristics of pediatric patients with suspected CNS infection evaluated with MEP.

Materials and Methods

A total of 230 pediatric patients who were followed up for the suspicion of CNS infection at the Eskişehir Osmangazi University Medical Faculty, Department of Pediatrics and underwent CSF analysis with BioFire® FilmArray®, MEP (bioMérieux, Utah, USA) between January 2018 and December 2021 were included in the study. Using MEP, *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, *Escherichia coli*, cytomegalovirus (CMV), enterovirus (EV), herpes simplex virus type I (HSV-1), herpes simplex virus type II (HSV-2), human herpesvirus 6 (HHV-6), human parechovirus (HPeV), varicella zoster virus (VZV), and *Cryptococcus neoformans* were identified in CSF. Epidemiological, clinical, and laboratory findings of the patients were retrospectively evaluated. The study was started after the approval of the ethics committee of Eskişehir Osmangazi University Faculty of Medicine (Date: 30.03.2022, Number: 21).

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 28.0 for Windows (SPSS, Chicago, IL, USA) was used for statistical analyses. Continuous variables were expressed as median (minimum-maximum). After assessing normal distribution using the Kolmogorov-Smirnov test, the parameters were compared among the groups using the Mann-Whitney U test for continuous variables and a Chi-square test for categorical variables. P values< 0.05 were considered to be statistically significant.

Results

CSF samples were taken from 230 cases with the preliminary diagnosis of CNS infection. According to CSF microscopy, biochemistry, culture and MEP results, 115 of them were diagnosed with CNS infection. The study population with an median age of 42 months (range= 1-192 months) consisted of 65 (56%) male and 50 (44%) female pediatric patients who were being followed up with CNS infection. The most common symptoms were fever, loss of appetite, malaise, altered consciousness, headache, and vomiting. The most common physical examination findings were nuchal rigidity, Kernig's and Brudzinski's sign positivity, and bulging fontanelle, in decreasing order of frequency (Table 1). Ventriculoperitoneal shunt was present in 16 cases (13%), cochlear implant in two (1.7%), and history of head trauma in two (1.7%). According to CSF findings, pleocytosis was detected in 38 cases (33%), relatively increased percentage of neutrophils in 29 (25%), decreased glucose levels in 20 (17%), and elevated protein levels in 57 (49%) cases. CSF culture revealed *Streptococcus epidermidis* in two cases, and *S. agalactiae*, *Enterococcus faecium*, *S. pneumoniae*, *S. haemolyticus* and *Pseudomonas aeruginosa* were identified in one case for each of the indicated microorganisms. MEP revealed viral pathogens (HHV-6, EV, VZV, CMV) in 23 and bacterial pathogens (*S. pneumoniae* in

Table 1. Clinical and epidemiological features of the patients

	n= 115
Sex, n (%)	
Male	65 (56%)
Female	50 (44%)
Median Age	42 (1-192%)
Symptoms, n (%)	
Fever	79 (69%)
Loss of Appetite	56 (49%)
Confusion	41 (35%)
Malaise	48 (42%)
Seizure	21 (19%)
Vomiting	29 (26%)
Headache	19 (16%)
Physical Examination Findings	
Nuchal Rigidity	15 (13%)
Kernig/Brudzinski Sign	9 (8%)
Fontanel Bulging	7 (6%)
Laboratory Findings	
Elevated CRP	67 (58%)
Leukocytosis	59 (51%)
Neutrophilia	36 (31%)
Lymphopenia	22 (19%)
Treatment	
Antibiotherapy	93 (81%)
Acyclovir	8 (7%)
Ganciclovir	1 (0.8%)

CRP: C-reactive protein.

four, *H. influenzae* in one, *S. agalactiae* in one case) in six cases (Table 2). MEP detected a viral agent in 23 and a bacterial pathogen (*S. pneumoniae* in three, *H. influenzae* in one) in four of the 27 cases in which no pathogen was detected with CSF culture. Growth of pathogenic microorganisms was detected in the conventional CSF culture of five cases in which no causative agent was detected by MEP (five of them non-panel agents). In two cases, the same pathogenic microorganisms (*S. pneumoniae* and *S. agalactiae*) were detected with both MEP and CSF culture. While 32% of the cases were hospitalized in the intensive care unit, the median of hospital stay was 14 (1-120) days. The most commonly used antimicrobial agents were ceftriaxone/cefotaxime, vancomycin and acyclovir. Empirical antibiotherapy was initiated in eight cases and was discontinued in the early period when a viral agent was detected as a result of MEP. Five cases developed complications of hydrocephalus and subdural effusion. No patient died due to CNS infection.

Discussion

With the worldwide use of pneumococcal and *H. influenza* type b vaccinations, changes have occurred in the prevalence of agents causing CNS infection (2,3). Hasbun et al. have reported that the most common bacterial agents causing CNS infection in children were enteroviruses (54%); however, bacterial agents were detected in only 13% of all cases (7). In studies conducted in our country to detect the viral etiology

Table 2. Cerebrospinal fluid characteristics of the patients

	n= 115
Pleocytosis, n (%)	38 (33%)
Low CSF Glucose Level, n (%)	20 (17%)
Elevated CSF Protein Level, n (%)	57 (49%)
Pathogenic Microorganisms Identified in Cerebrospinal Fluid Culture Media	7 (6%)
Pathogens also Identified with MEP	
S. pneumoniae	1 (0.8%)
S. agalactiae	1 (0.8%)
Pathogens not Screened with MEP	
P. aeruginosa	1 (0.8%)
S. epidermidis	2 (1.7%)
E. faecium	1 (0.8%)
S. haemolyticus	1 (0.8%)
Pathogenic Microorganisms Identified with MEP	29 (25%)
Human herpes virus-6	10 (8%)
Enterovirus	8 (7%)
S. pneumoniae	4 (3.4%)
Varicella zoster virus	3 (2.6%)
Cytomegalovirus	2 (1.7%)
Haemophilus influenzae tip b	1 (0.8%)
Streptococcus agalactiae	1 (0.8%)

CSF: Cerebrospinal fluid, MEP: Meningitis/encephalitis panel.

of acute meningitis and encephalitis in children, Törün et al. have identified the most common viral agents causing CNS infections as EV (25%), adenovirus (22.9%), and HHV-6 (22.9%) while Akkaya et al. have reported EV (29.5%), adenovirus (22.7%), and HSV type I (22.7%) (8,9). In multicenter studies by Ceyhan et al., it has been reported that *N. meningitidis* (54.8%), *S. pneumoniae* (29.6%), and *H. influenzae* type b (15.6%) were the most common bacterial agents causing CNS infections in children in our country between 2005-2012 and 2015-2018 (3,10). In our study, the most common viral agents detected with MEP were HHV-6 (8%) and EV (6%), which is consistent with the literature data.

Many studies have been conducted comparing molecular tests such as MEP with conventional methods used for the identification of pathogenic microorganisms such as CSF culture, microscopic examination, Gram staining and CSF biochemistry in the diagnosis of CNS infection. Waldrop et al. have conducted a study on 1.625 patients with suspected CNS infection and reported that they identified pathogenic microorganisms including viral (n= 88; 74%), bacterial (n= 27; 23%), and fungal (n= 4; 3%) agents in a total of 119 cases using MEP. They have stated that they observed growth of a bacterial agent in CSF culture of only nine of these 27 cases identified by MEP (11). Similarly, a study by Ekambaram et al. has identified bacterial agents using MEP in 53 of 355 children with a preliminary diagnosis of CNS infection based on the identification of a causative agent in CSF samples. Among them, bacterial growth was identified in CSF culture media of only 22 cases while in 29 cases, MEP could not reveal the presence of any causative agent. Again, in the same study, non-panel agents such as *S. aureus, S. epidermidis,* and *Pseudomonas* were found in the CSF culture of 19 cases in which no agent was detected by MEP (12). On the other hand, Messacar et al. have detected a causative agent in 102 (13%) of a total of 805 CSF samples studied with MEP including bacterial agents identified in 20 (2%) CSF samples. In five of these 20 cases, any bacterial growth was not detected in the CSF culture, and the researchers were able to identify the causative agent by Gram staining in only four cases. While any pathogenic agent could not be detected by MEP in two cases, non-panel (*S. aureus)* growth could be revealed in the CSF culture (13).

In our study, the causative agent was detected with MEP in 29 of 230 cases, with a detection rate of 12.6%, similar to the study data reported by Messacar et al. Again, consistent with the literature, viral agents were detected in 23 (79%) and bacterial agents in 6 (21%) of the 29 cases. In our study, as in previous studies, bacterial growth was detected in CSF culture of only two of six cases in which bacterial agents were identified by MEP. There was growth of non-panel pathogenic bacteria in the CSF culture of five cases in which agents were not identified by MEP. This indicates that molecular methods such as MEP are insufficient for detecting non-panel agents such as *S. epidermidis*, *P. aureginosa*, and *enterococci* despite its higher sensitivity in identifying pathogenic microorganisms relative to CSF culture.

Although MEP is a very useful method in the detection of viral pathogens, it is not successful in distinguishing between latent and active infection or viral chromosomal integration, especially for viruses such as HHV-6, CMV, and VZV, which can remain in cells for a long time without causing an active infection (8,14,15). Although CMV was detected in two, VZV in three, and HHV-6 in 10 cases according to MEP, only two cases whose clinical and radiological findings were compatible with CNS infection were treated with acyclovir and one case with ganciclovir.

The main limitations of our study were the small number of cases, the retrospective study design, the inability to perform both cell cultures for viral agents and confirmatory agent-specific PCR tests.

Conclusion

In conclusion, the most common etiological agents in CNS infections in children are viral agents, primarily HHV-6 and enteroviruses, followed by bacterial agents such as *S. pneumonie* and *H. influenzae*. Molecular tests such as meningitis/encephalitis panels are important for the diagnosis of CNS infections and they should be used together with culture and microscopic tests due to their advantages such as screening many factors at once, evaluating both viral and bacterial agents and yielding rapid results.

Ethics Committe Approval: This study was obtained from Eskişehir Osmangazi University of Medicine Deanery Local Ethics Committee (Decision no: 21, Date: 30.03.2022).

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References

1. *Prober CG, Srinivas NS, Mathew R. Central Nervous System Infections. In: Kliegman R, Stanton B, St. Geme J, Schor N (eds). Nelson Textbook Of Pediatrics. 20th ed. Philadelpia: Elsevier Inc, 2016:2936-48.*
2. *Feigin RD, Curter WB. Bacterial Meningitis Beyond The Neonatal Period. Feigin RD, Cherry JD, Harrison RE, Kaplan SL (eds). Feigin & Cherry's Textbook of Pediatric Infectious Diseases, 2009. https://doi.org/10.1016/B978-1-4160-4044-6.50042-X*

3. *Ceyhan M, Ozsurekci Y, Tanır Basaranoglu S, Guler N, Sali E, Keser Emiroğlu M, et al. Multicenter hospital-based prospective surveillance study of bacterial agents causing meningitis and seroprevalence of different serogroups of Neisseria meningitidis, Haemophilus influenzae type b, and Streptococcus pneumoniae during 2015 to 2018 in Turkey. mSphere 2020;5:e00060-20. https://doi.org/10.1128/mSphere.00060-20*
4. *Brouwer MC, Tunkel AR, Van De Beek D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. Clin Microbiol Rev 2010;23(3):467-92. https://doi.org/10.1128/CMR.00070-09*
5. *Houlihan CF, Bharucha T, Breuer J. Advances in molecular diagnostic testing for central nervous system infections. Curr Opin Infect Dis 2019;32(3):244-50. https://doi.org/10.1097/QCO.0000000000000548*
6. *Ramanan P, Bryson AL, Binnicker MJ, Pritt BS. Syndromic panel-based testing in clinical microbiology. Clin Microbiol Rev 2018;31(1):1-28. https://doi.org/10.1128/CMR.00024-17*
7. *Hasbun R, Wootton SH, Rosenthal N, Balada-Llasat JM, Chung J, Duff S, et al. Epidemiology of meningitis and encephalitis in infants and children in the United States, 2011-2014. Pediatr Infect Dis J 2019;38(1):37-41. https://doi.org/10.1097/INF.0000000000002081*
8. *Törün SH, Kaba Ö, Yakut N, Kadayıfçı EK, Kara M, Yanartaş MS, et al. Multicenter prospective surveillance study of viral agents causing meningoencephalitis. Sci Rep 2021;11(1):7216. https://doi.org/10.1038/s41598-021-86687-0*
9. *Akkaya O, Güvenç Hİ, Güzelant A, Kaya M, Yüksekkaya Ş, Opuş A, et al. Investigation of the causative agents of meningitis by real-time PCR method. Türk Mikrobiyol Cem Derg 2017:47(3);131-7. https://doi.org/10.5222/TMCD.2017.131*
10. *Ceyhan M, Gürler N, Ozsurekci Y, Keser M, Aycan AE, Gurbuz V, et al. Meningitis caused by Nesisseria meningitidis, Hemophilus influenzae type b and Streptococcus pneumoniae during 2005-2012 in Turkey. Hum Vaccin Immunother 2014;10:2706-12. https://doi.org/10.4161/hv.29678*
11. *Waldrop G, Zucker J, Boubour A, Radmard S, Green DA, Thakur KT. Clinical significance of positive results of the BioFire cerebrospinal fluid film array meningitis/encephalitis panel at tertiary medical center in the United States. Arch Pathol Lab Med 2022;146(2):194200. https://doi.org/10.5858/arpa.2020-0380-OA*
12. *Ekambaram M, Nabower A, Rajbhandari P, Eisenberg J, Goodrich N, Ampofo K, et al. Evaluation of discordant results between film array meningitis/encephalitis panel and conventional testing in pediatric patients: A multisite retrospective cohort study. J Pediatric Infect Dis Soc 2022;11(4):134-41. https://doi.org/10.1093/jpids/piab126*
13. *Messacar K, Palmer C, Gregoire L, Elliott A, Ackley E, Perraillon MC, et al. Clinical and financial impact of a diagnostic stewardship program for children with suspected central nervous system infection. J Pediatr 2022;244:161-8.e1. https://doi.org/10.1016/j.jpeds.2022.02.002*
14. *Clark DA. Clinical and laboratory features of human herpesvirus 6 chromosomal integration. Clin Microbiol Infect 2016;22(4):333-9. https://doi.org/10.1016/j.cmi.2015.12.022*
15. *Feyzioğlu B, Yavru S, Özdemir M. Laboratory diagnosis of pediatric herpesvirus infections of the central nervous system by a multiplex polymerase chain reaction assay and intrathecal antibodies. J Pediatr Infect Dis 2018:13(3);178-84. https://doi.org/10.1055/s-0038-1639616*