Research Article

Frequency of Herpes Simplex Virus Types 1 and 2 and Cytomegalovirus in Perilymph and Peripheral Blood Samples of Cochlear-Implanted Children Using the Polymerase Chain Reaction Method

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Highlights

- The Cytomegalovirus (CMV) may be associated with sensorineural hearing loss
- The IgG antibody against CMV is positive in most of cochlear-implanted children

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ABSTRACT

Background and Aim: Inner ear infection with some viruses may be one of the possible causes of Sensorineural Hearing Loss (SNHL). This study aims to determine the frequency of Herpes Simplex Virus 1 and 2 (HSV-1, HSV-2) and Cytomegalovirus (CMV) in perilymph and peripheral blood samples of cochlear-implanted children.

Methods: In this cross-sectional study, 30 children with severe-to-profound SNHL (aged 1.1–5 years) underwent cochlear implantation surgery. During surgery, their perilymph and peripheral blood samples were collected. The samples were analyzed separately for the presence of herpes HSV-1, HSV-2, and CMV by real-time Polymerase Chain Reaction (PCR) method. The load of IgG and IgM antibodies against these viruses was determined using the Enzyme-Linked Immunosorbent Assay (ELISA) method.

Results: The frequency of CMV in perilymph samples was 16.7% (5 patients) and in peripheral blood samples was 3.3% (1 patient). The IgG antibody against CMV and HSV-1 was positive in 80% and 46.7% of the patients, respectively. The IgM antibody against CMV was positive in 10%. The mean IgM serum antibody load against HSV-1, HSV-2, and CMV was 2.70, 1.70, and 5.47, respectively, and the mean IgG antibody load against these viruses was 56.07, 2.50, and 23.67, respectively.

Conclusion: The IgG test is positive in cochlear-implanted children with CMV in their perilymph samples, and the CMV genome is not present in their peripheral blood. This may indicate the previous presence of this virus in the ear and its role in hearing loss.

Keywords: Sensorineural hearing loss; perilymph fluid; cytomegalovirus; herpes simplex

Introduction

Sensorineural Hearing Loss (SNHL) is a common type of hearing loss and can cause severe disability and social problems in the effected individuals and impose a cost burden on the individual and the society [1]. Viral infections are among the causes of SNHL. Recently, studies have been conducted on the role of Cytomegalovirus (CMV) in causing congenital and acquired hearing loss [2-4]. Although the presence of viruses in the inner ear may be related to the hearing loss, it is more important to find the viruses that remain in the inner ear after the initial infection and can be caused by gradual destruction or reactivation of the causes. Possible SNHL [5, 6]. After genetic diseases, infections are the most important cause of SNHL in children, and viruses such as Herpes Simplex Virus (HSV) and CMV not only cause infection and damage to the inner ear during the perinatal period, but also they can cause progressive or sudden SNHL [7-9]. Viruses cause hearing loss through various mechanisms, including direct damage to hair cells and the organ of Corti and even to the immune system [10, 11].

Reaching the inner ear to confirm the presence and tendency of viruses is not easy. Few studies have been done in this field [12]. CMV and HSV viruses are from the human Herpesviridae family that have a high tendency to involve nervous organs. CMV is the largest member of the family and has double-stranded DNA [13]. After the initial infection, which usually occurs at the beginning of life or during the embryonic period, CMV is hidden in the tissues and can become active again later, especially in immunocompromised patients and cause tissue damage. Hearing loss caused by congenital infection with is usually bilateral and severe and is accompanied by other neurological symptoms. Probably, infection with this virus after birth can lead to severe and profound hearing loss, even in adulthood. Treatment of CMV infection with drugs such as Ganciclovir can stop the process of inner ear damage and even improve hearing [10]. Hearing loss caused by HSVis unilateral or bilateral and is usually moderate to severe. HSV-1 is more associated with hearing loss and neurological complications than HSV-2. Congenital HSV infection can be prevented by avoiding delivery through an infected birth canal and taking medications such as acyclovir [11, 14]. In SNHL, the inner ear is the most important site of involvement, which is usually not accessible in living people; however, during cochlear implantation surgery, perilymph fluid sampling is possible without any additional damage [15, 16].

Recently, some studies have been conducted on the role of CMV in causing congenital and acquired hearing loss, but there is scant research to isolate viruses from the inner ear of living patients [17, 9]. Thus, this study aims to elucidate the role of congenital CMV infection in hearing loss with unknown etiology in children without genetic abnormalities.

Methods

This is a cross-sectional study. Thirty children with severe to profound bilateral SNHL (aged 1.1–5 years old) referred to Be'sat Hospital in Hamadan, Iran, during 2018–2019 were included in the study. The exclusion criteria were SNHL following meningitis, SNHL following direct trauma, hearing loss at low frequencies, and being candidates for electric-acoustic stimulation using a short electrode array. After obtaining written consent from the patients or their parents, blood and perilymph samples were taken during the cochlear implantation surgery (no fees were charged from for the surgery). Perilymphatic fluid was collected under the microscope and before electrode placement by inserting a spinal needle (with a diameter of 0.4 mm and a length of 90 mm) into the round window. The perilymph and blood samples were transported to the virology laboratory of Hamadan University of Medical Sciences, and kept at a temperature of 2–8°C for 30 minutes before performing real-time PCR. The samples were prepared after treatment with a suitable buffer to remove blood in the perilymph fluid, as well as citrated plasma after separation to extract the genetic material and genome. After this step, the DNA extraction was carried out according to the manufacturer's instructions using the high pure viral DNA/RNA commercial kit (Roche Diagnostics, Switzerland). In this regard, 400 µL of binding buffer was added to 200 µL of samples in a microtube. The mixture was transferred to a filter tube and centrifuged at 8000 xg for one minute. 500 µL of binding buffer was added again and the tube was centrifuged. Then, 450 µL of wash buffer was added in two steps with centrifugation at 8000 xg for one minute after each step. Finally, 50 µL of elution buffer was added, followed by a final centrifugation at 8000 xg for

one minute. The eluted DNA was stored at –80°C for PCR. DNA purity was determined using a NanoDrop (Thermo, USA) by assessing the ratio of absorbance at 260 nm and 280 nm, which usually ranges from 1.8 to 2. Furthermore, the AmpliSens diagnostic kit was used to detect each HSV-1, HSV-2, and CMV separately using real-time PCR. We assessed two qualitative variables: one was the PCR result of perilymph for CMV (positive or negative), and the other was the IgM or IgG test result using Enzyme-Linked Immunosorbent Assay (ELISA) method (Euroimmune, Germany) (positive or negative). We examined the relationship between these two variables using Fisher's exact test.

Results

Of 30 cochlear-implanted children who underwent the surgery in this study, 14 were male (46.67%) and 16 (53.33%) were female. Their mean age was 2.83 years, ranging from 13 months to 5 years. Nine patients (30%) had<2 years of age, 15 (50%) had 2–4 years of age, and 6 (20%) were 5 years old. By performing the PCR on the perilymph fluid of the patients, CMV was observed in 5 cases (16.7%), but HSV-1 and HSV-2 were not observed in the inner ear of any patients. Also, by performing PCR on patients' serum samples, one case (3.3%) of CMV was reported; HSV-1 and HSV-2 were not observed in the peripheral blood samples of any patients. According to the results, the mean IgM serum antibody load against HSV-1, HSV-2, and CMV in patients was 2.70, 1.70, and 5.47, respectively, and the mean serum IgG antibody load against these viruses was 56.07, 2.50, and 67.23, respectively (Table 1).

In examining the patients' serum levels, 14 patients (46.7%) had IgG antibodies against HSV-1, 24 patients (80%) had IgG antibodies against CMV, and three patients (10%) had IgM antibodies against CMV. None of the patients were positive for IgG and IgM antibodies against HSV-2. In all 5 patients whose CMV genome was found in their perilymph, the IgG was positive for this virus. Moreover, due to the non-parametric distribution of antibody load and that only in five patients, the PCR test was positive for CMV on the perilymph fluid, Mann-Whitney test was used to compare the average load of IgM and IgG antibodies and no significant difference was observed between the two groups. Moreover, the Fisher's exact test results showed no significant relationship of IgM (p=0.433) and IgG (p=0.553) with PCR of perilymph for CMV (Table 2, Figures 1 and 2).

Discussion

In the present study, based on PCR results, CMV genome was found in the perilymph fluid of five cochlear-implanted children (16.7%) and in the peripheral blood of one child (3.3%). In Sugiura et al., study, CMV was isolated from the perilymph of one (20%) in five patients who underwent cochlear implantation [18]. The infection with CMV is mostly subclinical and can affect all organs of the body, including the inner ear [19]. The virus mainly hides in the nervous system, and is reactivated after

Table 1. Examination of serum IgG and IgM antibodies against herpes simplex viruses 1 and 2 and cytomegalovirus in cochlear implanted patients

Variable	Mean	SD	Minimum	Maximum
IgM				
HSV-1	2.70	1.64	1	8
HSV-2	1.70	1.47	0	7
CMV	5.47	6.92	0	33
IgG				
HSV-1	70.56	57.89	1	126
HSV-2	2.50	2.44	0	9
CMV	67.32	41.92	1	121

HSV; herpes simplex virus, CMV; cytomegalovirus

Table 2. Releation between IgG and IgM serum antibodies against cytomegalovirus, according to the polymerase chain reaction results on the perilymph fluid of the cochlear implanted patients

Anti-cytomegalovirus CMV Ab	PCR of perilymph for cytomegalovirus		p
	Negative (n=25)	Positive (n=5)	
IgM			
Positive	2	1	
Negative	23	4	0.433
Total	25	5	
IgG			
Positive	19	5	
Negative	6	0	0.553
Total	25	5	

CMV; cytomegalovirus, PCR; polymerase chain reaction

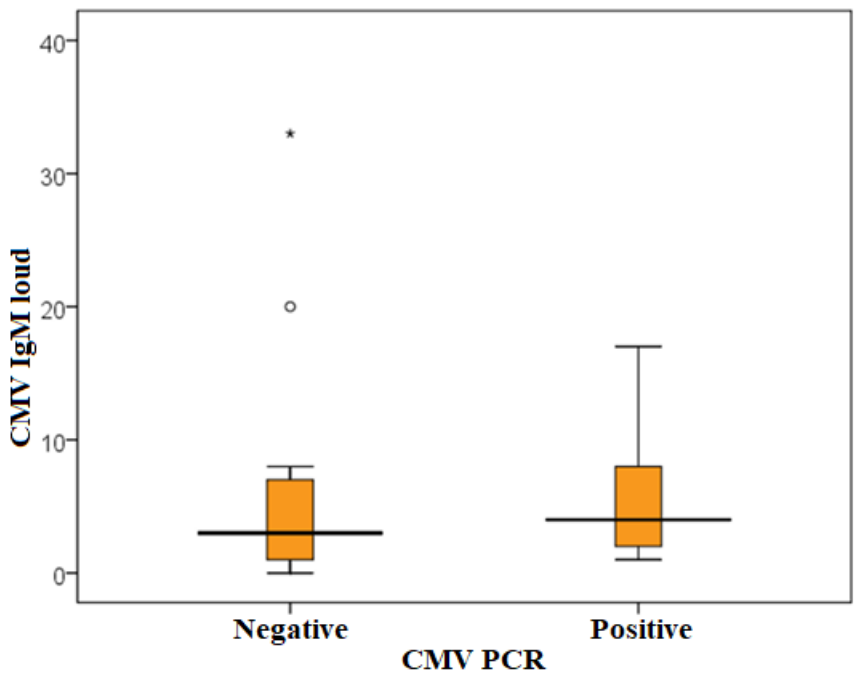

Figure 1. Frequency distribution of IgM antibody load against cytomegalovirus according to polymerase chain reaction results on perilymph fluid of cochlear implanted patients. CMV; cytomegalovirus, PCR; polymerase chain reaction

congenital infections or under certain conditions, such as immune system changes [20]. Viruses are involved in the development of some ear diseases, including Meniere's disease [21], otosclerosis [22] and SNHL. CMV is one of the viruses that can cause endolymphatic hydrops with inner ear damage [23, 24]. In addition, CMV infection is one of the possible causes of SNHL and tinnitus [25]. In another study by Sugiura et al. in 2004 on the samples obtained from the perilymph of 14 patients during cochlear implantation surgery, two patients (14.3%) who had a history of congenital CMV infection symptoms were positive for CMV [26]. Martinez-Gomez et al. in a review study reported that 14% of the patients with SNHL had a history og CMV infection [27].

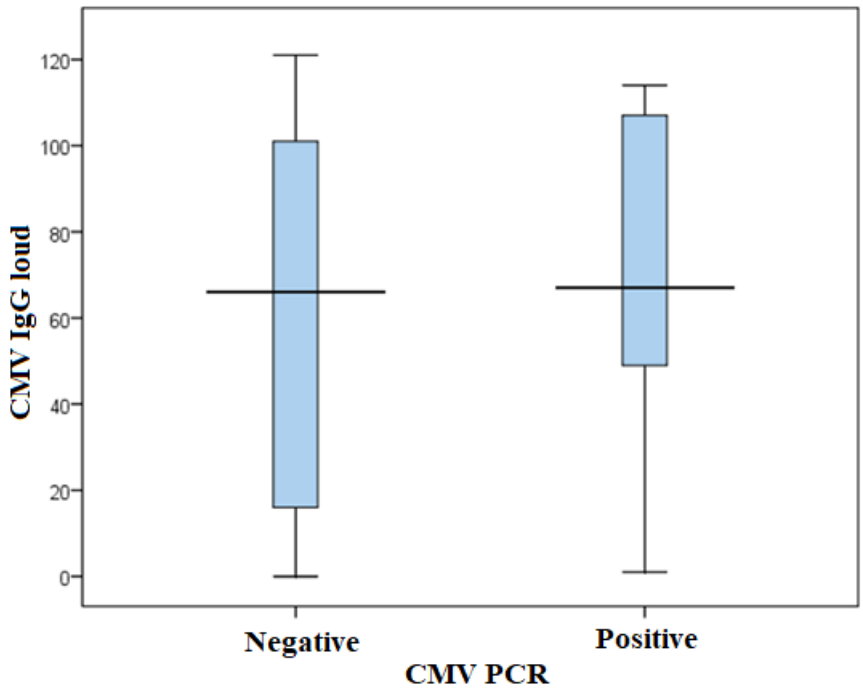

Figure 2. Frequency distribution of IgG antibody load against cytomegalovirus according to polymerase chain reaction results on perilymph fluid of cochlear implanted patients. CMV; cytomegalovirus, PCR; polymerase chain reaction

In the current study, 10% of the patients had IgM positive against CMV, indicating active infection or a recently acquired disease. In addition, 80% of them had IgG antibodies against CMV, which indicates having a history of disease in the past or congenital infection. The PCR results showed that the prevalence of CMV infection in peripheral blood and perilymph fluid samples were 3.3% and 16.7%, respectively, while the serum of none of five patients with CMV in the perilymph was positive for this virus. This may indicate a possible role of CMV infection in SNHL. Considering that four of five patients with CMV in their perilymph fluid had no evidence of new infection with this virus (IgM antibodies were negative against CMV), probably the inner ear is one of the organs where this virus hides and causes further damage. It should be noted that in these five patients, serum IgG antibodies against CMV was positive, indicating previous history of infection with this virus and confirming the PCR result of these patients.

In a study by Noorbakhsh et al. on 18 children with no observed hearing loss, CMV DNA was detected in the perilymph fluid of three patients (16.7%) using the PCR method [28]. In de Vries et al.'s study on a perilymph samples taken from 29 children during cochlear implantation, the CMV genome was obtained in one case (3.45%) using the PCR [29]. In Di Nardoa et al.'s study on the perilymph fluid of 36 patients undergoing cochlear implantation, the CMV genome was found in two patients and HSV-1 genome was in one patient. The PCR results showed that the serum of all patients was negative [11]. The findings of our study regarding the frequency of CMV in the perilymph fluid samples are consistent with the results of de Vries et al. [29], but it is lower than in Noorbakhsh et al.'s study. The reason for this discrepancy in the results may be due to the fact that the children with cochlear implantation were examined in our study, while in the study by Noorbakhsh et al. [28], the patients with unexplained hearing loss participated. In our study, none of the patients were HSV positive in PCR or antibody assay. Regarding HSV-1, the PCR results for peripheral blood and perilymph fluid were negative, but 46.7% of the patients had IgG antibodies against HSV-1, while there was no IgM antibody test positive. Consistent with the results of our study, the HSV genome was not found in the perilymph of patients in the study by Sugiura et al. [26].

Considering that in most of the studies, including our

study, CMV has been the most important common pathogen isolated from the inner ear of patients with SNHL, it seems that infection with this virus is related to hearing damage [6]. Probably, this virus hides in the inner ear after the initial fetal infection or later and causes continued damage to the cochlear.

To our knowledge, this is the first study that investigates three viruses of HSV-1, HSV-2, and CMV in the perilymph fluid and blood samples of cochlear-implanted children by the real-time PCR method and determines the level of IgG and IgM antibodies against these viruses. However, the lack of a control group to compare the frequency of infections due to methodological problems was one of the limitations of this study .Also, since the infants are not routinely screened for CMV and HSV presence in Iran, it was not possible to properly distinguish congenital infections with these viruses from acquired ones. Moreover, considering the high costs of genetic testing and the unknown nature of some genes involved in disease, it was not possible to conduct genetic examination on all patients and determine the genetic causes of SNHL [30]. It is recommended that the genetic evaluation of the children with SNHL was conducted in future studies to find the genes involved in SNHL and make a more accurate assessment of the impact of infectious virus. In addition, considering that conducting a study on the perilymph of living people, including patients who are candidates for cochlear implantation is not possible, it is recommended to systematically review all studies on the viruses causing SNHL, including CMV. Considering CMV infection in the perinatal period and later, which causes irreparable neurological damage, necessary measures should be taken to convince the Iranian health system to develop screening programs for this virus in the country.

Conclusion

In this study, by collecting serological and molecular information for three viruses of Herpes simplex virus-1, Herpes simplex virus-2, and Cytomegalovirus (CMV) in causing hearing loss from perilymph and blood samples of cochlear-implanted children, CMV was identified as the possible causative agent of this disease. The IgG test is positive in cochlear-implanted children with CMV genome present in their perilymph, and CMV genome is not present in their peripheral blood. This may confirm the previous presence of the virus in the ear and its role in hearing loss. It may also be related to congenital CMV infection in these children.

Ethical Considerations

Compliance with ethical guidelines

Ethical approval was IR.UMSHA.REC.1399.1061.

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Authors' contributions

FF: Study design, interpretation of the resultsand drafting the manuscript; MVR: Interpretation of the results, and drafting the manuscript; HN: Interpretation of the results and drafting the manuscript; ES: Interpretation of the results and drafting the manuscript; RN: Statistical analysis, FAJ: Study design, acquisition of data, interpretation of the results.

Conflict of interest

There is no conflict of interest between the authors.

References

1. Bubbico L, Bellizzi S, Ferlito S, Maniaci A, Leone Guglielmotti R, Antonelli G, et al. The Impact of COVID-19 on Individuals with Hearing and Visual Disabilities during the First Pandemic Wave in Italy. Int J Environ Res Public Health. 2021;18(19):10208. [DOI:10.3390/ijerph181910208]

2. Prosser JD, Holmes TW, Seyyedi M, Choo DI. Congenital cytomegalovirus (CMV) for the pediatric otolaryngologist. Int J Pediatr Otorhinolaryngol. 2021;148:110809. [DOI:10.1016/j.ijporl.2021.110809]

3. Smiechura M, Strużycka M, Konopka W. Congenital and acquired cytomegalovirus infection and hearing evaluation in children. Otolaryngol Pol. 2014;68(6):303-7. [DOI:10.1016/j.otpol.2014.04.003]

4. Fowler KB, Boppana SB. Congenital cytomegalovirus (CMV) infection and hearing deficit. J Clin Virol. 2006;35(2):226-31. [DOI:10.1016/j.jcv.2005.09.016]

5. Karimi-Boroujeni M, Zahedi-Amiri A, Coombs KM. Embryonic Origins of Virus-Induced Hearing Loss: Overview of Molecular Etiology. Viruses. 2021;13(1):71. [DOI:10.3390/v13010071]

6. Shi X, Liu X, Sun Y. The Pathogenesis of Cytomegalovirus and Other Viruses Associated with Hearing Loss: Recent Updates. Viruses. 2023;15(6):1385. [DOI:10.3390/v15061385]

7. Harris S, Ahlfors K, Ivarsson S, Lernmark B, Svanberg L. Congenital cytomegalovirus infection and sensorineural hearing loss. Ear Hear. 1984;5(6):352-5. [DOI:10.1097/00003446-198411000-00006]

8. Rezaei A, Sariri R. Periodontal Status, Salivary Enzymes and Flow Rate in Passive Smokers. Pharmacologyonline. 2011;3:462-76.

9. Eisen MD, Ryugo DK. Hearing molecules: contributions from genetic deafness. Cell Mol Life Sci. 2007;64(5):566-80. [DOI:10.1007/s00018-007-6417-3]

10. Cohen BE, Durstenfeld A, Roehm PC. Viral causes of hearing loss: a review for hearing health professionals. Trends Hear. 2014;18:2331216514541361. [DOI:10.1177/2331216514541361]

11. Di Nardo W, Anzivino R, Cattani P, Santangelo R, De Corso E, Paludetti G. Herpes simplex virus-1 and cytomegalovirus DNAs detection in the inner ear of implanted patients with non-congenital infection. Acta Otolaryngol. 2017;137(8):791-6. [DOI:10.1080/00016489.2017.1293292]

12. Chebib E, Vauloup-Fellous C, Benoit C, Noël Petroff N, Van Den Abbeele T, Maudoux A, et al. Is CMV PCR of inner ear fluid during cochlear implantation a way to diagnose CMV-related hearing loss? Eur J Pediatr. 2023;182(1):375-83. [DOI:10.1007/s00431-022-04691-6]

13. Tayyar R, Ho D. Herpes Simplex Virus and Varicella Zoster Virus Infections in Cancer Patients. Viruses. 2023;15(2):439. [DOI:10.3390/v15020439]

14. Sauerbrei A, Wutzler P. Herpes simplex and varicella-zoster virus infections during pregnancy: current concepts of prevention, diagnosis and therapy. Part 1: herpes simplex virus infections. Med Microbiol Immunol. 2007;196(2):89-94. [DOI:10.1007/s00430-006-0031-0]

15. Zamani P, Soleymani Z, Rashedi V, Farahani F, Lotf G, Rezaei M. Spoken and Written Narrative in Persian-Speaking Students Who Received Cochlear Implant and/or Hearing Aid. Clin Exp Otorhinolaryngol. 2018;11(4):250-8. [DOI:10.21053/ceo.2017.01011]

16. Gohari N, Emami SF, Mirbagheri SS, Valizadeh A, Abdollahi N, Borzuei M. The Prevalence and Causes of Auditory Neuropathy/Dys-synchrony (AN/AD) in Children with Hearing Impairment. Indian J Otolaryngol Head Neck Surg. 2019;71(1):71-5. [DOI:10.1007/s12070-018-1494-1]

17. Xia W, Yan H, Zhang Y, Wang C, Gao W, Lv C, et al. Congenital Human Cytomegalovirus Infection Inducing Sensorineural Hearing Loss. Front Microbiol. 2021;12:649690. [DOI:10.3389/fmicb.2021.649690]

18. Sugiura S, Yoshikawa T, Nishiyama Y, Morishita Y, Sato E, Hattori T, et al. Detection of human cytomegalovirus DNA in perilymph of patients with sensorineural hearing loss using real-time PCR. J Med Virol. 2003;69(1):72-5. [DOI:10.1002/jmv.10263]

19. Vancíková Z, Dvorák P. Cytomegalovirus infection in immunocompetent and immunocompromised individuals--a review. Curr Drug Targets Immune Endocr Metabol Disord. 2001;1(2):179-87. [DOI:10.2174/1568005310101020179]

20. Dupont L, Reeves MB. Cytomegalovirus latency and reactivation: recent insights into an age old problem. Rev Med Virol. 2016;26(2):75-89. [DOI:10.1002/rmv.1862]

21. Schuknecht HF, Suzuka Y, Zimmermann C. Delayed endolymphatic hydrops and its relationship to Meniére's disease. Ann Otol Rhinol Laryngol. 1990;99(11):843-53. [DOI:10.1177/000348949009901101]

22. McKenna MJ, Kristiansen AG, Haines J. Polymerase chain reaction amplification of a measles virus sequence from human temporal bone sections with active otosclerosis. Am J Otol. 1996;17(6):827-30.

23. Kamei T. Delayed endolymphatic hydrops as a clinical entity. Int Tinnitus J. 2004;10(2):137-43.

24. Lobo D, Tuñón M, Villarreal I, Brea B, García-Berrocal JR. Intratympanic gadolinium magnetic resonance imaging supports the role of endolymphatic hydrops in the pathogenesis of immune-mediated inner-ear disease. J Laryngol Otol. 2018;132(6):554-9. [DOI:10.1017/S0022215118000749]

25. Dean NJ, Pastras C, Brown D, Camp A. Are viral-infections associated with Ménière's Disease? A systematic review and meta-analysis of molecular-markers of viral-infection in case-controlled observational studies of MD. PLoS ONE. 2019;14(11): e0225650. [DOI:10.1371/journal.pone.0225650]

26. Sugiura S, Yoshikawa T, Nishiyama Y, Morishita Y, Sato E, Beppu R, et al. Detection of herpesvirus DNAs in perilymph obtained from patients with sensorineural hearing loss by real-time polymerase chain reaction. Laryngoscope. 2004;114(12):2235-8. [DOI:10.1097/01.mlg.0000149466.80703.55]

27. Martinez-Gomez E, Perez-Carpena P, Flook M, Lopez-Escamez JA. A Systematic Review on the Association of Acquired Human Cytomegalovirus Infection with Hearing Loss. J Clin Med. 2020;9(12):4011. [DOI:10.3390/jcm9124011]

28. Noorbakhsh S, Farhadi M, Daneshi A, Mohamadi S, Tabatabaei A. Viral infections detected by serology and PCR of perilymphatic fluid in children with idiopathic sensorineural hearing loss. East Mediterr Health J. 2011;17(11):867-70.

29. de Vries JJ, Vesseur A, Rotteveel LJ, Korver AM, Rusman LG, Wessels E, et al. Cytomegalovirus DNA detection in dried blood spots and perilymphatic fluids from pediatric and adult cochlear implant recipients with prelingual deafness. J Clin Virol. 2013;56(2):113-7. [DOI:10.1016/j.jcv.2012.10.008]

30. Noorbakhsh S, Farhadi M, Haghighi F, Minaeian S, Hasanabad MH. Neonatal screening for congenital cytomegalovirus infection in Tehran, Iran, using Guthrie cards. Iran J Microbiol. 2020 Jun;12(3):198-203. [10.18502/ijm.v12i3.3236]

31. Kashani A, Shariatpanahi E, Ayubi E, Emami SF. The Best Users of Cochlear Implants.Indian J Otolaryngol Head Neck Surg. 2023. [DOI:10.1007/s12070-023-04073-w]