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EPIDEMIOLOGY OF OROFACIAL CLEFTS

Doctoral (Ph.D.) – thesis

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I. INTRODUCTION

Birth defects – or by according to the World Health Organization's term: congenital anomalies – are structural, functional and/or biochemical-molecular defects present at birth whether detected at that time or not. Among different categories of birth defects, congenital abnormalities (abbreviation: CAs), i.e. structural-morphological defects represent the largest group. The causes of congenital abnormalities can be classified into three main groups: genetic, environmental and multifactorial origin.

Congenital abnormalities include several categories with different origin. First, we have to differentiate the isolated and multiplex CAs. The dissertation includes mainly the analysis of isolated or non-syndromic orofacial clefts (abbreviation: OFCs). In his pioneer work Paul Fogh Andersen demonstrated that isolated cleft lip with or without cleft palate (abbreviation: CL±CP) and posterior cleft palate only (abbreviation: CPO) are distinct entities with different genetic background, therefore it is important to analyze them separately. A comprehensive epidemiological study of OFCs based on Hungarian data would be very important, since a complete analysis was done so far only for neural tube defects and eye CAs.

The first and one of the largest CA registries, the population-based data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities, 1980-1996, seemed to be appropriate to study the origin of OFCs and different epidemiological and statistical methods.

II. OBJECTIVES

The main objective of the study is to find the factors that play an import role in the origin of orofacial clefts and to promote the primary prevention. OFCs are among the most common birth defects encountered in humans and the most extensively studied congenital abnormalities. Due to their visibility these malformations may cause serious psychiatric problems for the families of affected children. There is a well-known worldwide variation in the prevalence of cases with OFCs at birth that alters between 0,45 – 3,62 per 1000. In Hungary the prevalence of OFCs is around 2,02 per 1000, namely it occurs in every 500th birth. Our knowledge about etiology of CL±CP and CPO is still lacking, therefore studies

dealing with the primary prevention of OFCs are very important. The etiology of nonsyndromic oral clefts remains to be completely understood, but today's best evidence suggests that these birth defects are multifactorial in origin with both genetic and environmental causative factors.

We aimed to analyze the association between the isolated orofacial clefts and following exposures: acute maternal diseases during the critical period of CL±CP and CPO, chronic maternal diseases during pregnancy, maternal drug use in the critical period of OFCs, maternal employment status (as an indicator of socioeconomic status), and the effect of folic acid supplementation in early pregnancy. We examined the large population-based data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities, 1980-1996, to study the possible association between different exposures and OFCs. Our hypothesis was that some maternal diseases (primary chronic diseases) might be risk factors for isolated OFCs. However, this teratogenic effect of maternal diseases may be restricted by appropriate medical treatment. We presumed that drugs might have a limited role in the origin of isolated CL±CP and CPO, and sometimes the underlying disease is responsible for the increased risk.

Although folic acid supplementation reduces the risk of neural tube defects, the role of folic acid in the origin of CL±CP and CPO and other CAs is still controversial. Therefore, the next objective of our studies was to study the role of folic acid in the origin of OFCs and to check the possible association between maternal employment status and periconceptional folic acid supplementation.

There are several epidemiological methods that could be used in the epidemiology of birth defects. The second objective of my dissertation is to describe the statistical methods and models, which can be used efficiently in the epidemiology of OFCs and other CAs. I would like to describe the advantages, disadvantages, costs of different methods and to compare the following designs: animal studies, clinical trials, cohort studies, case-control design, cross-sectional analyses, case-crossover and case-time-control methods. There are several decisions, which should be discussed and considered before the data analysis in order to lower bias. In the planning phase of our studies we discussed the following problems: convenient critical period, potential confounding factors, choice of reference group, conditional vs. unconditional regression, multiple comparison, gene-environmental and other interactions.

III. MATERIALS AND METHODS

The Hungarian Congenital Abnormality Registry (abbreviation: HCAR) was founded in 1962, following the World Health Organization's recommendations of 1970. The Hungarian Case-Control Surveillance of Congenital Abnormalities (abbreviation: HCCSCA) was established in 1980 and is based on HCAR. The main objective of the HCCSCA is to detect the causes of CAs.

Cases with CAs were selected for the HCCSCA from the population-based HCAR. Liveborn infants, stillborns and malformed fetuses from electively terminated pregnancies in the second or third trimester were included in the case group. Selection of cases was based on three criteria: (1) Cases with CAs reported to the HCAR in the first 3 months after birth or pregnancy termination were selected for the HCCSCA. This short interval between the birth or pregnancy termination and data collection increases the accuracy and effectiveness of the information about the history of pregnancies and can reduce the recall bias. 77% of cases were reported during this time window to the HCAR. (2) Three mild isolated CAs such as congenital dislocation of the hip based on Ortolani click, congenital inguinal hernia, and major hemangioma were excluded from the HCCSCA. (3) Third, CA-syndromes of Mendelian or chromosomal origin (except Down syndrome) were also not included in the HCCSCA because they have known, non-teratogenic origin.

In general two controls without CAs were matched with every case from HCCSCA according to sex, birth week and district of parents' from the National Birth Registry of the Central Statistical Office. Three population controls were chosen for each case between 1986 and 1992 in order to increase the statistical power. However, after 1992 it was no financial support for the third control. The procedure of data collection in the HCCSCA was changed in 1997; therefore in present dissertation I evaluated only the data set of 17 years between 1980 and 1996.

There were three sources of information in the HCCSCA regarding different maternal and paternal exposures during pregnancy: retrospective maternal information, medically recorded data and supplementary data collection in the course of home visits. Finally, the HCCSCA included 22843 CA cases and 38151 population controls. Between 1980-1996 96.3% (84.4% from reply, 11.9% from home visit) of cases and 83.0% (82.6% from reply, 0.4% from home visit) of controls were evaluated. Finally, 1,374 CL±CP cases and 601 CPO cases, 38,151 population controls without any defects and 20,868 patient controls with other

defects were compared. The statistical analyses of the data were performed using SAS version 9.1 (SAS Institute, Cary North Carolina, USA). PROC LOGISTIC and PROC PHREG procedures were used to estimate the risk.

In epidemiology of CAs the main task is to avoid bias. I tried to find solutions to reduce bias during the planning and analyses of studies. In the planning phase of our studies we discussed the following problems: the definition of critical period of CL±CP and CPO; potential biases and confounding factors and how can be treated during the analyses; choice of convenient reference groups (population and patient controls); use of conditional or unconditional logistic regressions; multiple comparisons; gene-environmental and other interactions.

The critical period of isolated CL±CP is between 49 and 64 gestational days, calculated from the first day of the last menstrual period; thus, its critical period is between 7.0 and 9.1 weeks, i.e., during the last week of the second and the first week of the third gestational months. The critical period of isolated CPO covers 70–99 gestational days, i.e., between 10.0 and 14.1 weeks, and overlaps with the last 2 weeks of the third and first 2 weeks of the fourth gestational months.

First, the occurrence of acute maternal diseases during the critical period and the occurrence of chronic diseases overall were compared among the case and control groups, and adjusted odds ratios (abbreviation: ORs) with 95% confidence intervals (abbreviation: 95% CIs) were calculated by unconditional multiple logistic regression model. The ORs were adjusted for maternal age, birth order, maternal employment and marital status. Next, further detailed analyses were done for maternal influenza, common cold, depression, diabetes mellitus and angina pectoris. The objective of the next study was to investigate the possible association between nausea and vomiting in pregnancy and congenital abnormalities, especially OFCs.

The prevalence of different drug use during the critical period of CL±CP and CPO was compared between the study groups, and crude ORs with 95% CIs were calculated. We used unconditional logistic regression models to estimate the adjusted ORs with 95% CIs for the association of CL±CP and CPO and maternal drug use. Potential confounders were identified separately for five groups of medications: antifevers, antimicrobial/anti-inflammatory drugs, sedatives and drugs for pregnancy complications, hormones, and other drugs. The critical period was extended to second-fourth gestational months during the comparison of CPO cases with patient controls in order to cover the critical period of most CAs. Next, the possible associations between medically recorded drug treatments during the critical period of OFCs

were analyzed in order to reduce the potential recall bias. Finally, we studied OFCs following exposure to phenytoin, phenobarbital, and diazepam in a case-time-control study.

Finally, the major features of mothers (maternal age, birth order, maternal employment and marital status) were described in the two, CL±CP and CPO groups and in the population and patient controls. The distribution of employment status of mothers was compared among different types of OFCs, in addition to cases with OFC and population and patient controls. Adjusted ORs with 95% CI were calculated in a multiple logistic regression model. The ORs were adjusted for maternal age, birth order, use of folic acid/multivitamin and paternal employment status. High dose (6 mg) of folic acid use amid the mothers was compared among the four study groups: two types of OFC, population controls and patient controls. A statistical test for heterogeneity of the odds ratios across the maternal employment status classes was performed when OFC groups were compared with population and patient controls.

IV. RESULTS AND DISCUSSION

1. Maternal diseases

Maternal diseases were differentiated into two categories. The first category included acute maternal diseases, i.e., those that occurred during pregnancy in a relatively short period. Acute diseases could have a possible association with CAs only if they occurred during the critical period of the particular CA studied. Thus, the occurrence of acute maternal diseases was evaluated during the second to third months for cases of isolated CL±CP and during the third to fourth months of gestation in the group of isolated CPO. The second category included chronic maternal diseases, for which, in general, onset occurred before conception and continued during pregnancy; thus, they can have their possible teratogenic effect in the critical period for both isolated CL±CP and CPO.

Among acute diseases maternal influenza, common cold (mostly with secondary complications), orofacial herpes, and gastroenteritis occurred more frequently in the mothers of cases with CL±CP than in the mothers of either population or patient controls. The prevalence of bronchitis (OR and 95% CI: 2,3 ; 1,0-5,3) and cystitis (OR and 95% CI: 1,7 ;

1,1-2,5) was higher in the group of CL±CP cases than in the group of population controls, but not in the group of patient controls.

There was a higher prevalence of influenza (OR and 95% CI: 1,6 ; 1,0-2,6), common cold (OR and 95% CI: 1,5 ; 1,0-2,2), and cystitis (OR and 95% CI: 2,1 ; 1,2-3,5) in the mothers of children with CPO than in the mothers of population controls. Three diseases, influenza (OR and 95% CI: 1,6 ; 1,1-2,3), sinusitis (OR and 95% CI: 3,5 ; 1,4-8,8), and bronchitis (OR and 95% CI: 2,2 ; 1,0-4,7), occurred more often in the mothers of cases with CPO than in the mothers of patient controls.

The prevalence and distribution of chronic maternal diseases were similar in isolated CL±CP and CPO. Thus, these groups were combined as an OFC group for the comparison to population and patient controls in order to increase the number of exposed. Epilepsy (with population controls OR and 95% CI: 3,5 ; 2,0-6,3, with patients controls OR and 95% CI: 2,7 ; 1,5-4,8) and angina pectoris (with population controls OR and 95% CI: 11,9 ; 4,8-29,4; with patients controls OR and 95% CI: 6,0 ; 2,5-14,3), were more prevalent in the mothers of OFC cases than in both population and patient controls. Depression and other forms of affective psychiatric disorders occurred more frequently in the group of mothers of OFC cases than in the population controls (OR and 95% CI: 2,0 ; 1,3-3,1).

To assess whether maternal insulin-treated diabetes constitutes a risk factor for congenital abnormalities we have analyzed the data of HCCSCA. Our data indicate that maternal pre-gestational diabetes seems to be strongly associated with more, specific congenital abnormalities. Therefore, it is not appropriate to use the patient control group as reference during the analysis of diabetes and OFCs.

During the analysis of maternal influenza the tests for effect modification suggest that the use of antifever drugs in the second and/or third month of pregnancy was an effect modifier; therefore, the analyses were repeated with the variable of interest stratified. We repeated the analysis in mothers with and without maternal antifever treatment in the critical period. According to our results orofacial clefts showed a higher OR for maternal flu if the mothers were not treated by antifever drugs (CL±CP OR and 95% CI: 3,3 ; 2,4-4,7, CPO OR and 95% CI: 2,5 ; 1,4-4,5), but we did not find a significant association for orofacial clefts when maternal flu was treated by antifever drugs (CL±CP OR and 95% CI: 1,4 ; 0,8-2,5, CPO OR and 95% CI: 1,3 ; 0,6-2,9).

The results of stratified analysis of common cold by antifever drugs in the critical period of OFCs improve our earlier results, namely that antifever treatment is an effect modifier. Antifever drugs were able to prevent the possible teratogenic effect of the common

cold (CL±CP OR and 95% CI: 1,4 ; 0,9-2,2, CPO OR and 95% CI: 1,2 ; 0,6-2,4). In contrast, we found a positive association between common cold and OFC when the antifever therapy was not present (CL±CP OR and 95% CI: 2,9 ; 2,4-3,6, CPO OR and 95% CI: 1,7 ; 1,2-2,5).

I attempted to differentiate the effect of panic disorder and the related drug (mostly bensodiasepines) treatments for CL±CP. Panic disorder in mothers without antipanic drugs had a higher OR (OR and 95% CI: 3,1 ; 1,4-6,9) than mothers with antipanic drug treatments (OR and 95% CI: 1,5 ; 0,7-3,2).

The objective of a further study was to investigate the possible association between nausea and vomiting in pregnancy and congenital abnormalities. Cases with CL±CP and CPO had mothers with a lower prevalence of severe nausea and vomiting in pregnancy compared to population controls. In the group of CL±CP the adjusted OR and 95% CI was 0,5 ; 0,4-0,7, while in case of CPO OR and 95% CI was 0,5 ; 0,3-0,9.

Previously, only the possible association between the common cold of the mother and OFC in the offspring had been reported in the evaluation of acute maternal diseases. Our study indicated an association between influenza and some other acute maternal infectious disease and isolated CL±CP and CPO. However, for the evaluation of acute maternal diseases, it is necessary to consider and differentiate the possible effects of pathogens, fever (hyperthermia) and medications. We raise the hypothesis that increased risks for isolated OFCs by these maternal diseases could be due to underlying fever. A main finding of our study is that we were able to show the protective effect of antifever therapy for maternal flu–induced CL±CP and CPO. Orofacial clefts showed a higher OR for maternal flu if the mothers were not treated by antifever drugs, but we did not find a significant association for orofacial clefts when maternal flu was treated by antifever drugs. Similar results occurred during the stratified analysis of common cold. Thus, it is important to consider preventing the potential teratogenic effect of maternal flu by vaccination in mothers who plan a pregnancy and by starting antifever therapy as soon as possible after the diagnosis of maternal flu during pregnancy.

Among chronic maternal diseases, epilepsy showed an obvious association with isolated OFCs. Epilepsy itself and the related use of antiepileptic drugs were proved previously as etiologic factors in the origin of CAs among offspring. However, in general, antiepileptic drugs (e.g., phenytoin) cause multiple CAs, including OFCs. Epilepsy itself without anticonvulsant treatment was reported as the cause of isolated OFCs; however, only later was the teratogenic effect of antiepileptic drugs indicated.

Although mothers of infants with OFCs showed more depression and other similar psychiatric (e.g., manic-depressive or panic) diseases, however it is difficult to differentiate the effect of such maternal diseases, treatment and lifestyle factors. However, this association between maternal depression and OFCs was not seen in the informative offspring of mothers with panic disorder who were treated with antipanic drugs. Further studies are needed to clarify whether or not the increased rate of occurrence of OFCs in mothers with panic disorders is due to a direct biological effect.

The possible association between maternal coronary artery disease (angina pectoris) and isolated OFCs is striking and needs further studies. In our dataset only 6 CL±CP and 2 CPO mothers had medically recorded angina pectoris, and the details of these diagnoses were not available. I could no find significant association between angina pectoris and other CAs. The question is whether it is a causal association, or can be explained by related drug treatments and lifestyle factors, unevaluated confounders or by chance. Due to the small number of exposed I was not able to stratify by related drug treatments, however drugs used for the treatment (e.g. nitrates) do not seem to be teratogenic and they do not have known relation with OFCs. On the other hand we cannot exclude the triggering effect of some potential confounding factors during the study pregnancy (e.g. smoking). Our hypothesis is a gene-environmental interaction in the origin of isolated orofacial cleft based on the common genetic predisposition for both angina pectoris and OFCs and triggering factors, mainly smoking. Unfortunately, we were not able to test the gene-environmental interactions in our dataset. Thus, further studies are needed to test our hypothesis or to generate a more reasonable explanation for this possible association.

Nausea, vomiting is the most common pregnancy complication. Our study suggested an inverse association between severe nausea and vomiting and risk of OFCs. Some studies showed protective effect of nausea and vomiting in pregnancy for OFCs, however others did not find an association. Therefore, further studies are needed to clarify this inverse association.

2. Drugs

There are five drugs: amoxicillin, diazepam, thiethylperazine, oxprenolol, and phenytoin, which were used more frequently by CL±CP case mothers than by population and malformed control mothers. On the other hand, aminophenazone (OR and 95% CI: 1,7 ; 1,2-2,4), metamizole caffeine drotaverine (Quarelin, Chinoin; OR and 95% CI: 1,8 ; 1,0-3,2) and phenobarbital (OR and 95% CI: 2,1 ; 1,2-3,8) were used more frequently in the group of cases with CL±CP than in the group of population controls but not in the group of malformed controls.

Two drugs, oxytetracycline (with population controls OR and 95% CI: 4,3 ; 1,5-12,0; with patient controls OR and 95% CI: 2,6 ; 1,2-5,7) and carbamazepine (with population controls OR and 95% CI: 13,7 ; 3,9-47,5; with patient controls OR and 95% CI: 8,4 ; 2,4-29,8), were used more frequently by mothers of cases with CPO than by the mothers of population and malformed controls. Five drugs, paracetamol (OR and 95% CI: 3,7 ; 1,1-12,0), chlordiazepoxide (OR and 95% CI: 2,9; 1,1-7,9), promethazine (OR and 95% CI: 1,4 ; 1,0-1,9), allylestrenol (OR and 95% CI: 1,4 ; 1,0-1,8), and oxprenolol (OR and 95% CI: 3,6 ; 1,1-11,7), were used more frequently by CPO mothers than by population control mothers but not by malformed control mothers.

Next, the medically recorded treatments of the previously found teratogenic ‘‘candidate’’ drugs during the critical periods of CL±CP or CPO were compared between the case and malformed control groups. Choosing the malformed controls as reference group may decrease the recall bias. Previously found associations were confirmed in amoxicillin (OR and 95% CI: 5,4; 1,9-15,4), oxprenolol (OR and 95% CI: 2,8 ; 1,2-6,6), phenytoin (OR and 95% CI: 4,4 ; 2,1-9,1), and thiethylperazine (OR and 95% CI: 2,1 ; 1,2-3,7) in the group of CL±CP and carbamazepine (OR and 95% CI: 8,4 ; 2,4-29,8) and oxytetracycline (OR and 95% CI: 2,6; 1,2-5,7) in the group of CPO.

We do not know about the potential teratogenic effect of penicillin derivatives. There was no significant difference in the frequency of major and mild congenital abnormalities among children of women treated with amoxicillin in a randomized controlled trial. Further, the Collaborative Perinatal Project did not suggest an association between penicillin derivatives and congenital abnormalities, but the data for amoxicillin were not evaluated separately. Thus, the possible CL±CP and CPO inducing effect of amoxicillin needs further study.

Oxprenolol is used for the treatment of hypertension during pregnancy. Oxprenolol crosses the placenta (Gallery, 1985) but no congenital abnormalities attributable to oxprenolol have been reported. However, experience during the critical period treatment is lacking.

Considering CPO, we found a higher use of oxytetracycline during the critical period of this congenital abnormality than in the mothers of malformed controls. Nearly all oxytetracycline treatment was medically recorded because this drug is considered as a human teratogenic chemical.

A previous case-control study based on Hungarian data showed a higher rate of CL±CP in infants born to mothers with thiethylperazine treatment during the first trimester of pregnancy, a finding that was confirmed in this study. Thiethylperazine caused a higher rate of OFC in mice and rats as well (Szabo and Brent, 1974).

A weak association was found between diazepam and CL±CP, which was not confirmed at the evaluation of medically recorded drug uses. The detailed analysis of a previous case-control study also did not indicate the teratogenic potential including the OFCs inducing effect of diazepam in nonpsychiatric patients.

We studied OFCs following exposure to phenytoin, phenobarbital, and diazepam in case-time-control design, since this study design helps avoid differential recall bias, confounding, and selection bias due to non-response. Numbers of discordant pairs were too small for analyses of OFCs in relation to phenytoin use. However, during the analysis of phenobarbital we found an OR and 95% CI of 1,4 ; 0,5-4,2 in CL±CP group, and 0,6 ; 0,1-3,9 in CPO group. Statistically significant case-time-control odds ratios were seen for diazepam, and we found an increased risk of CL±CP (OR and 95% CI: 1,8 ; 1,1-2,8), but not for CPO (OR and 95% CI: 0,7 ; 0,3-1,9).

We aimed to investigate whether folic acid supplementation in early pregnancy modifies the association between the prevalence of congenital abnormalities in the offspring and maternal use of carbamazepine, phenobarbital, phenytoin, and primidone. The results indicate that the risk of congenital abnormalities in children exposed in utero to antiepileptic drugs is reduced but not eliminated by folic acid supplementation. In the group of CL±CP for antiepileptic drugs with folic acid the OR and 95% CI was 2,4 ; 0,9-5,9 and without folic acid the OR and 95% CI was 3,0 ; 1,7-5,2. The OR and 95% CI of CPO among patients with folic acid was 1,1 ; 0,2-8,2, while without folic acid the OR and 95% CI was 2,6 ; 1,0-6,3.

Our study confirmed the well-known teratogenic effect of two anticonvulsant drugs: phenytoin and carbamazepine. Our results indicate that the risk of OFCs in children exposed to antiepileptic drugs is reduced but not eliminated by folic acid supplementation.

In conclusion, the findings of our studies showed that drugs taken in the critical period of CAs might have only a limited role in the origin of isolated CL±CP and CPO.

3. Maternal employment status

The proportion of professionals and managerials was lower, while the proportion of unskilled workers, housewives and others was significantly higher in the mothers of cases with both OFCs compared with the population control group. However, the comparison of CL±CP and CPO with patient control groups did not show any difference in the employment status of mothers.

The possible association between the socioeconomic status and OFCs has been mentioned several times but as Mossey and Little stated: "The overall conclusion of socioeconomic status in OFC is not well studied". More studies found a higher rate of OFC in couples with unemployment, poor housing and unskilled workers. In contrast, a small study in the United Kingdom, found no association between cleft lip or cleft palate and socioeconomic status, however this study included only 44 cases of CL±CP and 29 cases of CPO.

Our findings are consistent with the previously published data. These studies used different criterions and indicators of SES at the evaluation of socioeconomic status in the origin of isolated OFC therefore it is not possible to make a valid comparison. Due to a common criterion for the description of socioeconomic status including employment status does not exist it would be needed to achieve an international consensus in this field. There is a well-known worldwide variation in the prevalence of cases with CL±CP and CPO at birth, and this variation could be partly explained by the different socioeconomic status of different populations.

4. Folic acid

In the next step, high dose (6 mg) of folic acid use amid the mothers was compared among the four study groups. The folic acid use demonstrated the highest level in the

population control group and the lowest level in the CPO group. There was no difference between two orofacial cleft group and patient controls. Folic acid use was significantly higher in the population control group as in CL±CP cases (OR and 95% CI: 0,8; 0,7-0,9), and in CPO cases (OR and 95% CI: 0,8; 0,7 - 0,9). We were interested particularly in the proportion of folic acid supplementation during pregnancy by maternal employment status. Professionals showed a significantly lower level of folic acid use in both groups of CL±CP and CPO cases than in the population control group. The lower use of folic acid by mothers in the professional category was also confirmed by the comparison of CL±CP cases with the patient controls. The skilled-worker mothers of CPO cases had also a lower folic acid use compared with both population and patient control groups. The results of test of heterogeneity (in case of CL±CP $p = 0,02$, in CPO group $p = 0,01$) indicate that the odds ratios were significantly different among the maternal employment status classes.

Although folic acid supplementation reduces the risk of neural tube defects, the role of folic acid in the origin of OFCs and other CAs is still controversial. First, Tolarova presented that the high dose of folic acid combined with other vitamins is able to decrease significantly the occurrence of CL±CP. A previous Hungarian study showed that the high dose of folic acid can reduce the birth prevalence of OFCs and in our study we found a low use of folic acid even in the category of professionals. Educational campaigns and public health information should highlight the important role of folic acid supplementation in the prevention of OFC and some other CAs.

V. CONCLUSIONS

A. It is very important to choose the best statistical method for the analysis of CAs and to reduce the possible biases during the study planning and analyses.

B. The use of specified critical periods of different congenital abnormalities seems to be more scientific-based than the previously accepted methods for the evaluation of different exposure time windows. The previous use of first trimester concept is misleading. Thus this new and feasible approach is recommended for the controlled epidemiological studies in the future.

C. A much higher rate of isolated OFCs was found in the children of mothers with coronary artery disease (angina pectoris). The possible association between maternal angina pectoris and isolated OFCs is striking and needs further study.

D. The results indicated an association between maternal influenza and some other acute maternal infectious disease and isolated CL±CP and CPO. Our hypothesis it that increased risks for isolated OFCs by these maternal diseases could be due to underlying fever. Thus, it is important to consider preventing the potential teratogenic effect of maternal flu by vaccination in mothers who plan a pregnancy and by starting antifever therapy as soon as possible after the diagnosis of maternal flu during pregnancy. This teratogenic effect of maternal flu during pregnancy may be restricted by appropriate medical treatment.

E. Some maternal diseases (primary chronic diseases, e.g. epilepsy) are risk factors for the pathogenesis of isolated OFCs. However, this teratogenic effect of maternal diseases during pregnancy may be restricted by appropriate medical treatment and periconceptional folic acid supplementation.

F. Our study suggested a negative, inverse association between severe nausea and vomiting during pregnancy and risk of OFCs.

G. The findings of our studies showed that drugs might have only a limited role in the origin of isolated CL±CP and CPO, and in most cases the underlying disease is responsible for the increased risk.

H. Our results confirmed the well-known teratogenic effect of some anticonvulsant drugs. The risk of OFCs in children exposed to antiepileptic drugs is reduced but not eliminated by folic acid supplementation.

I. The high dose of folic acid use in early pregnancy may reduce the risk of OFCs, and the teratogenic effect of some maternal diseases and drugs may be reduced by folic acid supplementation.

J. A lower level of folic acid supplementation occurred in the professional mothers of cases with both types of OFC compared with the population control group.

K. The proportion of professionals and managerials was lower, while the proportion of unskilled workers and housewives was significantly higher in the mothers of cases with OFC compared with the population control group.

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VII. PUBLICATIONS

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VIII. PRESENTATIONS

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    XII. Primer Prevenciós Fórum, Fodor József Országos Közegészségügyi Központ, Budapest, 19. May 2005.
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