Molecular Analysis in Diagnostic Procedure of Hearing Impairment in Newborns

Sanja Zaputović, Tea Štimac, Igor Prpić, Vesna Mahulja-Stamenković, Igor Medica¹, Borut Peterlin²

Department of Obstetrics and Gynecology, Rijeka University Hospital Center, Rijeka, Croatia; ¹Outpatient Pediatric Clinic Pula, Pula, Croatia; and ²Division of Medical Genetics, Department of Obstetrics and Gynecology, University Medical Centre, Ljubljana, Slovenia

Aim
To determine the proportion of newborns diagnosed with hearing impairment through the hearing impairment screening program in newborns, and the frequency of 35delG/GJB2 mutation as a cause of hearing impairment. The results of the study imply the integration of the mutation analysis in the neonatal screening program.

Methods
Evoked otoacoustic emission (E-OAE) screening program was performed among 6019 newborns at the Department of Obstetrics and Gynecology, Rijeka University Hospital Center, between October 2002 and December 2004. Newborns diagnosed with hearing impairment were re-examined after three weeks and if abnormal responses persisted, the diagnosis was evaluated by auditory brainstem evoked response (ABER) testing. Children with confirmed diagnosis were examined by allele-specific polymerase chain reaction to identify the presence of 35delG/GJB2 mutation.

Results
After the first and second stage of screening, 86 newborns were suspect of having hearing impairment. ABER confirmed the diagnosis of hearing impairment in 14 children. Molecular analysis revealed 35delG/GJB2 mutation in 2 of 8 children analyzed. The mutation was homozygous in one, and heterozygous in the other child.

Conclusion
Neonatal hearing impairment screening is useful for early diagnosis of hearing impairment. It should be complemented with the 35delG/GJB2 mutation analysis, because the identification of the mutation and the etiologic diagnosis might improve the medical treatment and genetic counseling of patients and families with hearing impairment.

Hearing impairment is the most common sensory disorder with the incidence of 1/1,000 neonates and prevalence that increases with age (1,2). Considering the complexity of the structure of the hearing apparatus and the overall mechanism of the hearing process, the etiology of hearing impairment is heterogeneous (3). In half of the cases the cause is genetic – deafness may be part of a syndrome or it occurs as an independent entity. In 80% of children, genetic hearing impairment is non-syndromic, inherited as autosomal recessive disorder (4,5). Hearing impairment has unfavorable effect on speech development and the overall development of a child. Considering its frequency and sociologic impact, hearing impairment is an important public health problem. To allow for early diagnosis and early speech rehabilitation, efforts have been made to include the hearing impairment screening in neonatal screening program by using evoked otoacoustic emission...
(E-OAE), which is the method of choice. In Croatia, a universal national program for neonatal hearing screening was introduced in 2000, and the prevalence of non-syndromic inherited bilateral hearing impairment greater than 40 dB has been established to be 0.9/1,000 children (6).

In children diagnosed with hearing impairment through neonatal hearing impairment screening program, further diagnostic evaluation such as molecular genetic analysis is necessary to detect the etiology of hearing impairment. In various studies of Caucasian populations, the mutation 35delG in GJB2 gene has been identified as the most common, accounting for up to 80% of cases with non-syndromic autosomal recessive inherited hearing loss (4,5,7-11). The frequency of heterozygous carriers has been established in different European populations, with the highest occurrence in Greece (3.5%) and Italy (3.1%) (9-12).

We evaluated the results of audiological screening by E-OAE testing in newborns born at the Department of Obstetrics and Gynecology, Rijeka University Hospital Centre, between October 2002 and December 2004. We also assessed the results of mutation analysis 35delG/GJB2 performed in children diagnosed with hearing impairment through the screening program.

**Patients and Methods**

The neonatal hearing impairment screening program included all newborns born at the Department of Obstetrics and Gynecology, Rijeka University Hospital Centre, between October 2002 and December 2004. Newborn screening program by the E-OAE (Audix, Bio Logic, Mundelein, IL, USA) was performed, and risk factors for hearing impairment were recorded by use of a questionnaire focused primarily on family history data. Risk factors included in utero infection, Apgar score 0-4, positive family history, malformation known to include sensorineural or conductive hearing loss, birth weight <1,500 g, mechanical ventilation, bacterial meningitis, ototoxic medications, hyperbilirubinemia requiring exchange transfusion.

The inner ear was stimulated by a click sound, which produces the spin-off effect: healthy hair cells emit a low-level sound recorded in the external canal by a sensitive microphone. The E-OAE was performed before the children were discharged from the hospital. Cases suspected of hearing impairment were re-examined after three weeks. If there was still no response recorded, the newborns were referred to the audiologist and tested by the auditory brainstem evoked response (ABER) testing. In cases of abnormal results of ABER testing, molecular genetic evaluation was performed to identify the presence of the 35delG/GJB2 mutation by allele-specific polymerase chain reaction (PCR) method and PCR method based on the principle of PCR-mediated site-directed mutagenesis followed by a BsiYI digestion (13).

**Results**

In the study period, 6,094 neonates were born at the Department of Obstetrics and Gynaecology, Rijeka University Hospital Center. Newborn screening program by the E-OAE (Audix, Bio Logic, Mundelein, Illinois) was performed in 6,019 newborns. Seventy-five (1.2%) newborns were not screened because they were moved to another hospital or had died during the early neonatal period. The risk factors were identified in 46 screened newborns. Out of 6,019 screened newborns, 6,655 had normal screening test results ("pass"), whereas 364 were either unilateral or bilateral non-responders. At the second stage of the screening performed three weeks later, 340 newborns were tested again. Of them, 86 had abnormal screening results and were referred to an audiologist. Out of 74 newborns tested with ABER, 14 were diagnosed with hearing impairment (Table 1). In these 14 children, molecular genetic analysis was suggested and accepted by the parents in eight cases (Table 2).

Mutation 35delG/GJB2 was found in two children. One child had a homozygous mutation and bilateral hearing impairment, without any risk factor present, and the other had a heterozygous mutation with unilateral hearing impairment and positive family history for hearing impairment.

**Table 1.** Results of universal newborn hearing screening program

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Evoked otoacoustic emission in screening:</th>
<th>Auditory brainstem evoked response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total</td>
<td>6,019 (100)</td>
</tr>
<tr>
<td></td>
<td>&quot;pass&quot;</td>
<td>5,655 (93.9)</td>
</tr>
<tr>
<td></td>
<td>&quot;refer&quot;</td>
<td>364 (6.1)</td>
</tr>
</tbody>
</table>

E-OAE, evoked otoacoustic emission; ABER, auditory brainstem evoked response.

Zaputović et al: Diagnostic Procedure of Hearing Impairment in Newborns
Discussion

In our study of the frequency of hearing impairment diagnosed at neonatal screening program, the diagnosis was confirmed in 14 out of 6,019 screened newborns. In two out of eight newborns with definitive diagnosis, the involvement of the mutation 35delG/GJB2 as the cause of hearing impairment was found.

Undetected, hearing impairment has a clear impact on speech and language development and thus on communication skills and social life. Early identification of hearing impairment is the most important for the maximum enhancement of linguistic and communicative abilities in deaf children or children with severely impaired hearing (14). Family history of hearing loss is usually an indicator of a genetic cause, but in many cases genetic cause is chance isolated. Furthermore, a strong influence on hearing impairment is exerted by environmental causes, such as prematurity, in utero infection, low Apgar scores, malformation, mechanical ventilation, bacterial meningitis, ototoxic medications, and hyperbilirubinemia. The appropriate intervention must begin before 6 months of age because of the influence of auditory stimuli on the developing brain.

Hearing loss is the most common birth defect that affects 1 to 3 out of 1,000 newborns (15,16). It is important for newborns to be screened for hearing impairment within the first month of life, which is the first stage of screening (17). Within the first three months, the second-stage screening must be conducted and definitive diagnosis of hearing impairment/loss confirmed by an audiologist (18). E-OAE and ABER methods are objective diagnostic methods as they do not require patient response.

In our study, the first-stage screening revealed 6.1% of children as non-responders. These results are in accordance with literature (17,19). Interestingly, in 46 children with risk factor(s), hearing impairment was not found. These children were followed up for over a year and hearing problems were not noticed. However, there is a possibility of later diagnosis of hearing impairment in these children (progressive or delayed sensorineural or conductive hearing impairment) (20). At the second-stage E-OAE screening after three weeks, 86 (1.4%) children still had positive results. Similar percentage has been reported elsewhere (3). Seventy-four of these children were referred to an audiologist and tested by ABER. Fourteen had positive results and were informed about the possibility of molecular genetic evaluation. This result is in agreement with the reported incidence of congenital hearing loss in Croatia (6).

Considering the results of mutation analyses in Caucassoid populations, when hearing impairment diagnosed, molecular analysis for the presence of 35delG/GJB2 mutation should be performed, especially where no risk factors are registered or an autosomal recessive way of inheritance is suspected.

Such an analysis would establish the etiologic diagnosis in up to 50% of congenitally deaf children whose siblings are also affected and in 10-40% of sporadic cases in European countries (4,5). Positive result would be of great importance in genetic counseling.

In our study, the parents of 8 children consented to molecular genetics testing for the presence of 35delG/GJB2 mutation. The mutation was found in two children – one homozygote, with bilateral hearing impairment and without any observed risk factor, the other heterozygote, with unilateral hearing impairment and positive family
history. The newborn with the mutation in heterozygous state was suspected to be a compound heterozygote with a second mutation in GJB2 gene different from 35delG.

Our results confirm the importance of neonatal screening for hearing impairment, the E-OAE method being the method of choice. The program has definitely proved its importance regarding early diagnosis and rehabilitation of childhood deafness. The involvement of the 35delG/GJB2 mutation in the etiology of hearing impairment in two out of eight analyzed newborns allow us to suggest the mutation analysis as an additional test in screening program. Such a molecular genetic screening would allow for informative genetic counseling.

Furthermore, mutation analysis has therapeutic implications, as the patients with hearing impairment due to the mutation have the best performance on rehabilitation after cochlear implantation (21).

References

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Correspondence to:
Sanja Zaputović
Department of Obstetrics and Gynecology
Rijeka University Hospital Center
Camberiæva 17
51000 Rijeka, Croatia
Izapotov@inetr.hr