Possible Indirect Association Between Maternal Recurrent Orofacial Herpes in Pregnancy and Higher Risk for Congenital Abnormalities

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Abstract

Objective: To study the possible association between recurrent orofacial herpes during pregnancy and risk for congenital abnormalities.
Material and Methods: The pregnancy of mothers with recurrent orofacial herpes and without orofacial herpes were compared in the population-based large data set of the Hungarian Case-Control Surveillance System of Congenital Abnormalities.
Results: Of the 22 843 cases, 429 (1.9%) were born to mothers with recurrent orofacial herpes. Of the 38 151 newborn infants, 574 (1.5%) had mothers with recurrent orofacial herpes during pregnancy (adjusted POR with 95% CI; 1.4, 1.2-1.6). This higher risk for congenital abnormalities was explained by the higher prevalence of recurrent orofacial herpes in the second and/or third gestational month (adjusted POR with 95% CI; 1.6, 1.3 and 2.0) and particularly by some specific congenital abnormalities. Pregnant women with recurrent orofacial herpes in the second and/or third gestational month had a higher risk for isolated limb deficiencies, cardiovascular malformations, cleft lip+palate, and multiple congenital anomalies. However, antifever drug treatments can prevent this higher risk for the above congenital abnormalities except cardiovascular malformations. On the other hand, neural-tube defects also showed an association with recurrent orofacial herpes in pregnant women without antifever drug treatment. Periconceptional high dose folic acid supplementation also prevented the higher risk for cleft lip+palate, cardiovascular malformations and neural-tube defects.
Conclusions: Recurrent orofacial herpes during pregnancy is associated with a higher risk of congenital abnormalities and it may be the indirect effect of high fever related maternal diseases which preceded and triggered the recurrence of orofacial herpes. However, this risk is preventable with antifever drug treatment and/or periconceptional folic acid supplementation in some congenital abnormalities.

Keywords: recurrent orofacial herpes, congenital abnormalities, high fever, folic acid

Özet

Gebelikte Maternal Tekrarlayan Orofasiyal Herpes ve Yükselmiş Konjenital Anomali Riski Arasındaki Olası Dolaylı Bağlantı

Amaç: Bu yazıda amaç, gebelikte maternal tekrarlayan orofasiyal herpes ve yükselmiş konjenital anomali riski arasındaki olası dolaylı bağlantıyı incelemektir.
Materyal ve Metot: Tekrarlayan orofasiyal herpesi olan ve olmayan annelerin gebelikleri Macaristan Konjenital Anomali Vaka-Kontrol ve Gözetim Merkezi'nin popülasyon bazlı geniş veri tabanında karşılaştırıldı.
Sonuçlar: Vakaların 22 843'ünün 429'u (%1.9) tekrarlayan orofasiyal herpesi olan annelerden dünyaya gelmiştir. Tüm 38 151 yenidoğanın 574'ünün (%1.5) annelerinde, gebeliklerinde tekrarlayan orofasiyal herpes vardı (düzeltimiş POR %95 CI; 1.4, 1.2-1.6). Yükselmiş anomali riski, ikinci ve üçüncü gestasyonel aylarda (düzeltilmiş POR %95 CI; 1.6, 1.3 ve 2.0) geçirilen tekrarlayan orofasiyal herpese bağlı olarak ortaya çıkmakta ve özellikle bazı spesifik konjenital anomalilerde yoğunlaşmaktaydı. İkinci ve/veya üçüncü gestasyonel ayında tekrarlayan orofasiyal herpes geçiren kadınlarda izole ekstremite defekt-

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leri, kardiyovasküler anomaliler, yarık dudak+damak ve multipl konjenital anomali riski daha fazlaydı. Ancak, kardiyovasküler bozukluklar hariç, yukarıda sayılan konjenital anomaliler için yükselen risk ateş düşürücü ilaç tedavileri ile önlenebilir. Diğer yandan nöral tüp defektlerinin gebelikte tekrarlayan orofasiyal herpes ile bağlantılı olduğu ve özellikle ateş düşürücü tedavi uygulanmadığında ortaya çıktıkları gözlenmiştir. Perikonsepsiyonal yüksek doz folik asit yüklemesi de yarık dudak+damak, kardiyovasküler anomali ve nöral tüp defektlerinin ortaya çıkma riskini düşürmektedir.

Tartışma: Gebelikte tekrarlayan orofasiyal herpes, konjenital anomali riskindeki artışla bağlantılıdır. Burada orofasiyal herpes öncesinde yaşanan ve herpesi tetikleyen yüksek ateşli maternal hastalıkların dolaylı bir etkisi söz konusu olabilir. Bununla beraber, bazı anomaliler açısından artmış risk, ateş düşürücü tedaviler ve/veya perikonsepsiyonal folik asit yüklemesiyle önlenebilir.

Anahtar sözcükler: tekrarlayan orofasiyal herpes, konjenital anomaliler, yüksek ateş, folik asit

Introduction

Herpes simplex virus 1 (HSV-1) and Herpes simplex virus 2 (HSV-2) are known as human pathogens. HSV-1 is normally associated with orofacial infections, whereas HSV-2 usually causes genital infections (1). However, both viruses are capable of causing either genital or orofacial infections (2). Furthermore, both viruses can cause neonatal herpes which is a perinatally acquired infection (3). In the majority of cases, neonatal HSV is acquired via the birth canal, but rare cases of intrauterine infections have been described, hence transplacental transmission of the primary infection is possible (4-9). These previous studies showed associations between microcephaly, hydroencephaly, eye defects and primary intrauterine herpes infections particularly caused by HSV-2.

In developed countries seroconversion has been reported in about 20% of children younger than 5 years with a second seroconversion peak (40-60%) at the age of 20-40 years (10). Thus, orofacial herpes commonly affects women of childbearing age including pregnant women. The purpose of our study was to evaluate the possible association between recurrent orofacial herpes during pregnancy and different structural birth defects, i.e. congenital abnormalities in the population-based large data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities between 1980 and 1996 (11).

Materials and Methods

Cases with congenital abnormalities were selected from the Hungarian Congenital Abnormality Registry (12) for the Hungarian Case-Control Surveillance of Congenital Abnormalities. Notification of cases with congenital abnormalities is mandatory for physicians in cases from the birth until the first birthday to the Hungarian Congenital Abnormality Registry. Most congenital abnormalities are reported by obstetricians (in Hungary practically all deliveries take place in inpatient obstetric clinics and birth attendants are obstetricians) or paediatricians (working at neonatal units of inpatient obstetric clinics as well as of various inpatient and outpatient paediatric clinics). Autopsy during the study period was obligatory for all infant deaths and was usually performed in stillborn fetuses. Pathologists sent a copy of the autopsy report to the Hungarian Congenital Abnormality Registry if defects were identified in stillborn fetuses or infant deaths.

Fetal defects diagnosed by antenatal diagnostic centres with or without termination of pregnancy have also been reported to the Hungarian Congenital Abnormality Registry since 1984 and it means that malformed fetuses diagnosed during the 2nd and 3rd trimester of pregnancy are included in the data set of the Hungarian Congenital Abnormality Registry. Minor anomalies (e.g. hydrocele, umbilical hernia, minor haemangioma) were recorded but not evaluated at the calculation of rates of cases with different and total congenital abnormalities in the Hungarian Congenital Abnormality Registry. The total (birth and fetal) prevalence of cases with congenital abnormality diagnosed from the second trimester of pregnancy to the end of the first postnatal year was 35 per 1000 informative offspring (liveborn infants, stillborn fetuses, malformed fetuses in the second and third trimester of pregnancy) in the Hungarian Congenital Abnormality Registry, 1980-1996 and about 90% of major congenital abnormalities were notified (13).

However, there were three selection criteria of cases from the Hungarian Congenital Abnormality Registry for the Hungarian Case-Control Surveillance of Congenital Abnormalities. First, only cases notified during the first three months after birth or pregnancy termination were selected for the Hungarian Case-Control Surveillance of Congenital Abnormalities, these cases comprised 77% of all congenital abnormalities in the Hungarian Congenital Abnormality Registry. This shorter time between birth or pregnancy termination and data collection increases the accuracy of information regarding the data of the study pregnancy without undue loss of power. Second, three mild isolated congenital abnormalities such as congenital dysplasia of the hip, congenital inguinal hernia, and major haemangioma were excluded from the Hungarian Case-Control Surveillance of Congenital Abnormalities. Third, congenital abnormality syndromes of Mendelian or chromosomal (i.e. known preconceptional and not teratogenic) origin were also not included in the Hungarian Case-Control Surveillance of Congenital Abnormalities.

Controls were newborn infants without congenital abnormalities, selected from the National Birth Registry of the Central Statistical Office for the Hungarian Case-Control Surveillance of Congenital Abnormalities. In general two newborn infants were matched with every case according to sex, birth week and district of parents' residence. However, three controls were chosen for each case between 1986 and 1992, because

we wanted to increase the number of controls from 1986, but unfortunately there was no financial support for the third control after 1992.

There were three sources of information regarding maternal disorders and related drug treatments.

Retrospective maternal information
A post-paid structured questionnaire together with an explanatory letter and a list of diseases and medicaments, and a printed informed consent were mailed to the mothers immediately after the selection of cases and controls. The questionnaire requested information on pregnancy complications, maternal diseases and medicine (drug and pregnancy supplement) taken during pregnancy according to gestational months. In order to standardize the answers, mothers were asked to read the enclosed list of diseases including OFH (orufacial herpes) and medications as a memory aid before replying. Case mothers were asked to give a signature for informed consent which authorized us to record the name and address of their children in the Hungarian Case-Control Surveillance of Congenital Abnormalities. (Name and address of controls were not recorded in the Hungarian Case-Control Surveillance of Congenital Abnormalities.)

Medically recorded data
Mothers were also asked to send the prenatal care logbook and other medical records (mainly discharge summaries) related to diseases and their treatment during pregnancy and their child's defects. Prenatal care was mandatory for pregnant women in Hungary (if somebody did not visit prenatal care, she did not get maternity grant and leave), thus nearly 100% of pregnant women visited prenatal care, on average, seven times. The first visit was between 7 and 12 gestational weeks.

The period between birth or pregnancy termination and return of "information package" (questionnaire, logbook, informed consent, etc.) was 3.5±1.2 and 5.2±2.9 months for cases and controls, respectively.

Supplementary data collection
Regional nurses were asked to visit and question all non-respondent mothers of cases, but only 200 non-respondent control mothers, as the ethics committee considered that this follow-up would be disturbing to the parents of healthy children (14). Regional nurses helped mothers to fill in the same questionnaire and they evaluated available medical documents.

The flow of cases from the Hungarian Congenital Abnormality Registry and controls from the National Birth Registry to the Hungarian Case-Control Surveillance of Congenital Abnormalities was published previously (15). Necessary data were collected for 96.3% of cases (84.4% from reply, 11.9% from visit), and for 83.0% of controls (82.6% from reply, 0.4% from visit). Prenatal logbooks were available in 88.4% of cases and in 93.8% of controls. Informed consent was signed and returned by 98.4% of case mothers. The name and address of subjects without signed informed consent were deleted in the Hungarian Case-Control Surveillance of Congenital Abnormalities.

The procedure of data collection in the Hungarian Case-Control Surveillance of Congenital Abnormalities was changed in 1997 because regional nurses visited and questioned all cases and controls; unfortunately, this data set has not been validated until now. It explains why the data set of 17-years between 1980 and 1996 is evaluated here.

The orofacial herpes was evaluated according to seven aspects. a) Two types: primary and recurrent orofacial herpes were differentiated. b) Source of information: (i) maternal information only, (ii) medical records only, (iii) both. c-d) onset and duration of orofacial herpes according to gestational month. e) The gestational age was calculated from the first day of the last menstrual period. Three time intervals were considered: (i) First month of gestation because it is before the organogenesis. The first two weeks are before conception while the third and fourth weeks comprise the pre- and implantation period of zygotes and blastocysts including omnipotent stem cells. Thus congenital abnormalities cannot be induced by environmental agents in the first month of gestation and it explains the "all-or-nothing effect" rule, i.e. total loss or normal further development. (ii) The second and third months of gestation; this is the most sensitive, the so-called critical period for major congenital abnormalities. (iii) The fourth through ninth months of gestation; i.e. pregnancy after the organforming period. f) Medication used during pregnancy including administrative route (oral, parenteral, topical), dose, and duration of treatment. g) Confounding factors; such as maternal age, birth order, marital and employment status as indicator of socioeconomic status, other maternal diseases and use of drugs and pregnancy supplements.

Statistical analyses
Statistical analyses were performed using the software package SAS version 8.02 (SAS Institute Inc., Cary, North Caroline, USA). First, the occurrence of recurrent orofacial herpes during the study pregnancy was compared between the two study groups (cases and controls) and crude prevalence odds ratios (POR) with 95% confidence interval (CI) were calculated. Second, frequency tables were made for the main maternal variables in order to describe the study groups of mothers with recurrent orofacial herpes and of mothers without orofacial herpes as reference. Third, the prevalence of pregnancy complications, other acute and chronic maternal diseases, drugs and pregnancy supplements used during the study pregnancy were compared between case and control mothers with recurrent orofacial herpes, and crude POR with 95% CI were evaluated. Fourth, the prevalence of recurrent orofacial herpes according to gestational period in 21 different congenital abnormality groups, including at least 3 cases born to mothers with recurrent orofacial herpes was compared with the frequency of recurrent orofacial herpes in their all matched control pairs, and adjusted PORs with 95%

CI were evaluated in a conditional logistic regression model. The latter PORs were adjusted for maternal age (<25 yr vs. 25-29 yr vs. 30 yr or more), birth order (first delivery vs. one or more previous deliveries), maternal employment status (professional-managerial-skilled worker vs. semiskilled worker-unskilled worker-housewife vs. others) and acute maternal diseases (as a dichotomous variable). Fifth, we estimated the association between the "candidate" congenital abnormalities and maternal recurrent orofacial herpes during the second and/or third months of pregnancy stratified by the use of antifever drugs and folic acid supplementation during the second and/or third months of pregnancy using the unconditional logistic regression models.

Results

Of the 22 843 cases, 435 (1.9%) were born to mothers with orofacial herpes during the study pregnancy. The number of births in Hungary was 2 146 574 between 1980 and 1996, while the number of evaluated newborn infants as controls was 38 151; i.e. 1.8% of all births in the study period. Of the 38 151 newborn infants, 577 (1.5%) had mothers with orofacial herpes during the study pregnancy.

Of the 435 case mothers, 429 (98.6%) and of the 577 control mothers, 574 (99.5%) reported recurrent orofacial herpes, thus only 6 case and 3 control mothers had primary orofacial herpes. These mothers were medically recorded in the prenatal logbook and they were excluded from the study. Recurrent orofacial herpes was recorded rarely in the prenatal care logbooks and discharge summaries, thus it was mostly reported by the mothers (81.0% of cases and 72.1% of controls). Finally 429 case and 574 control mothers with recurrent orofacial herpes were evaluated in the study (crude POR with 95% CI: 1.3, 1.1-1.4).

The maximum of recurrent orofacial herpes was found in the 3rd gestational month (21.1% in case and 17.2% in control mothers), followed by 2nd, 4th and 1st gestational month. The occurrence of recurrent orofacial herpes was rare (about 3%) in the last two months of gestation. There was no significant difference in the monthly distribution of recurrent orofacial herpes between case and control mothers. Of the 429 case and 574 control mothers, only 6 and 8 mentioned two or more recurrent orofacial herpes during the study pregnancy, respectively. The first recurrent orofacial herpes was evaluated at the analysis of possible association between recurrent orofacial herpes during pregnancy and congenital abnormalities.

Table 1. shows the basic characteristics of mothers with recurrent orofacial herpes and without orofacial herpes as reference. Mothers with recurrent orofacial herpes were somewhat older than referent mothers while mean birth order was lower (t=6.7; $p<0.001$) due to the larger proportion of primiparous. There was no significant difference in the mean maternal age (t=1.3; $p=0.20$) and mean birth order (t=0.7; $p=0.46$) between case and control mothers with recurrent orofacial herpes. We did not find difference in the proportion of unmarried pregnant women among the study groups. Maternal employment status showed some differences between mothers with recurrent oro-

Table 1. Basic characteristics of case and control mothers with recurrent orofacial herpes (OFH) or without OFH as reference

Variables	Case mothers				Control mothers			
	Without OFH (n=22 414)		With OFH (n=429)		Without OFH (n=37 577)		With OFH (n=574)	
	No.	%	No.	%	No.	%	No.	%
Continuous								
Maternal age (yr)								
<25	10 759	48.0	186	43.4	17 754	47.3	240	41.8
25-29	6 992	31.2	162	37.8	12 679	33.7	204	35.9
>29	4 663	20.8	81	18.9	7 144	19.0	128	22.3
Mean (SD)	25.5	(5.3)	25.7	(4.7)	25.4	(4.9)	26.1	(4.7)
Birth order								
1	13 614	60.7	300	69.9	22 328	59.4	414	72.3
≥ 2	8 800	39.3	129	30.1	15 242	40.6	158	27.7
Mean (SD)	1.6	(1.0)	1.4	(0.7)	1.6	(0.9)	1.4	(0.7)
Categorical								
Unmarried	1 269	5.6	16	3.7	1 471	3.9	18	3.1
Employment status								
Professionals	1 824	8.1	77	18.0	4 201	11.2	152	26.5
Managerial	4 849	21.6	119	27.7	9 969	26.5	165	28.8
Skilled worker	6 194	27.6	135	31.5	11 536	30.7	154	26.8
Semiskilled worker	3 820	17.0	49	11.4	5 740	15.3	43	7.5
Unskilled worker	1 495	6.7	8	1.9	1 853	4.9	6	1.1
Housewife	2 118	9.5	10	2.3	2 029	5.4	9	1.6
Others	2 114	9.4	31	7.2	2 249	6.0	43	7.8

Table 2. Prevalence of other diseases during the study pregnancy of mothers with recurrent OFH and mothers without OFH as reference

Maternal diseases	Case mothers				Control mothers				Difference between case and control mothers with OFH	
	Without OFH (n=22 414)		With OFH (n=429)		Without OFH (n=37 577)		With OFH (n=574)			
	No.	%	No.	%	No.	%	No.	%	POR	95% CI
Acute										
Influenza-common cold	4 798	21.4	169	39.4	6 855	18.2	207	36.2	1.15	0.89-1.49
Respiratory system	2 037	9.1	81	18.9	3 329	8.9	126	22.0	0.83	0.61-1.13
Digestive system	313	1.4	9	2.1	359	1.0	9	1.6	1.35	0.53-3.42
Urinary tract	1 555	6.9	27	6.3	2 250	6.0	44	7.7	0.81	0.49-1.33
Genital organs	1 635	7.3	37	8.6	2 837	7.5	48	8.4	1.03	0.66-1.62
Chronic										
Diabetes mellitus	55	0.2	1	0.2	52	0.1	0	0.0	-	-
Epilepsy	68	0.3	2	0.5	68	0.2	1	0.2	2.68	0.24-29.69

facial herpes or without orofacial herpes (χ^2_6=966.7; p<0.001). Recurrent orofacial herpes was more frequent among professional and managerial women, while less frequent in semi- and unskilled workers and housewives. In Hungary most housewives belong to the low socioeconomic class. The distribution of maternal employment status was also different between case and control mothers with recurrent orofacial herpes (χ^2_6=15.2; p=0.01).

The occurrences of pregnancy complications in mother with recurrent orofacial herpes were evaluated in our previous study (16). Severe nausea and vomiting, threatened preterm delivery and placental disorders were more frequent while pre-eclampsia was less frequent in pregnant women with recurrent orofacial herpes.

The prevalences of other maternal diseases are shown in Table 2. Among acute infectious maternal diseases, the occurrence of influenza-common cold with secondary complications and acute diseases of respiratory system (mainly tonsillitis) was 1.9 and 2.3 fold more frequent in mothers with recurrent orofacial herpes than in mothers without orofacial herpes, respectively. However, there was no difference in the occurrence of acute maternal diseases between case and control mothers with recurrent orofacial herpes. Chronic maternal diseases, such as insulin dependent diabetes mellitus and epilepsy did not show difference among the study groups.

Among frequently used drugs (in more than 2.5% either in case or control mothers with recurrent orofacial herpes)

Table 3. The use of frequently used drugs

Frequently used drugs	Case mothers				Control mothers				Difference between case and control mothers with OFH	
	Without OFH (n=22 414)		With OFH (n=429)		Without OFH (n=37 577)		With OFH (n=574)			
	No.	%	No.	%	No.	%	No.	%	POR	95% CI
Acetylsalicylic acid	1 059	4.7	35	**8.2**	1 485	4.0	39	**6.8**	1.22	0.76-1.96
Allylestrenol	3 428	15.3	53	12.4	5 284	14.1	73	12.8	0.97	0.66-1.41
Aminophenazone	964	4.3	37	**8.6**	1 207	3.2	37	**6.5**	1.37	0.85-2.20
Aminophylline	1 348	6.0	26	6.1	2 243	6.0	41	7.2	0.84	0.51-1.39
Ampicillin	1 591	7.1	53	**12.4**	2 542	6.8	82	**14.3**	0.85	0.58-1.23
Bromhexine	465	2.1	16	3.7	770	2.0	30	5.2	0.70	0.38-1.31
Clotrimazole	1 581	7.1	60	**14.0**	3 007	8.0	70	**12.2**	1.17	0.81-1.70
Diazepam	2 688	12.0	58	13.5	4 072	10.8	58	10.1	1.39	0.94-2.05
Dimenhydrinate	896	4.0	18	4.2	1 694	4.5	32	5.6	0.74	0.41-1.34
Drotaverine	1 996	8.9	57	13.3	3 432	9.1	49	8.6	**1.64**	**1.10-2.46**
Metamizole	1 452	6.5	72	**16.8**	2 044	5.4	77	**13.5**	1.30	0.92-1.85
Metronidazole	941	4.2	23	5.4	1 389	3.7	27	4.7	1.15	0.65-2.03
Penamecillin	1 553	6.9	43	**10.0**	2 191	5.8	55	**9.6**	1.05	0.69-1.60
Pholedrin	752	3.4	16	3.7	1 476	3.9	33	5.8	0.64	0.35-1.17
Prednisolone	195	0.9	16	**3.7**	378	1.0	15	**2.6**	1.44	0.71-2.95
Promethazine	3 571	15.9	77	18.0	5 924	15.8	101	17.7	1.02	0.74-1.42
Senna	475	2.1	31	7.2	906	2.4	31	5.4	1.36	0.82-2.28
Terbutaline	2 284	10.2	66	**15.4**	3 903	10.4	91	**15.9**	0.97	0.68-1.36

Bold numbers indicate significant association

Table 4. The use of pregnancy supplements

Pregnancy supplement	Case mothers				Control mothers				Difference between case and control mothers with OFH	
	Without OFH (n=22 414)		With OFH (n=429)		Without OFH (n=37 577)		With OFH (n=574)			
	No.	%	No.	%	No.	%	No.	%		
Iron	14 442	64.4	302	70.4	26 334	70.1	440	76.7	**0.77**	**0.75-0.80**
Calcium	1 794	8.0	45	10.5	3 541	9.4	85	14.8	**0.83**	**0.79-0.88**
Folic acid	11 034	49.2	245	57.1	20 397	54.3	378	65.9	**0.82**	**0.79-0.84**
Vitamin B6	1 952	8.7	61	14.2	3 973	10.6	113	19.7	**0.81**	**0.76-0.85**
Vitamin D	5 717	25.5	140	32.6	9 794	26.1	169	29.4	0.98	0.94-1.01
Vitamin C	867	3.9	48	11.2	1 615	4.3	66	11.5	**0.91**	**0.83-0.98**
Vitamin E	1 370	6.1	48	11.2	2 222	5.9	65	11.3	1.04	0.97-1.11
Multivitamins	1 288	5.7	50	11.7	2 437	6.5	89	15.5	**0.88**	**0.82-0.94**

Bold numbers indicate significant associations

(Table 3), antifever (acetylsalicylic acid, aminophenazone, metamizole) and antimicrobial (ampicillin, clotrimazole, metronidazole, penamecillin) drugs had a more frequent use in mothers with recurrent orofacial herpes than in pregnant women without orofacial herpes due to the higher prevalence of the previously mentioned acute maternal diseases. Among drugs used for the prevention or treatment of threatened preterm delivery, terbutaline showed a higher rate in mothers with recurrent orofacial herpes. However, only the use of spasmolytic drotaverine was more frequent in case mothers with recurrent orofacial herpes than in control mothers with orofacial herpes. Only 15 mothers with recurrent orofacial herpes were treated with acyclovir introduced in Hungary during the 1990s in the data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities. Mainly prednisolone was used for the treatment of recurrent oro-

Table 5. Association between maternal recurrent orofacial herpes (OFH) during the study pregnancy and different congenital abnormalities using the conditional logistic regression model

Study groups	In the 2nd and/or 3rd months				Anytime during pregnancy				Grand total
	No.	%	POR*	95% CI	No.	%	POR*	95% CI	No.
Isolated CAs									
Neural-tube defects	11	0.9	1.5	0.6-3.4	23	1.9	1.2	0.7-2.1	1 202
Cleft lip±palate	19	1.4	**2.5**	**1.2-5.0**	45	3.3	**2.3**	**1.4-3.5**	1 374
Posterior cleft palate	4	0.7	2.1	0.4-11.7	15	2.5	2.5	0.9-6.4	601
Esophageal atresia/stenosis	3	1.4	2.9	0.4-19.9	4	1.8	0.7	0.2-2.5	217
Pyloric stenosis	1	0.4	0.5	0.1-4.4	3	1.2	0.8	0.2-3.2	241
Rectal/anal atresia/stenosis	2	0.9	2.7	0.4-20.1	4	1.8	2.4	0.6-10.5	220
Obstructive urinary CAs	3	0.6	2.2	0.3-16.3	11	2.2	1.0	0.3-3.5	502
Hypospadias	12	0.4	1.0	0.5-2.0	35	1.2	0.9	0.6-1.3	3 038
Undescended testis	6	0.3	1.1	0.4-3.1	21	1.0	0.9	0.5-1.5	2 051
Exomphalos/gastroschisis	0	0.0	-	-	3	1.3	1.1	0.2-5.6	238
Hydrocephaly	0	0.0	-	-	4	1.3	0.7	0.2-2.4	314
Ear CAs	2	0.6	2.9	0.3-34.1	5	1.4	1.4	0.4-4.9	354
Cardiovascular CAs	44	1.0	**2.6**	**1.6-4.2**	100	2.2	**1.7**	**1.3-2.3**	4 479
CAs of genital organs	1	0.8	0.9	0.1-10.8	4	3.3	1.5	0.3-7.6	123
Clubfoot	5	0.2	0.5	0.2-1.3	28	1.2	0.9	0.5-1.4	2 424
Limb deficiencies	8	1.5	**6.5**	**1.7-25.3**	14	2.6	1.6	0.8-3.4	548
Poly/syndactyly	11	0.6	1.1	0.5-2.3	33	1.9	1.4	0.9-2.3	1 744
CAs of skeletal system	2	1.0	1.6	0.3-9.8	5	2.4	1.7	0.5-5.6	211
Diaphragmatic CAs	0	0.0	-	-	4	1.7	1.8	0.4-7.8	243
Other isolated CAs	14	1.0	1.5	0.7-3.0	32	2.3	**2.0**	**1.2-3.1**	1 370
Multiple CAs	15	1.1	**2.8**	**1.3-6.3**	36	2.7	**1.8**	**1.1-2.9**	1 349
Total cases	163	0.7	**1.6**	**1.3-2.0**	429	1.9	**1.4**	**1.2-1.6**	22 843
Total controls	191	0.5	referent		574	1.5	referent		38 151

*Matched analysis for each case and their all (1-3) matched controls when PORs were adjusted for maternal age, birth order, maternal employment status and use of folic acid in the 2nd and/or 3rd months of pregnancy

Bold numbers indicate significant associations

facial herpes in the study period, but there was no significant difference in the occurrence of this drug treatment between case and control mothers with recurrent orofacial herpes.

The use of pregnancy supplements such as folic acid and multivitamins is shown in Table 4. All pregnancy supplements were used more frequently by mothers with recurrent orofacial herpes than by mothers without orofacial herpes. However, it is worth mentioning that these pregnancy supplements, except vitamin D and E, were less frequently used by case mothers with recurrent orofacial herpes than by control mothers with recurrent orofacial herpes. The usual dose of folic acid was 3-9 mg, in general 6 mg.

The main objective of the study was to evaluate the possible association between recurrent orofacial herpes anytime during pregnancy, but particularly in the second and/or third gestational month and different congenital abnormalities (Table 5). Adjusted POR showed a higher risk for 4 congenital abnormality groups: isolated limb deficiencies, cardiovascular malformations, cleft lip±palate, and multiple congenital abnormalities, if maternal recurrent orofacial herpes occurred in the second and/or third gestational month. After the exclusion of pregnant women with recurrent orofacial herpes in the second and/or third gestational month from the total group of the above 4 congenital abnormalities, the rest did not show association between recurrent orofacial herpes and these "candidate" congenital abnormalities. Of the 32 cases with other isolated congenital abnormalties, 2 had microcephaly and 2 were affected with eye defects (buphthalmos and coloboma).

In the next step, we differentiated pregnant women with recurrent orofacial herpes according to the use or without the use of antifever drugs in the second and/or third gestational month in the 4 "candidate" congenital abnormality groups which showed association with recurrent orofacial herpes (Table 6). The previously mentioned associations were lost except in the group of cardiovascular malformations. Table 6 also shows neural-tube defects because this congenital abnormality group associated with recurrent orofacial herpes in the second gestational month if these pregnant women were not treated with antifever drugs before or during the time of recurrent orofacial herpes. However, it is necessary to mention that the numbers of pregnant women with recurrent orofacial herpes and with antifever treatment were limited.

Finally, we evaluated the possible association between recurrent orofacial herpes in the second and/or third gestational month and five candidate congenital abnormalities according to the supplementation of folic acid in the second and third gestational months (Table 6). If a woman used folic acid in the first gestational month (i.e. preconceptionally), it was continued in all women in the second gestational month. The supplementation of folic acid was able to prevent the risk for neural-tube defects, cleft lip±palate and cardiovascular malformations, but not for limb deficiencies and multiple congenital abnormalities associated with recurrent orofacial herpes.

Unfortunately, we were not able to analyse the possible synergistic effect of antifever drugs and folic acid due to the too low number of pregnant women with recurrent orofacial herpes in this subgroup, though at present the Hungarian Case-Control Surveillance of Congenital Abnormalities has the largest case-control data set in the world.

Table 6. Association between maternal recurrent OFH in the second and/or third gestational month and the so-called "candidate" CA groups stratified by the use of antifever drugs and folic acid in the second and/or third month of pregnancy using unconditional logistic regression model

Study groups	Total		No antifever drugs			Antifever drugs			No folic acid			Folic acid					
	No.	%	POR* (95% CI)			POR* (95% CI)			POR* (95% CI)			POR** (95% CI)			POR** (95% CI)		
Controls	191	0.5	Referent	166	0.5	Referent	25	1.9	Referent	123	0.5	Referent	68	0.6	Referent		
Isolated CAs																	
Neural-tube defects	11	0.9	**2.0 (1.1-3.6)**	10	0.9	**2.1 (1.1-4.0)**	1	1.6	1.0 (0.1-7.3)	9	1.0	**2.3 (1.2-4.6)**	2	0.7	1.2 (0.3-4.9)		
Cleft lip± palate	19	1.4	**3.1 (1.9-5.0)**	15	1.2	**2.9 (1.7-5.0)**	4	5.1	3.0 (0.9-9.1)	14	1.4	**3.4 (2.0-5.9)**	5	1.4	2.5 (1.0-6.3)		
Cardiovascular CAs	44	1.0	**2.2 (1.6-3.0)**	33	0.8	**1.9 (1.3-2.8)**	11	4.9	**2.8 (1.4-5.8)**	32	0.9	**2.3 (1.6-3.4)**	12	1.1	1.9 (1.0-3.4)		
Limb deficiencies	8	1.5	**3.2 (1.6-6.5)**	7	1.4	**3.3 (1.5-7.1)**	1	3.0	1.8 (0.2-13.6)	5	1.2	**2.9 (1.2-7.1)**	3	2.1	**4.0 (1.2-12.8)**		
Multiple CAs	15	1.1	**2.5 (1.5-4.3)**	11	0.9	**2.2 (1.2-4.1)**	4	4.8	3.0 (0.9-8.9)	8	0.8	1.9 (0.9-3.9)	7	2.2	**4.1 (1.8-8.9)**		

*PORs were adjusted for maternal age, birth order, maternal employment status and use of folic acid in the 2nd and/or 3rd months of pregnancy
Unmatched analysis for cases and all controls as reference
**PORs were adjusted for maternal age, birth order and maternal employment status
Unmatched analysis for cases and all controls as reference

Bold numbers indicate significant associations

Discussion

The major and unexpected finding of our study was a possible association between maternal recurrent orofacial herpes in the second and/or third gestational months and four congenital abnormality groups: isolated limb deficiencies, cardiovascular malformations, cleft lip±palate, and multiple congenital abnormalities. However, the parallel use of antifever drugs was able to prevent this risk in all but one congenital abnormality group, the exception was cardiovascular malformations. On the other hand, there was an association between neural-tube defects and recurrent orofacial herpes in the second gestational month of pregnant women without antifever drugs. In addition, the high dose of folic acid supplementation in the early pregnancy reduced the risk for neural-tube defects, cardiovascular malformations and cleft lip±palate due to recurrent orofacial herpes.

The strengths of the Hungarian Case-Control Surveillance of Congenital Abnormalities are (i) the large and (ii) population-based data set including 1 003 pregnant women with recurrent orofacial herpes in ethnically homogeneous European sample. This data set is based (iii) on matching of cases and controls, (iv) the knowledge of exposure time and (v) confounding factors. (vi) We were able to differentiate medically recorded primary and recurrent orofacial herpes, and only the latter was evaluated in the study. (vii) In addition the diagnoses of congenital abnormalities had good validity due to the medically reported cases, and these diagnoses were checked by experts in the Hungarian Congenital Abnormality Registry, furthermore new information from the questionnaire and recent medical examinations was helpful to exclude cases with misdiagnosed congenital abnormalities or to correct their diagnoses in the Hungarian Case-Control Surveillance of Congenital Abnormalities.

Of course, limitations of the data set need to be mentioned as well. (i) The major weakness of our study is that the diagnosis of most recurrent orofacial herpes was based on maternal self-reported information. However, maternal information is valid regarding their diseases during pregnancy (14). (ii) We were not able to identify HSV-1 and HSV-2, in addition to detect asymptomatic HSV infections because we had no serological data of our pregnant women. (iii) Response rate was similar in the group of cases and controls (84% vs. 83%) but all non-respondent case mothers while only 200 non-respondent control mothers were visited at home by regional nurses. However, the prevalence of recurrent orofacial herpes did not show differences between respondent and non-respondent families (14). (iv) In addition, there was a longer time between the end of pregnancy and return of information package in control mothers than in case mothers (t=84.4; $p<0.001$). (v) Thus, we have to consider the recall bias in control mothers. The birth of an infant with congenital abnormalities is a serious traumatic event for most mothers who therefore try to find a causal explanation such as diseases or drug uses during pregnancy. This does not occur after the birth of a healthy newborn baby. Recall bias might inflate an increased risk for congenital abnormalities. Our previous analysis showed that a case-control surveillance of this type may cause spurious association between drugs and congenital abnormalities with biased POR up to a factor of 1.9 (17). However, adjusted POR exceeded this level in 4 congenital abnormality groups. In addition, at the planning of our study design we wanted to limit recall bias by the evaluation of critical period of congenital abnormalities because we expect an underreporting of recurrent orofacial herpes in both the critical and non-critical periods of congenital abnormalities in the control group.

Pregnant women with recurrent orofacial herpes in our study had higher mean maternal age, but lower mean birth order, and a larger proportion of primiparous mothers with higher socioeconomic status (professional and managerial women with higher level of education), in addition they used more frequently folic acid and other pregnancy supplements. On the other hand, it is worth mentioning the higher occurrence of influenza-common cold with secondary complications, and acute infectious diseases of respiratory system mainly tonsillitis in our pregnant women with recurrent orofacial herpes. In our previous studies, 91.5%, 56.5% and 47.5% of mothers affected with influenza (15), common cold with secondary complications (18) and tonsillitis (19) during pregnancy had high fever (over 38.5°C), respectively.

In the first step of this project (16) we showed that mothers with recurrent orofacial herpes during pregnancy had a somewhat longer (0.4 wk) gestational age (adjusted t=2.7; $p=0.006$) but an obviously lower proportion of preterm births (3.5% vs. 9.3%) than pregnant women without orofacial herpes (adjusted POR with 95% CI=0.42, 0.27-0.65). However, there was no significant difference in the mean birth weight and rate of low birthweight between the two study groups.

The major unexpected finding of this study is an association between the recurrent orofacial herpes and some congenital abnormalities, though these congenital abnormalities did not show similarity with congenital abnormalities which were found to be associated with primary herpes virus, particularly HSV-2 intrauterine infections in previous studies (4-9). Thus, it would be necessary to explain this discrepancy. First of all, we have to differentiate the possible effect of pathogens (i.e. HSV-1 and/or HSV-2), the introductory hyperthermia due to fever related diseases which preceded and probably triggered recurrent orofacial herpes, medications used for the treatment of recurrent orofacial herpes and confounders.

As far as we know, herpes viruses did not induce congenital abnormalities which showed associations with recurrent orofacial herpes in the study (4-9).

The teratogenic effect of hyperthermia is well-known (20-23). Our previous study showed a higher prevalence of maternal influenza during the second and/or third months of pregnancy in women who later had offspring with cleft lip±palate, posterior cleft palate, neural-tube defects and cardiovascular malformations (15). A similar pattern of con-

genital abnormalities was found in the offspring of mothers with the common cold and secondary complications (18). Thus, fever was considered as the common denominator in the teratogenic effect of influenza and the common cold with secondary complications. In addition we confirmed the effect of hyperthermia (over 38.5°C) in the origin of neural-tube defects (24), cleft lip±palate (25) and multiple congenital abnormalities (26). On the other hand, we were able to show that antifever drugs reduced or prevented the congenital abnormality inducing effect of high fever in the previously mentioned studies (15,18,24-26), and now it was confirmed in this study connected with orofacial herpes. These findings are very important arguments for the human teratogenic effect of high fever.

Antifever and antimicrobial drugs were not used more frequently by case mothers than control mothers with recurrent orofacial herpes. In addition our previous studies showed that antifever drugs used in Hungary had no teratogenic effect (27). The occurrence of prednisolone treatment was also not significantly higher in the group of case mothers than in the group of control mothers with recurrent orofacial herpes. Our previous study did also not indicate an association between prednisolone and congenital abnormalities (28) which was used for the treatment of recurrent orofacial herpes. The teratogenic effect of acyclovir and other antiviral drugs was not found (29).

Known confounders were considered at the calculation of adjusted POR in the study. Nevertheless it is worth mentioning the use of pregnancy supplements. Folic acid (30) or folic acid-containing multivitamin (31) can reduce the first occurrence of neural-tube defects, while folic acid-containing multivitamins some other congenital abnormalities as well (32-34). Case mothers with recurrent orofacial herpes used less frequently high doses of folic acid (in general 6 mg) and folic acid-containing multivitamins in general and in the early postconceptional period than control mothers with recurrent orofacial herpes. However, these differences were considered at the calculation of adjusted POR. Another important finding of our study is that the high dose of folic acid reduced the hyperthermia related risk for some congenital abnormalities associated with recurrent orofacial herpes.

Our hypothesis for the explanation of these findings is that the higher occurrence of isolated limb deficiencies, cleft lip±palate, cardiovascular malformations, in addition neural-tube defects and multiple congenital abnormalities may be connected with the high fever due to some acute maternal diseases which preceded and triggered recurrent orofacial herpes. These congenital abnormalities are part of the hyperthermia induced spectrum of congenital abnormalities (35). On the other hand, immune suppression of pregnant women is also a well-known fact which plays a fundamental role in maintaining pregnancy. This immune suppression may explain the higher occurrence of the above fever related acute maternal infectious diseases in pregnant women. Recurrent orofacial herpes usually also develops in a suppressed state of the immune system (36-38). In addition, it is also well-known that there is an association between fever and recurrent orofacial herpes. Thus the order of events may be (i) pregnancy with suppressed state of immune system, (ii) in creased risk for high fever related acute infectious maternal diseases (iii) which trigger the recurrence of orofacial herpes and (iv) induce some congenital abnormalities.

The main message of our study is that the possible association between recurrent orofacial herpes and some congenital abnormalities probably is caused by the high fever and this risk can be reduced by antifever drugs in the above congenital abnormality groups except cardiovascular malformations. In addition, these high fever induced congenital abnormalities were prevented by the high dose of folic acid supplementation in neural-tube defects, cleft lip±palate and cardiovascular malformations. Thus it is necessary to use antifever therapy and folic acid supplementation with maternal fever related diseases during pregnancy which induce both recurrent orofacial herpes and some congenital abnormalities.

In conclusion, our study showed an association between recurrent orofacial herpes and some congenital abnormalities and it may be explained by high fever related diseases which trigger the recurrent orofacial herpes and induce congenital abnormalities. However, a major part of this teratogenic risk can be prevented by antifever therapy and folic acid supplementation.

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