PhD THESIS

MODERN CLINICAL, AUDIOLOGICAL AND GENETIC METHODS OF INVESTIGATION CONGENITAL SENSORINEURAL HEARING LOSS

SUMMARY

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congenital sensorineural hearing loss, connexin 26, liminar tonal audiometry, impedance-metry, audogram by game, acoustic otoemissions, early auditory evoked potential, genetic tests, autosomal recessive transmission, GJB2, prosthetic rehabilitation, cochlear implant.
I. IMPORTANCE OF THE ISSUE,
MOTIVATION OF THE PAPER

Congenital hearing loss is a major public health problem, being one of the most common congenital anomaly with an incidence of 1-3 per thousand live births; 1 in 300 children have mild congenital sensorineural deafness and 1 in 1000 children will present severe sensorineural hearing loss before adulthood.

In Romania, the age at which hearing impairment is detected varies between 3 and 4 years. Severe, permanent hearing loss, if not detected early, will influence and cause serious impact on speech development, language acquisition and cognitive development, with negative effects on emotional and social quality of life. Besides the negative impact on interpersonal communication, severe bilateral hearing loss affects educational progress, mental health, self-esteem and long-term employment opportunities.

These are the reasons that led to this clinical study in all aspects of diagnosis and treatment, conducted on patients who were presented to the Department of Audiology of Clinical Emergency Hospital Craiova.
II. MATERIAL AND METHOD

1. OBJECTIVES AND PURPOSE

   The objectives of this paper are as follows:
   a. A synthesis of information on congenital sensorineural hearing loss.
   b. Description of audiological diagnosis methods for the identification and quantification of cases with this type of hearing loss.
   c. Identification of the risk factors involved in etiopathogenesis of congenital sensorineural hearing loss.
   e. Evaluation of post-verbal auditory recovery.
   f. The purpose of this retrospective study is:
      a. To reveal the importance of an early detection of hypoacoustic persons, ideally within sensorimotor period (under 1 year) as this period is critical for the baby since it acquires important language traits.
      b. In this respect, therefore, it is necessary to introduce universal hearing screening programs for the newborns.
      c. To reveal the importance of using, depending on age, subjective tests especially those investigating auditory acuity targets to establish a diagnosis as early and accurate as possible to stage hearing loss. The whole approach in supporting the deaf patient depends entirely on the rigor of assessment aids.
      d. To reveal the high frequency of GJB2 gene mutation occurrence and hence the importance of genetic analysis which should be comprised in the usual range of tests for any hypoacoustic child and be associated with newborn hearing screening. All this could lead to an early detection of hearing deficits and a rapid therapeutic intervention.
2. MATERIAL AND METHOD

The complex issue of congenital sensorineural deafness has been approached in a clinical-statistical study conducted on a sample of 514 patients selected from all patients with hearing that were presented at Audiology Department of Emergency County Hospital Craiova between 2006 - 2010.

The criterion for inclusion in the study was the presence of sensorineural hearing loss with prelingual onset and without other episodes of ototoxic risk at that time, so congenital hearing loss.

For genetic study, subjects were recruited from the subgroup of 162 patients aged 0-10 years who were presented to the Department of Audiology in Clinical Emergency Hospital Craiova within 2006-2010 on the basis of certain inclusion criteria. Thus we obtained a subset of 87 patients, and genetic analysis for GJB2 gene mutation screening was performed on 26 patients in the Genetics laboratories in Bucharest, Cluj and Craiova.
III. SYNTHESIS OF THE MAIN RESULTS

Distribution by age and sex of the studied cases

Following the distribution of 514 patients with congenital sensorineural hearing loss for the period included in the study we noted the following incidence:

- in 2006 there were 186 patients with congenital sensorineural hearing loss;
- in 2007 there were 142 patients with congenital sensorineural hearing loss;
- in 2008 there were 78 patients with congenital sensorineural hearing loss;
- in 2009 there were 62 patients with congenital sensorineural hearing loss;
- in 2010 there were 46 patients with congenital sensorineural hearing loss.

Our results show an almost equal distribution of cases of congenital sensorineural hearing loss between the sexes, with a slight predominance of males to the females (51.36% male to 48.64% female). This result corresponds to specialty literature data, Morton, 2002, found predominance of recessive genetic deafness in males. Also, Russ / et al. 2003 show that genetic deafness X Link manifests in males.

Distribution by area of origin of patients

The study of the distribution of cases by area of origin showed a higher proportion of patients in urban areas (58.36% in urban area to 41.63% in rural area).

We can explain this share of urban area as follows:

- easier access to specialized medical services;
- diverse and qualitative medical service;
- higher degree of information and medical practice;
- sometimes archaic mentality in rural areas regarding medical assistance.

Distribution by age

Studying age groups of the 514 cases we found that the dominant age group is the one between 11 and 20 years with 34.44%, followed by the group between 0 and 10 years with 31.52%. This distribution is explicable, given the hindrances encountered in the educational system, beginning with the secondary educational system, so there are several factors, Besides family, involved in hearing loss detection as the hypoacoustic children do not come in time to the clinics.

Distribution of audiological investigations conducted in the first phase on the studied group

Analyzing the cases, distribution of audiological investigations carried out in the first stage was as follows (Figure 1):

- in 50 cases there was performed acoustic impedance, ie 8.87%.
• in **47** cases there was performed audiogram by game, associated with acoustic impedance, ie **8.33%**;
• in **34** cases there was performed tonal audiogram associated with acoustic impedance, ie **6.03%**;
• in **383** cases there was performed tonal audiogram, ie **67.91%**.

This distribution is shown in the following histogram:

[Graphic 1. Distribution of cases by audiological investigations]

Liminar tonal audiometry in patients who could perform it was the reference point for the diagnosis and staging the hearing loss.

In the acoustic imittance in all cases tympanogram was type A, which indicates a normal middle ear function, and we could estimate the level of the auditory acuity by determining the thresholds of acoustic stapedian reflex through stimulation with pure tone stimuli and white noise.

We found that we needed to combine subjective investigation (audiogram by game or liminar tonal audiogram) with objective investigation (acoustic imittance) both in young patients and in those who lacked cooperation to establish, in the first stage, an accurate audiological diagnosis. In some cases, the detection of profound hearing loss, difficulties were encountered due to psycho-emotional and intellectual deficits induced by deafness.

I was particularly interested in the patients aged 0 - 10 years old, because the whole medical and educational approach depends on the precocity of auditory assessment of the children with verbal auditory disability. By the age of 30 months we performed only acoustic imittance and determined the thresholds of the stapedian reflex.

After the age of 6 years we performed audiogram by game combined with acoustic imittance to have a highly accurate assessment. We also made liminar tonal audiogram, and in patients who had difficulties in cooperation with us we associated liminar tonal audiogram with acoustic imittance.

Audiological investigations are difficult in this age group and call for a lot of patience as well as the audiologist’s ability to adapt to each case.

This age group was assessed every year within our study and we combined subjective investigation (audiogram by game or liminar tonal audiogram) with acoustic imittance audiological to have an accurate diagnosis.
**Distribution of the revaluated patients belonging to age 0-10**

We started from the premise of having a very early auditory evaluation of children with auditory-verbal disabilities as the entire medical and educational approach depends on it, and that is why the patients belonging to this age group were assessed in a subsequent stage by objective hearing tests, acoustic otoemissions and auditory evoked potentials performed in IFACFORL Bucharest.

Distribution of the **revaluated patients belonging to age 0-10** was as follows (Figure 2):

![Graph 2](image)

**Graphic 2**

Within the 5 years of research we compared the possibility of assessing the patients in the age group 0-10 years by modern objective investigations, namely AEO and PAEP, and we determined an upward trend in the number of the cases investigated.

We were particularly interested in the age group 0-10 years, subgroups under 1 year and 1-2 years, because this period is very important in learning the basics of the language. Within the 5 years of study we determined an increase in the number of patients who were reinvestigated by PRB and PAEP.

**Distribution of patients by the degree of hearing loss**

We have showed that the highest percentage of patients consists of those with profound sensorineural hearing loss - 194 patients, representing 37.7% of all patients.

Then there come the cases with severe hearing loss - 86 patients, representing 16.7%. Next place is occupied by cases of average sensorineural hearing loss - 76 patients, representing 16.7%. 75 patients had bilateral cophosis, ie 14.5% of cases.

This dominance of large hearing loss is explained by the fact that the hearing loss is more easily noticed by the patient or his entourage and is highly disturbing, resulting in patient’ presence in the clinics. Corroborating all this with genetic data, we know that autosomal recessive forms represent 80% of total non-syndromic congenital deafness (Smith / VanCamp, 2007). From the point of view of phenotypic expression, this type of hearing loss is usually sensorineural, and its severe forms are mostly common (Schrijver, 2004).

In the age group 0-10 years, the distribution of patients according to the degree of hearing loss was about the same as per all ages, hence profound sensorineural hearing loss was predominant.
Statistically we determined a direct, strong link between the hearing loss and language acquisition, a higher degree of hearing loss is in inverse relationship with the degree of language acquisition. This correlation is consistent with the dominant symptom which makes the patient see the physician; we showed that in the subgroups aged less than 1 year, 1-2 and years the dominant alerting symptom was the lack of language which made the parents take the child to the physician.

**Distribution of patients by personal and family medical history**

We considered the following risk factors in etiopathogenesis of sensorineural hearing loss:

- prematurity;
- Rh incompatibility;
- prolonged neo-natal jaundice;
- obstetrical trauma.

Prematurity ranked in the first place as a risk factor - 59 cases, followed by prolonged neonatal jaundice in 6 cases and Rh incompatibility in 3 cases. 1 case presented obstetrical trauma in personal history and in the case of 3 patients we could not find information on personal history. Specialized studies [23,74,128] rank prematurity first among the risk factors. Another risk factor found in the study of Woolf NK et al. (1990) is cytomegalovirus infection, a factor that we have not met our cases.

The trend in developed countries is to introduce universal screening in medical practice because about 50% of congenital hearing loss present no risk factors at birth, as evidenced in our study. [11,56,93]. If the screening program included only those children with risk factors at birth, we are expected to lose 50% of children with congenital hearing loss, according to the study published by Coplan J. in 1987.

From family history of the patients in the studied group we determined the following:

- normal parents and siblings from audiological point of view;
- a parent with sensorineural hearing loss, normal siblings from audiological point of view;
- both parents with sensorineural hearing loss, normal siblings from audiological point of view;
- siblings with sensorineural hearing loss, normal parents from audiological point of view;
- parents and siblings with sensorineural hearing loss.

The results show that 346 patients have normal parents and siblings from audiological point of view, therefore 75% of cases have no risk factor in direct hereditary collateral line. 84 patients had a heavy heredity, hypoacoustic siblings and/or parents and siblings, which represents 16.34% of cases. The same number of patients – 84 could not provide information about family history, ie 16.34% of cases. Data from the study by Hernandez-Herrera R. et al. (2007) also noted the absence of risk factors in direct hereditary collateral line in a close percentage (72%).

We followed the same aspects of family history in the group consisting of patients aged 0-10 years. We also found in this age group a predominance of the cases without a family history of hearing loss, which supports the need for a universal hearing screening for newborns.
Distribution of cases by associated genetic syndromes

Analyzing associated genetic syndromes in the studied group, we determined the following distribution:

- 489 patients do not have associated genetic syndromes, which corresponds to 95.13%;

We analyzed the patients in the age group 0-10 years following the presence of associated genetic syndromes.

We found that 153 patients, representing 94.44% did not have associated genetic syndromes, the remaining 9 patients presenting this association, which represents 5.56%.

Of genetic syndromes that may be associated with congenital hearing loss, in our group we met cranio-cephalic abnormalities in 4 patients, Down syndrome in 3 patients and 2 cases of ear malformations.

The influence of genetic factors in congenital hearing loss in the studied group emphasizes the importance of ante-natal screening and prenatal genetic consultation if there is family history of congenital hearing loss. In our study, congenital genetic syndromes were statistically lower than a family history of congenital hearing loss.

Distribution of genetically investigated cases by gender and age

In the group of 26 patients selected according to the established criteria for genetic testing the gender distribution was as follows:

- 20 female patients, representing 76.9%
- 6 male patients, representing 23.1%.

Distribution by age groups in the studied group was as follows:

- 3-6 years: 8 patients, representing 30.7%
- 7-10 years: 18 patients, representing 69.3%.

Mean age of genetically investigated patients was 8 years old. Unfortunately, most patients analyzed were diagnosed with sensorineural hearing loss in a late stage. Multiple reasons, such as lack of a screening program in maternity, less informed parents and educators for early detection, lack of high standard audiological test equipment in ENT surgeries (particularly for the young) explains a late diagnosis of congenital sensorineural hearing loss and implicitly, late genetic investigations.

Distribution of genetically investigated cases by the presence and frequency of the mutation

The studied mutation of GJB2 gene was 35delG, which is the most common mutation of this gene in the Caucasian population. [51,136]. The frequency of this mutation in our group is:

- 6 children showed mutation 35delG in homozygous form (35delG/35delG), which represents 23.1%
- 5 children showed mutation 35delG in heterozygous form (wt/35delG), which represents 19.2%
- 15 children had normal genotype (wt / wt), which is 57.7%.
The frequency of homozygous types in the studied group was of 23.1%. Gasparini et al. (2000) considers 35delG mutation most common to many populations in southern, northern and central Europe (from 28-63%). Among the closest European countries, Hungary has a frequency of this mutation of 38% [59], Poland a frequency of 35.2% [142] and Turkey a frequency between 15 and 23.5% [51]. In homozygous children this is certainly the cause of hearing loss.

In the case of heterozygous children, 35delG children mutation (representing 19.2% in the study group) it is necessary to continue genetic investigations in the sense of searching all GJB2 mutations (sequencing the gene), even if they are more rare than 35delG mutation. This is in accordance with specialty studies [44,45,125] which determined that the association of this mutation with a non-35delG for connexin 26 or with a mutation for connexin 30 (Digene transmission) are surely responsible for the hearing loss. We recommend the testing of 35delG mutation on the other family members. Concerning the children who were tested for 35delG mutation and presented normal genome of wt/wt type, representing 57.7% in the study group, we can determine that this is not the cause of their hearing loss, without excluding other unknown or uninvestigated genetic causes.
We determined that in the case of the 6 children from the studied group, who had homozygous genotype for 35delG mutation, hearing loss was severe in 50% of cases, profound in 33.33% of cases and even cophosis in one case. In conclusion, we can say that all homozygous cases studied (representing 100%) have severe or profound hearing loss, or even cophosis. These data correspond to those obtained by Cryns in th K.et al. (2004), who reported a significant concordance between the genotype of GJB2 gene and the degree of hearing loss.

The 5 children who have heterozygous genotype for 35delG mutation have a lower degree of hearing loss: 20% have moderate hearing loss, 40% have severe hearing loss and 40% have profound hearing loss, according to studies. [33.83].

Unlike the studies mentioned, in the case of the 15 genetically investigated patients where we obtained a normal genotype, the degree of hearing loss varies from severe, profound and cophosis; thus it is a decrease in auditory acuity. The data obtained can be considered in accordance with the study published by RL Snoeckx et al. (2005), which asserts the existence of a correlation between the degree of the hearing loss and the extent of mutation, but which also shows the existence of a significant variability of the phenotype within the same genotype. This variability can be explained by both the effect of modifying genes and the effect of environmental factors that can generate incomplete penetrance and variable expression.

We must also mention that in all the 11 hypoacoustic who showed GJB2 gene mutation for connexin26, either hetero or homozygous, hearing loss affects all frequencies and is generally stable, which is consistent with the data obtained by Cohn ES.et al. (1999).

In conclusion, taking into consideration the above-mentioned study (by Cohn ES.et al. (1999) we can say that genetic analysis of this mutation, frequently involved in the development of neurosensory hearing loss have to enter the routine series of prenatal tests, directing subsequent genetic investigations and genetic counseling, even a genetic mutation screening, as Ryan M. et al. (2003), Schmmenti LA et al. (2004) asserted.
Distribution of cases by methods of recovering auditory function

In the studied group the methods used to recover auditory function were as follows:

- unilateral prosthesis;
- bilateral prosthesis;
- cochlear implant.

The methods used detect congenital sensorineural hearing loss have no effect unless they are followed by measures to recover auditory deficit, auditory prosthesis or cochlear implant. In the studied group we determined that in 237 cases the audiological diagnosis was followed by audio prosthetic treatment. Conventional hearing prosthesis was the solution to auditory rehabilitation in most cases of sensorineural hearing loss: 176 patients had bilateral prosthesis and 61 patients had unilateral prosthesis. In 5 patients the treatment consisted of cochlear implant.

We were particularly interested in the recovery of hearing deficit in patients belonging to the age group 0-10 years, because research demonstrated the existence of 'auditory deprivation' and its irreversible effects on auditory-verbal rehabilitation possibilities. It is a well known fact that the period the patient was deprived of sound information has a decisive influence on his auditory-verbal performance after prosthetic treatment as well.

In the studied group, 84 patients received prosthetic rehabilitation treatment: 78 patients had bilateral prosthesis, 2 patients had unilateral prosthesis and 4 patients received cochlear implants. 72 patients did not have any prosthesis for various reasons: low auditory deficit, parents’ refusal to accept prosthesis, considering it a kind of stigma, parents belonging to the deaf community, parents with cophosis.

We studied the correlation between the degree of hearing loss and the method used to recover auditory function.

We found that with the increase of auditory deficit degree it also increases the number of the patients benefiting from prosthetic treatment. The number of patients with mild and average hearing loss exceeds the number of patients who receive hearing prosthesis. Most cases of patients appealing to auditory prothetic treatment are patients with profound sensorineural hearing loss.

The 4 patients who received cochlear implants are aged between 1 and 2 years and reviewed by objective audiologic methods OEA and PEAP.

From the statistical point of view we determined a direct link between revaluation methods, the complexity of these revaluation methods in patients belonging to the age group 0-10 years are directly related to the complexity of therapeutic methods.

We found that with increasing auditory deficit degree the number of patients receiving prosthetic treatment grows. In cases of mild and average hearing loss the number of non-prosthetic patients exceeds the number of patients who received hearing aid. Most cases of patients who appealed to prosthetic auditory treatment are patients with profound sensorineural hearing loss.
**Distribution of cases by post-therapy evolution**

We compared the post-therapy evolution in terms of the improvements we observed in language acquisition.

We analyzed the post-therapy evolution of the-studied group and found that 200 patients had a favorable outcome and in 126 patients the post-therapy evolution had a stationary character. Specialized studies in this area, Moller M. (2000) show that the development of language is far better for hypoacoustic children diagnosed by the age of 6 months and receiving hearing aids or cochlear implants than the children whose diagnosis is established later.

Statistically, there is a strong, direct connection between revaluation methods, whose complexity is directly related to post-therapy evolution (to favorable condition).

The large number of patients with stationary character of the post-therapy evolution indicates that there were cases in our study in which hearing loss was not diagnosed within an early stage and thus phenomenon of "auditory deprivation" occurred.

On the other hand, patients and parents should understand that prosthetic treatment must be combined with speech therapy exercises, and the treatment of hypoacoustic patients should be multidisciplinary, including audiologist, pediatrician, speech therapist and, not least, experienced psychologist in behavior management of children with hearing deficiency (Popescu R 2001).
IV. CONCLUSIONS

1. Hearing impairment due to congenital deficiency of Corti or auditory neural pathways with prelingual onset is an important issue of study by approaching a pathology with serious repercussions on the social integration of the individual.

2. In our clinical-statistical prospective study there were detected 514 patients with congenital sensorineural hearing loss that were presented to the Department of Audiology in Clinical Emergency Hospital Craiova and who met the selection criteria.

3. Studying the factors involved in the etiology of congenital hearing deficiencies revealed the existence of prematurity, Rh incompatibility and genetic deficiencies, 35delG mutation in homozygous form within GJB2 gene structure at the rate of 23.1% of the cases investigated genetically.

4. The dominant age group was within 11-20 years (34.44%), followed by the one within 0-10 years (32.52%), with an increasing tendency towards patients belonging to the age group 0-10 years.

5. Gender distribution of the cases studied corresponds to a nearly equal distribution of cases of congenital sensorineural hearing loss between the sexes, with a slight predominance of males (51.36% male to 48.64% female) and a higher proportion of patients in urban area (58.36% urban to rural 41.63%).

6. The dominant symptom was hearing loss, except for the age group 0-10 years where parents were alarmed by the children's incapability of speaking and consulted the physician.

7. Audiological investigation methods were represented by subjective methods (liminar tonal audiogram and audiogram by game) and objective methods impedance-metry in the first stage, followed by retesting child by acoustic otoemissions and early auditory potentials.

8. Liminar tonal audiogram was the reference point for the diagnosis and staging of hearing loss, dominating audiological investigations conducted in the first stage.

9. It is very important to apply the correct test protocols. The study showed that it is necessary, depending on the age and psycho-emotional status of each patient, to combine subjective methods with objective audiological investigation in order to establish the audiological diagnosis as early and accurately as possible. The study demonstrates the superiority of association objective audiological tests with genetic testing.

10. Depending on the degree of hearing loss, sensorineural hearing loss predominated in patients with profound (37.7%) and low auditory thresholds at all frequencies (35.97%).

11. Audiological diagnosis was followed by prosthetic audio treatment. Most cases of patients who used the auditory prosthetic treatment suffered from profound sensorineural hearing loss. Treatment efficiency was conditioned by precocity of diagnosis and multidisciplinary approach to case.
12. Bilateral auditory prosthesis was the most widely used therapeutic option: 176 patients - 34.24%, 5 patients - 0.97% received cochlear implant.

13. We consider it is necessary that patients with genetic deficiencies be included in a consultation and genetic counseling program for early therapeutic intervention.

14. Congenital sensorineural hearing loss remains an open pathology chapter, especially in terms of the development of genetic tests, tests used for screening hearing loss and able to provide information on educational approach and treatment modalities.
V. SELECTIVE BIBLIOGRAPHY


