PhD thesis

Etiological factors in the origin of congenital abnormalities of external ear

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Introduction

As a clinical otorhinolaryngologist, treating my patients makes me truly delighted. My best-loved field/subspecialty in otorhinolaryngology is reconstructive head and neck surgery in which, one of the most challenging task is the surgical treatment of ear abnormalities. However, I always felt it would be worth understanding the development of these malformations and helping their prevention. We know that the surgical management of ear CAs cannot warrant the most pleasant - aesthetical - outcomes. In this case, the expectation/sentence "prevention is better than surgical treatment" is especially true. This is exactly why I have chosen etiology of congenital abnormalities of the external ear as the target of my PhD research.

In chapter *Introduction* of my PhD dissertation, I intended to demonstrate my knowledge of teratology and genetics. The *Objectives* aim the possible causal factors of disease source of the external ear CAs through the examination of the connection between the epidemiological characteristics, the characteristic models, teratogenic and genetical causes, maternal diseases, the maternal pharmacotherapy and its high risk. Our research results could be understood on the basis of the part *The study materials and methods*. In section *The most important findings of our studies* I only briefly summarized the most important results of my research because all the details can be found in the attached publications. Accordingly this is why I only mention - in part *References* - those of my publications on which my dissertation is based because the references to other authors are in these articles. Finally, in section *Conclusions* I sum up the principal theoretical findings and the practical attainments of our research. With the concurrence of my supervisor, Dr. Ferenc Bánhidy, I intended to publish the related articles in international field-specific (medical) journals. As the congenital abnormalities of external ear are not popular research fields, it was difficult to find specialists who could supervise and criticize my work. Nevertheless, the editors of the chosen international journals helped the final manuscript to be published with their useful hints and reflections. In addition, with respect to the modern principles of epidemiology, they also assessed my investigations based on a Hungarian population. I have already given – and I am intending to give more presentations about my results at national as well as international congresses, moreover I will publish them in Hungarian articles as well.

According to my ambitions, my study results will not only help me at enhancing the quality of my professional skills, but they will contribute to the – Hungarian and global – understanding of the origin of ear abnormalities.

Objectives

The etiology of congenital abnormalities (international abbreviation: CAs) of external ears is less known, therefore the chance for the prevention of this medium frequency CA-group is limited. We, clinicians, do our best to correct these CAs with surgical and other methods, but we know: there is only one optimal medical solution and it is their prevention. Thus, the aim of my PhD work was to support the knowledge regarding the possible causes of external ear CAs.

1. Assessing the prevalence of CAs at birth in Hungary.

Our aim was to describe the main characteristics of the Hungarian cases who were born with isolated or multiplex external ear abnormalities. Evaluating the frequency of external ear abnormalities at birth in Hungary means a population-based, case-control study which unveils the role of possible etiological factors in the development microtia/anotia.

2. Evaluating the external ear Cas

We evaluated the unclassified multiplex congenital abnormalities containing microtia/anotia as CA component in order to clarify the characteristic patterns of the other, associating CAs, and to attempt to make a so-called registry diagnosis based on the properties of the associated CAs, and to impel the foundation of an international registry for the unclassified CAs containing microtia/anotia.

3. Revealing the genetic and teratogenic factors causing external ear CAs.

4. Evaluating the association between maternal diseases during pregnancy and the children's higher risk to have external ear CA.

5. Evaluating the association between maternal drug-use during pregnancy and the children's higher risk to have external ear CA.

The study materials and methods

The internationally – both in size and in quality - unique dataset of the Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA) was used in these studies, which is available to be studied by every specialist. Cases with various CAs were selected from the Hungarian Congenital Abnormality Registry. Controls, i.e. newborns without any CA, were identified in the National Birth Registry of the Central Statistical Office. In general two controls were matched to each case according the sex and birth week of cases and geographical region of their parents/mothers. In 1996, the way the data was collected was

changed, thus in this PhD dissertation I evaluated only the dataset of the 17-year-long period from 1980 to 1996. Cases suffering from CAs were selected from Hungarian Congenital Abnormality Registry (HCAR), and are classified according to the newest international standards.

HCAR was founded in 1964 when the Ministry of Health ordered the physicians to report the identified CAs at the delievery or in the first year after the delivery. From 1970, the registration was done at the National Epidemiology Institute and was headed by Dr. Andrew Czeizel.

Reporting the cases with CAs took place from 3 sources: (I) by obstetricians (in Hungary, practically all the deliveries take place in inpatient obstetric clinics and birth attendants are obstetricians), (II) paediatricians (working at neonatal units of inpatient obstetric clinics as well as of various general and special surgical, cardiologic, orthopaedic, oto-rhino-laryngologic, etc. inpatient and outpatient paediatric clinics). (III) in cases of death: autopsy was obligatory for all infant deaths, and the autopsy reports were available at the departments of pathology. Beside these 3 most important sources, (theoretically) every practicing physician (IV) would have to report cases with CAs when they diagnose them, and most of the doctors do so. Since 1984, another source helped HCAR because by that time prenatal diagnostic centers had been established in Hungary, and the fetal defects diagnosed by them were also reported. Thus, the cases can be live- or stillborn fetuses, or – after fetal diagnostics – fetuses from elective termination of pregnancy in the second or third trimester.

There were three main criteria at selecting the cases with CA from the HCAR for the HCCSCA: (1) cases notified to the HCAR within not more than three months after birth or elective termination of pregnancy. This short interval between the diagnosis and the report increased the accuracy of data recording, and diminished the recall bias regarding maternal self-reported information. Thus 77% of reported CAs were selected into HCCSCA from HCAR, the rest had only mild CAs. (2) cases with three mild CAs as congenital dysplasia of the hip based on Ortolani click, congenital inguinal hernia, major hemangioma were not selected from HCAR and (3) cases with CA- syndromes caused by major gene mutations or chromosomal aberration (with preconception origin – except for Down-syndrome) were also excluded. Thus, cases with identified MCA-syndromes including ear CAs were excluded from the study.

The selection of the control group was performed in accordance with the Hungarian Central Statistical Office. The cases in the control group had no CAs, and each CA case had 2

matched controls adjusted by gender, week of delivery and by the geographical location of the parents.

The necessary maternal and birth data, in addition information regarding exposures were collected form 3 sources: (I) Medically recorded prospective data based on the prenatal maternity logbook and other medical documents. (II) Retrospective self-reported maternal information was based on structured questionnaires filled-in by the mothers. (III) Non-respondent mothers were visited at home by regional nurses and the latter ones obtained the necessary data.

At diagnosing and classifying the external ear CAs, I considered the recent international recommendations. In the first step, cases with isolated external ear CA and cases with multiple CA including ear CA, were separated because of their different etiology and clinical severity. In the second step, I divided the isolated external ear CAs into 4 groups (i) mild microtia or type II, (ii) severe microtia or type III, (iii) anotia and (iv) complex ear CA, when anotia/microtia was associated with CAs of middle ear. (Type I microtia, as minor anomaly was excluded from the study, as well as other minor anomalies of external ear.) In the third step, multiple ear CAs were analyzed according to the number of component CAs and I attempted to establish the so-called registry-diagnosis of well-known CA-syndromes on the basis of characteristic combination of component CAs.

At the analysis of exposures I preferred the medically recorded prospective data, and these exposures were only evaluated in the second and/or third gestational month of pregnancy, i.e. the critical period of external ear CAs.

The most important findings of our studies

I. The prevalence at birth and classification of external ears CAs.

The evaluation of the dataset of the Hungarian Congenital Abnormality Registry showed that the at-birth prevalence of cases with isolated ear CAs was 0.30 per mil during the study period while the rate of cases with multiple CA was 0.18 per mil. The total recorded birth prevalence of ear CAs proved to be 0.48 per mil in Hungary, so this CA-group belongs to the category of medium frequency CAs.

However, the detailed analysis of this dataset unfortunately indicated the incompleteness of the case reports with ear CAs as well as frequent misdiagnoses. In addition, multiple CAs

were rarely indentified as CA-syndromes because the previously delineated multiple CA-entities are not known by most clinicians.

Thus finally I evaluated the dataset of the Hungarian Case-Control Surveillance System with well-controlled cases. Of 354 cases with isolated external ear CA, 74 (20.9%) had mild microtia, 236 (66.7%) cases were affected with severe microtia, only 20 (6.8%) cases were diagnosed as anotia, and 20 cases had complex ear CAs. Of 156 cases with unclassified multiple CA, 48 (30.8%) were identified by registry diagnosis as known CA-syndrome.

II.The characteristics of external ear CAs in newborns.

Cases with microtia had a male predominance (53.2%), but the sex ratio of anotia (50.0%) corresponded to expected rate. However, there was an obvious male excess in cases with complex ear CAs (70.0%) and this male dominance may be associated with their etiology. Cases with multiple ear CAs had also a strong male excess (65.4).

The gestation age of cases with isolated external ear CA (39.3 weak) did not show a significant deviation from the gestation age of the control group (this sample represented well the Hungarian population figures) but the average birth weight (3 120 g) was significantly lower than that of the controls. These data obviously indicated an intrauterine fetal growth restriction. The birth data of cases with multiple ear CAs are difficult to evaluate because their fetal development is not determined by ear CAs but by severe other component CAs.

The cases with isolated microtia were mostly unilateral (97.2%) with some excess of the right side. A similar but less obvious unilateral phenotype could be seen in cases with anotia (91.7%). However, about two-third (63.2%) of cases with complex ear CA had bilateral manifestation. Cases with multiple ear CA showed also a predominance of unilateral manifestation (62.2%), though this proportion was far from the nearly 100 % unilateral manifestation of microtia. However, these cases also had right side predominance.

III. New findings in the etiological research of isolated external ear CAs

Cases with isolated CAs were born more frequently from mothers with higher birth order and lower socio-economic status. Thus their poor familial conditions may have some connection with the origin of isolated external ear CAs.

The teratogenic effect of drugs used by the mothers during pregnancy could not be proved.

Among the maternal diseases, I could prove the role of diseases associated with high fever such as seasonal influenza and common cold with secondary complications in the origin of isolated external ear CAs. In addition, maternal otitis media seems to be associated with the higher risk of complex ear CAs. But the teratogenic effects of these diseases could be reduced by appropriate treatment.

I was the first to be able to show that the isolated external ear CAs have multifactorial origins/causes. The multifactorial etiology is based on the interaction of genes (polygenic predisposition/liability) and triggering environmental factors. The expected rate of recurrence within the family in the first degree relatives of cases could be estimated on the basis of Galton-rules, and this expected rate did not differ significantly from the observed rate in their families.

IV. New findings in the etiological research of multiple ear CAs

Cases with multiple ear CAs were also born frequently to mothers with higher birth order and lower socio-economic status. Thus the poor family conditions may also play a role in the origin of multiple ear CAs.

All kinds of drug use during pregnancy were evaluated in the mothers of cases with multiple ear CA. One well-known drug, hydroxyethylrutosidea (Venorutin) after oral administration during the second and/or third gestational month of pregnancy was associated with 9 times higher risk of multiple ear CAs. This association was strongly in parallel with the fact that this multiple ear CA had a specific pattern of component CAs, so it is a CA-syndrome. The teratogenic human drugs – in general – induce special CA-syndromes such as phenytoin-Diphedan, valproate, roaccutane, etc.

The high fever associated seasonal influenza and common cold with secondary complications showed also an obvious association with the higher risk of multiple ear CAs. Thus high fever is a specific etiological factor for both isolated and multiplex ear CAs.

Among other maternal diseases, varicella disease during pregnancy was associated with a higher risk of multiple ear CAs. One of the principal symptoms of this CA-syndrome is the secondary microtia caused by scarification of the pinnas.

Conclusions

According to our study, only a minor part of isolated external ear CAs showed a familial pattern (i.e. first degree relative having congenital ear abnormalities). However, case reports

about autosomal dominant and recessive inheritance were published, but now it seems to be clear that only a very small proportion of cases with external ear CAs is caused by genetic factors. Based on the familial pattern, the idea of multifactorial origin arises, with the contribution of polygenic predisposition and environmental factors. The observed rate of cases did not differ significantly from the expected rate based on the multifactorial model. Thus, the isolated external ear CA is regarded to have multifactorial origin.

In the group of cases with multiplex ear CAs, we could not observe any familial patterns, contrariwise, the syndromes consisting of congenital disorders were caused by major mutant genes and teratogenic agents. We did not include cases with chromosome-aberrations in the study because this was one of the exclusion criteria. Based on our so-called "registry-diagnosis", the theory came up that syndromes consisting of multiplex congenital abnormalities were caused by major mutant genes which showed mostly autosomal dominant inheritance. The observed cases were results of new mutations as none of the parents or the brothers/sisters were affected. Because of the immunization phenomenon, the most of teratogenic factors, such as fetal varicella disease do not recur in brothers/sisters.

As for summary, the origin of isolated external ear CAs can be explained by the multifactorial model (interaction of genes-environmental factors). This congenital abnormality can be triggered by high fever (can be prevented with antipyrectics) associated with maternal diseases during pregnancy – on the basis of a polygenic predisposition.

Possible ways to prevent external ear CAs

The mothers of both cases with isolated external ear CA and cases with multiple ear CA had a lower socio-economic status, therefore social factors may play a role in the origin of these CAs. Thus, an improvement of standard of living for pregnant women may associate with a lower risk of ear CAs. However, this question belongs to social affairs rather than to medicine.

High fever related maternal diseases have an important role in the origin of both isolated external ear CA and multiple CAs. However, our study showed that this high risk is preventable with appropriate antifever drugs. The high risk of complex CAs due to maternal otitis media can also be reduced by appropriate antibiotic treatment. These estimations underline the fact that the exaggeration of drug teratogenicity, and the treatments skipped because of this may cause more problems than the rare – truly – teratogenic drugs do. Thus,

we need a better knowledge regarding the balance between the benefit and risk of drug treatments during pregnancy.

The association between the higher risk of congential abnormalities in the children and the maternal use of antiepileptic drugs is well known. It can be clearly seen from our data that microtia/anotia also belongs to antiepileptics-associated congenital abnormalities. After studying multiplex ear CAs, I proved that oral use of hydroxyethylrutosidea (Venoruton) in the $2^{nd}/3^{rd}$ month of pregnancy rises the risk of a specific CA containing microtia/anotia as the most important component. Accordingly, this drug has to be classified into the group of human teratogenic medications which has 45 entity, and its use should be avoided in the 2^{nd} and 3^{rd} gestational month.

Finally, there is another maternal disease related to higher risk of multiple ear CAs and it is varicella. In the past, nearly all children were infected thus they had an immunological protection against this disease during pregnancy. Recently we have had a growing number of pregnant women without previous varicella disease, and – unfortunately - vaccination. Thus, it is necessary to recommend to all the prospective pregnant women without previous varicella or vaccination to be vaccinated against varicella. This preventive action can avoid the fetal varicella disease/fetopathy associated with multiple ear CAs.

The main findings of our studies confirmed the main idea of modern medicine: prevention is much more successful than treatment with dubious results.

Publications containing the results of my PhD Thesis:

1. Paput L, Bánhidy F, Czeizel AE. Prevalence at birth of congenital abnormalities of external ears in Hungary. *Cent Eur J Med* 2011; 8: 341-348.
2. Paput L, Bánhidy F, Czeizel AE. Distribution of associated component abnormalities in cases with unclassified multiple ("syndromic") anotia/microtia *Int J Pediat Otolaryng* 2011; 75: 639-647.
3. Paput L, Bánhidy F, Czeizel AE. Maternal characteristics and birth outcomes of pregnant women who had offspring with congenital ear abnormalities – a population-based case-control study. *J Mat-Fetal Neonat Med* 2011; 24: 1-8.
4. Paput L, Bánhidy F, Czeizel AE. Association of drug treatment in pregnant women with the risk of external ear congenital abnormalities in their offspring: A population-based case-control study. *Congenit Anom* 2011; 51: 126-137.
5. Paput L, Czeizel AE, Bánhidy F. Association of maternal diseases in pregnant women with the risk of isolated ear abnormalities in their offspring - a population -based case-control study. *Cent Eur J Publ Health*, 2011; 19: 128-134.
6. Czeizel AE, Dudas I, Paput L, Bánhidy F. Prevention of neural-tube defects with periconceptional folic acid, methylfolate or multivitamins? *Ann Nutr Metab 2011; in press*, 33776, DOI 101159000330776.
7. Paput L, Falvai J, Bánhidy F. Az anotiat és microtiat kisérő többszörös fejlődési rendellenességek eloszlása. *Hung Med J, 2011;* 35:1399- 1416.

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