Evaluation of cytomegalovirus DNA in perilymphatic fluid in patients with sensorineural hearing loss using PCR technique

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ABSTRACT

Purpose: Congenital cytomegalovirus (CMV) infection is the most common congenital infection in developing countries with sensorineural hearing loss (SNHL) being the most important of its long-term sequelae. The aim of this research was to evaluate the amount of CMV DNA in perilymph of patients with SNHL undergoing cochlear implant (CI) procedure by Polymerase chain reaction (PCR) technique.

Materials and methods: This cross-sectional and prospective study was conducted in a group of patients with ages ranging from 1 to 7 years. The perilymph was extracted preoperatively using a Yale spinal needle after opening of the round window. 25 microliters of perilymph was extracted and stored at -80 degrees centigrade. PCR technique was employed to detect CMV DNA. The data was collected and statistically analyzed.

Results: A sample size of 41 patients with bilateral profound SNHL was selected. After cochlear implantation, CMV DNA evaluation was performed which was found positive in 18 patients (43.9%) and negative in 23 patients (56.09%) with a statistically significant relationship. No statistical relationship was found with gender, age and other comorbid disorders. 3-6 months’ follow-up was individually performed to compare Categories of Auditory Performance (CAP) and Speech Intelligibility Rating (SIR) tests in CMV DNA positive and negative patients, yielding different results which were statistically insignificant.

Conclusion: The early detection of CMV infection with real time PCR technique enables us to perform viral screening tests in patients with SNHL.

Keywords: SNHL, cytomegalovirus, perilymphatic fluid, cochlear implant

INTRODUCTION

Incidence of CMV infection at birth is about 1 in 200 births (1) and is the most common of TORCH group of diseases causing congenital infections during pregnancy. Babies born with congenital CMV infection may or may-not manifest symptoms at birth. About 90-95% of babies with congenital CMV infection will not have clinically apparent symptoms at birth. Both symptomatic and asymptomatic infants might later develop sequelae, which are more frequent and severe in the symptomatic infants (2,7). Sequelae of CMV infection include SNHL, mental retardation, vision loss, cerebral palsy and seizures (3). The most common of the CMV infection sequelae is SNHL (10-15%) and is defined as the loss of hearing either due to cochlear or retro cochlear lesions (8). Hearing loss is a condition which may be caused by either hereditary or acquired disorders. Infections, ototoxic drugs, meningitis, measles, encephalitis, chicken pox, mumps, head trauma, and intense sounds can be cited from among the acquired causes (4,5,6). CMV infection in adults has also been reported to cause SNHL. But this problem is relatively different from that of children and is often seen along with diseases such as leukemia and lymphoma, whereas in children the disease occurs locally and systemically (9).
Although SNHL is known to be one of the most important sequelae of congenital CMV infection, most of the causes of SNHL are unclear. Among the most important reasons for the causes being unknown are the lack of access to the 21-day neonatal blood samples required for the diagnosis of congenital CMV infection and lack of access to cochlear fluid for the detection of CMV in alive children (10).

30% to 50% of the children who suffer from SNHL have symptomatic CMV infection and 8% to 12% of them are asymptomatic (11). Small minority of newborns who fail their newborn hearing screening tests have congenital CMV (3%-8%). The great majority of newborns with congenital CMV infection pass their newborn hearing screen (93%). Hearing loss due to congenital CMV infection has no pathognomonic features in the audiometry tests. It is seen as unilateral high frequency loss as well as bilateral hearing loss. Both progressive and fluctuating hearing losses have been reported in CMV infection (12). Recent studies have shown that 21% of the cases show SNHL at birth and 25% up to 4 years of age. Thus an assessment of the hearing thresholds is required every six months. If changes in hearing thresholds are noticed, the evaluation is done every three months (8). The diagnosis of congenital CMV infection in the first 3 weeks of birth can be done from the urine and saliva of the neonate. The fastest diagnostic technique for detecting CMV is the PCR (9) and provides results in almost 24 hours. Cytotoxic effect (CPE) and detection of early antigen fluorescent foci (DEAFF) are other diagnostic techniques which require 2-4 weeks for results.

The first case study of inner ear CMV was reported in 1969 in a patient who had died from the infection. Ten years later in 1979, the virus was extracted from perilymph of an alive patient, and CMV DNA was first extracted in January 2003 by Sugiura et al. (5) using Real Time PCR (RT PCR). The purpose of this study was to evaluate the prevalence of CMV DNA in perilymph of patients with severe to profound SNHL who were candidates for cochlear implant (CI). The other goal was to investigate the effects of CMV infection on verbal clarity and auditory perception at 3 and 6 months after having undergone CI surgery.

### MATERIALS & METHODS

This prospective and cross-sectional study was conducted on patients undergoing surgery for CI in Ahvaz CI Center from March 2017 to March 2018. About 25 microliters of perilymph sample was drawn peroperatively from the cochlea through round window using Yale spinal needle attached to a syringe and immediately preserved at -80°C. In all cases auditory brainstem evoked response (ABER), otoacoustic emissions (OAE) and pure tone audiometry (PTA) were performed as per the American Academy of Otolaryngology guidelines. CMV DNA was extracted by industrial kit and extraction column and traced by PCR method. DNA extraction was done using ABI 5700 sequence detection system. The primer and probe were set according to the guide for CMV DNA detection. SPSS® Statistics 22 was used to provide percentages, mean and standard deviation of the group under study. Chi-square test was used to compare CMV DNA levels among different groups. Independent t-test was used to compare the mean of the two groups. The final analysis of the data was done using logistic regression and P < 0.05 was considered as significant.

### RESULTS

CMV DNA evaluation was performed in 41 patients who underwent CI procedure (Figure 1). Out of those, 18 patients were found to be positive for CMV DNA (43.9%) and 23 (56.09%) patients were negative for CMV DNA showing a statistically significant relationship.

Mean, standard deviation and CMV comparison after cochlear implantation in terms of age is presented in Table 1 by using independent t-test. As it is observed in Table 1, after cochlear implantation the mean and standard deviation of age in patients with positive CMV DNA were 3.4 and 1.4 respectively. In patients who were negative for CMV DNA, mean and standard deviation of age were 3.5 and 1.8 respectively.

In order to compare CMV in terms of age, with regard to the normal distribution of age variable, based on Kolmogorov-Smirnov test in positive CMV patients (P = 0.350, Z = 0.0211), and in negative CMV patients (P=0.387, Z = 0.182) and the equality of variances based on Levene’s test (P= 0.222, F=1.54), the independent t-test was used for independent groups. The results of independent t-test also showed that age could not create a significant difference in CMV of patients after cochlear implantation (p = 0.869).

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<tr>
<td></td>
<td>P.value</td>
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<td>Positive</td>
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<tr>
<td>Negative</td>
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</tr>
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<td>Mean</td>
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<td>SD</td>
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**Table 1: Mean and Standard Deviation of Positive and Negative CMV DNA in Terms of Age**

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Frequency, Percentage and CMV comparison after cochlear implantation in patients with SNHL based on gender, accompanying disorder, CAP and SIR at 3 and 6 months have been reported by using the Chi-square test in Table 2. As it is observed in Table 2, the 44.4% of girls were positive for CMV DNA and 43.9% of girls were negative for CMV DNA after CI (P=0.753, X² =0.099).

It is also observed that 72% of the patients who were positive for CMV DNA at the time of CI had no other accompanying disorder. 11.1% had one accompanying disorder and 16.7% had two accompanying disorders.

In patients who were negative for CMV DNA at the time of CI, 65.2% had no accompanying disorder, 21.7% had one accompanying disorder, 4.3% had two accompanying disorders, 4.3% had four accompanying disorders (P=0.432, X² = 3.9).

In patients who were positive for CMV DNA the CAP scales at 3 months after CI were as following: 22.2% were in category 1, 44.4% were in category 2, 27.8% were in category 3 and 5.6% were in category 5.

In patients who were negative for CMV DNA, CAP scale at 3 months after CI were as following: 18.2% were in category 0, 45.5% were in category 1, 13.6% were in category 2, 33.3% were in category 3, 33.3% were in category 4, 11.1% were in category 5, and 5.6% were in category 7 (P=0.183, X² = 7.5).

Patients who were positive for CMV DNA CAP scales at 6 months after CI were as following: 16.7% were in category 0, 4.5% were in category 1, 13.6% were in category 2, 18.2% were in category 3, 36.4% were in category 4 and 13.6% were in category 5 (P=0.477, X² = 5.5).
Patients who were positive for CMV DNA, SIR at 3 months after CI were as following: 94.4% were in category 1 and 5.6% were in category 3.

Patients who were negative for CMV DNA, SIR at 3 months after CI were as following: 90.9% were in category 1 and 9.1% were in category 2 (P=0.238, $X^2=2.78$).

Patients who were positive for CMV DNA, SIR at 6 months after CI were as following: 72.2% were in category 1, 22.2% were in category 2 and 0% were in category 3.

Patients who were negative for CMV DNA, SIR at 6 months after CI were as following: 72.8% were in category 1, 17.2% were in category 2 and 9.1% were in category 3 (P=0.401, $X^2=2.94$).

The results of investigating CMV DNA positive patients versus CMV DNA negative patients show that there is a difference in verbal resolution and auditory perception, but no statistically significant difference has been observed probably due to the time period of less than one year in this research.

**DISCUSSION**

Di Nardo et al. (2011) reported one patient with an unknown cause of hearing loss who was referred for CI. CMV infection and anti-CMV immunoglobulins were detected positively in patient but perilymph which was examined for CMV DNA during CI was found negative (13). Noyola et al. (2000) conducted a 6-year case study as a case report on children with congenital CMV infection and measured the relationship between urinary cytomegalovirus levels over time and the rate of physical milestones or neurological milestones of children, neurodevelopment and progression of SNHL. No significant correlation was found between the cytomegalovirus in urine and the rate of development of children and cognitive development and neural development, but its effect on the progression of SNHL was significant (14). Davis et al. (1969) reported a case died because of congenital CMV infection that had CMV in inner ear autopsy (15). In a retrospective case series Bauer et al. (2005) analyzed perilymph and blood samples using real time PCR for presence and quantification of CMV in six patients with SNHL undergoing CI procedure (16). All six patients had negative blood results for the virus but four patients were positive for CMV in the perilymph. In a study by Noorbakhsh et al. (2011), CMV and herpes simplex virus were found to be 16.7% in perilymph fluid. In our study, CMV DNA in perilymphatic fluid of patients with idiopathic sensory neural hearing loss were 43.9% using PCR technique (17). In the study, there was no relationship of CMV DNA with age and sex. In CMV DNA positive patients, there was a more difference in verbal clarity and auditory perception after CI compared to cytomegalovirus negative patients, but statistically not significant that probably due to the time period of evaluation being less than one year. The study emphasizes the need for longer periods of follow-up of patients.

**CONCLUSION**

The early detection of CMV infection with real time PCR technique enables us to perform viral screening tests in patients with SNHL. A better understanding of the causes of sensory neural hearing loss is important for identifying high-risk children who need early otologic follow-up and who may be candidates for anti-viral therapies. There is still a lot of work to do, but we hope to increase awareness of the CMV disease burden.

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