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ORIGINAL ARTICLE

Is there a reduction of congenital abnormalities in the offspring of diabetic pregnant women after folic acid supplementation? A population-based case-control study

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ABSTRACT **The objective of the present study was to estimate the preventive effect of folic acid for structural birth defects (i.e. congenital abnormalities [CAs]) in the offspring of pregnant women with diabetes mellitus type 1 (DM-1). The occurrence of medically recorded DM-1 in pregnant women who had malformed fetuses/newborns (cases) and delivered healthy babies (controls) with or without folic acid supplementation was compared in the population-based Hungarian Case-Control Surveillance System of Congenital Abnormalities. The case group included 22 843 offspring, and there were 79 (0.35%) pregnant women with DM-1, while the control group comprised of 38 151 newborns, and 88 (0.23%) had mothers with DM-1. Case mothers with DM-1 associated with a higher risk of total rate of CAs in their offspring (OR with 95% CI: 1.5, 1.1–2.0) compared to the total rate of CAs in the offspring of non-diabetic case mothers. This higher risk can be explained by four specific types/groups of CAs: isolated renal a/dysgenesis; obstructive CA of the urinary tract; cardiovascular CAs; and multiple CAs, namely caudal dysplasia sequence. However, there was no higher rate of total CAs in the children of pregnant women with DM-1 after folic acid supplementation; in addition, neural-tube defect and renal a/dysgenesis did not occur. However, this benefit cannot be explained by the CA reduction effect of folic acid during the critical period of major CAs. In conclusion, there was a certain reduction in maternal teratogenic effect of DM-1 after folic acid supplementation during pregnancy, but the explanation of this effect requires further study.**

Key Words: congenital abnormality, diabetes mellitus type 1, folic acid, population-based case-control study, pregnancy

INTRODUCTION

Maternal diabetes mellitus (DM) during pregnancy associates with a higher risk of certain structural birth defects (i.e. congenital abnormalities [CAs]) in the offspring (Moore 2004). The spectrum of DM-related isolated CAs (Molsted-Pedersen *et al*. 1964; Becerra *et al*. 1990; Nielsen *et al*. 2005) includes neural-tube defects (Milunsky *et al*. 1982), cardiovascular CAs, particularly transposition of great vessels, double outlet right ventricle, and common truncus (Ferencz *et al*. 1990; Loffredo *et al*. 2001), kidney CA (renal a/dysgenesis), obstructive CAs of the urinary tract, congenital limb deficiency (mainly a/dysplasia of femoral head), CAs of spines (Rusnak and Driscoll 1965; Martinez-Frias *et al*. 1998; Greene 1999; Sheffield *et al*. 2002) and a specific multiple CA, the so-called caudal dysplasia sequence (Kucera *et al*. 1965; Passarge and Lenz 1966).

Three main types of DM are differentiated, such as type 1 (DM-1), with the previous term juvenile-onset DM or insulin dependent DM (IDDM); type 2, with the previous term adult-onset DM or non-insulin dependent DM (NIDDM); and gestational DM. Our previous study showed a 1.5-fold higher risk of CAs in the offspring of pregnant women with DM-1 (Bánhidy *et al*. 2010). However, the risk of CAs in the offspring of pregnant women with overt DM prior to conception was four to eight times higher in the previous studies (Moore 2004); thus, our previous study may reflect recent progress in the specific medical care of diabetic pregnant women (Bánhidy *et al*. 2010). The objective of this study was to check whether recently introduced periconceptional folic acid supplementation may contribute to the reduction of CAs in the offspring of pregnant women with DM-1, because folic acid diminished diabetes-induced embryotoxicity in rats (Gareskog *et al*. 2006). The population-based dataset of the Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA), 1980–1996 (Czeizel *et al*. 2001) seemed to be appropriate to reply to this question.

MATERIALS AND METHODS

Subjects: cases and controls

Cases were selected from the dataset of the Hungarian Congenital Abnormality Registry (HCAR), 1980–1996 (Czeizel 1997) for the HCCSCA as described previously (Czeizel *et al*. 2001; Bánhidy *et al*. 2010).

Two main categories of cases with CAs were differentiated: isolated (only one organ is affected) and multiple (concurrence of two or more CAs in the same person affecting at least two different organ systems) (Czeizel 2009b). The total (birth + fetal) prevalence of cases with CA diagnosed from the second trimester of pregnancy through the age of one year was 35 per 1000 informative offspring (live-born infants, stillborn fetuses and electively terminated malformed fetuses) in the HCAR, 1980–1996 (Czeizel 1997), and about 90% of major CAs was recorded in the HCAR during the 17 years of the study period (Czeizel *et al*. 1993).

Controls were indentified and selected from the National Birth Registry of the Central Statistical Office for the HCCSCA. Controls were defined as newborn infants without CA. In general, two controls were matched to every case according to sex, birth week, and district of parents' residence.

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Collection of maternal, particularly exposure data

Prospective and medically recorded data

Mothers were asked to send us the prenatal maternity logbook and other medical records, particularly discharge summaries in an explanatory letter. Prenatal care was mandatory for pregnant women in Hungary (if a pregnant woman did not visit prenatal care clinic, she did not receive a maternity grant and leave); thus, nearly 100% of pregnant women visited prenatal care clinics, on an average of seven times during each pregnancy. The first visit was between the 6th and 12th gestational weeks when obstetricians recorded all pregnancy complications, maternal diseases and related drug prescriptions in the prenatal maternity logbook. The protocol of Hungarian prenatal care includes checking the blood glucose level of pregnant women.

Retrospective maternal information

A structured questionnaire, along with a list of drugs, pregnancy supplements and diseases, plus a printed informed consent form were also mailed to the mothers immediately after the selection of cases and controls. Mothers were asked to fill in the questionnaire after reading a list of medicinal products and diseases as a memory aid and provide a signature for the informed consent form.

The mean ± SD time elapsed between birth or pregnancy termination and return of the 'information package' (questionnaire, logbook, etc.) in our prepaid envelope was 3.5 ± 1.2 and 5.2 ± 2.9 months in the case and control groups, respectively,

Supplementary data collection

Regional nurses were asked to visit all non-respondent case mothers at home to help mothers to fill in the questionnaire, evaluate available medical records and obtain data regarding the lifestyle (smoking and drinking habits, and elicit drug use during the study pregnancy) through a cross-interview of mothers and their male partners or relatives if they were living with them. Regional nurses could visit only 200 non-respondent and 600 respondent control mothers as part of two validation studies (Czeizel *et al*. 2003; Czeizel and Vargha 2004) because the committee on ethics considered that it would be disturbing to the parents of all healthy controls to be visited. Regional nurses used the same method as in non-respondent case mothers.

The necessary information was available in 96.3% of cases (84.4% from reply to the mailing, 11.9% from the nurse visit) and in 83.0% of the controls (81.3% from reply, 1.7% from visit).

Gestational time was calculated from the first day of the last menstrual period. The critical period of most major CAs is in the second and/or third gestational month (Czeizel 2008; Czeizel *et al*. 2008).

Related drug treatments and use of folic acid or folic acid containing multivitamin supplements were also evaluated (Czeizel 2009a). Only one type of 3 mg folic acid tablet was available in Hungary during the study period. Among other potential confounding factors, maternal age, birth order, and marital and employment statuses as indicators of socio-economic status (Puho et al. 2004) were evaluated.

Diagnostic criteria of DM-1

DM-1 was recorded in the prenatal maternity logbooks in all pregnant women in the HCCSCA, and all pregnant women with DM-1 were treated with insulin, because pregnant women with DM-1 had low to absent insulin level and acute or subacute appearance of symptoms of DM-1, thus, they needed insulin treatment for life. The onset of DM-1 is predominantly under 30 years of age with a peak of 9 years in general in non-obese patients who are prone to ketosis (Moore 2004).

Pregnant women with specified diagnosis of DM-2 or unspecified DM diagnosis before the conception of the study pregnancy without insulin treatment were excluded from the study. The diagnosis of gestational DM was based on the recognition of DM during the study pregnancy; these pregnant women were also excluded from the study.

Statistical analysis

SAS version 8.02 (SAS Institute, Cary, NC, USA) was used for statistical analyses of data. First, the main maternal variables were evaluated in case and control pregnant women using Student's *t*-test for quantitative while χ^2 was used test for categorical variables at the comparison of pregnant women with DM-1 and without DM as reference. The prevalence of other maternal diseases, drug intake and pregnancy supplement, particularly folic acid and folic acid containing multivitamins during pregnancy, in addition to pregnancy complications were compared between the group of case and control mothers with DM-1 using odds ratios (OR) with 95% confidence intervals (CI). We compared the occurrence of DM-1 during the study pregnancy in specific CA groups of cases and in all matched controls, and adjusted OR with 95% CI were evaluated in conditional logistic regression models. Confounding variables were analyzed by comparing the OR for DM-1 in the models with and without inclusion of the potential confounding variables. Maternal age (<20 years, 20–29 years, and 30 years or more), birth order (first delivery or one or more previous deliveries), employment status, and maternal hypertension (yes/no) were included in the model as potential confounders. Finally, the distribution of specified CA-groups was evaluated in the offspring of pregnant women with DM-1 according to the folic acid/multivitamin supplementation during the study pregnancy.

RESULTS

The case group included 22 843 offspring with CA, and there were 79 (0.35%) pregnant women with DM-1. The control group comprised 38 151 newborns without CA, and 88 (0.23%) had mothers with DM-1 during the study pregnancy.

Of 79 diabetic case mothers, 40 (50.6%), while of 88 diabetic control mothers, 54 (61.4%) had folic acid supplementation during the study pregnancy (OR with 95% CI: 0.65, 0.33–1.25). The use of folic acid was lower in non-diabetic case (49.3%) and control (54.4%) mothers. Of 40 diabetic case mothers, 35 (87.5%), while of 54 diabetic control mothers, 49 (90.7%) had medically recorded folic acid supplementation in the prenatal maternity logbooks. These proportions were much higher than in non-diabetic pregnant women (about 67%). However, of 40 case mothers, only 23 (57.5%), while of 54 control mothers, only 36 (66.7%) had folic acid supplementation during the periconceptional period, including the critical period (i.e. the second and/or third gestational months) of major CAs. Our validation study showed that 89% and 11% of diabetic women used one or two folic acid tablets, respectively; therefore, the estimated daily dose was 3.3 mg. There was no difference in case and control diabetic mothers in this aspect. The distribution of folic acid users was different in non-diabetic pregnant women: 22%, 69% and 9% of women used one, two and three folic acid tablets, respectively; therefore, the estimated daily dose was 5.6 mg. Folic acid containing micronutrient combinations, so-called multivitamins were

also planned to be evaluated in the study, however, these multivitamins were not used by 79 case diabetic women while of 88 control diabetic mothers, 7 (8.0%) used multivitamins, therefore diabetic pregnant women with multivitamin supplementation were excluded from this analysis.

The characteristics of pregnant women with DM-1, as well as with or without folic acid supplementation are shown in Table 1. The mean maternal age was higher in the DM-1 groups than in the reference sample, with a somewhat higher mean birth order. The distribution of employment status indicated a better socioeconomic status of pregnant women with DM-1.

Folic acid supplemented diabetic mothers were somewhat younger with lower mean birth order than diabetic mothers without folic acid use. The proportion of professional women was three times higher in diabetic case mothers with folic acid use than in diabetic case mothers without folic acid supplementation; however, a similar difference was not seen in the subgroups of diabetic control mothers.

Among other maternal diseases, only essential hypertension showed a higher prevalence in case and control diabetic pregnant women (16.5% and 18.2%) than in non-diabetic pregnant women (6.9%). Thus, the use of antihypertensive drugs, beyond insulin, showed an obvious difference between diabetic and non-diabetic pregnant women.

The estimation of risk for different CAs in the offspring of pregnant women with DM-1 compared to the rate of CAs in the offspring of non-diabetic pregnant women is shown in Table 2. Pregnant women with DM-1 had a higher risk of total rate of CAs in their offspring (OR with 95% CI: 1.5, 1.1–2.0), explained mainly by three isolated CA groups: isolated renal a/dysgenesis, obstructive CA of the urinary tract (including 2 cases with cystic dysplasia), cardiovascular CA (including 12 cases with ventricular septal defect, but the second most common CA was transposition of great vessels in 5 cases) and by the group of multiple CAs.

However, the major objective of the study was to check whether folic acid supplementation during pregnancy can reduce the risk of DM-related CA; therefore, pregnant women with DM-1 were differentiated into two subgroups: with or without folic acid supplementation during the study pregnancy (Table 2). The total rate of CAs was not higher in the offspring of diabetic pregnant women with folic acid supplementation during pregnancy (OR with 95% CI: 1.1, 0.7–1.7). There was no offspring with neural-tube defect and renal a/dysgenesis in the folic acid supplemented subgroups, and only one case was affected with obstructive CAs of the urinary tract and cleft lip with or without cleft palate. The risk of cardiovascular CAs and multiple CAs has remained higher in the subgroups of diabetic pregnant women with folic acid supplementation. However, the total rate of CAs was significantly higher in the offspring of diabetic pregnant women without folic acid supplementation (OR with 95% CI: 1.7, 1.1–2.7), explained mainly by the higher rate of renal a/dysgenesis, obstructive CAs of urinary tract, cleft lip with or without cleft palate and cardiovascular CAs.

Finally, it would be necessary to evaluate the effect of folic acid according to the time of supplementation because the critical period of major CAs is during the second and/or third gestational month; therefore, a beneficial preventive effect of folic acid can be expected only with the use of folic acid during this time window. Table 3 shows the effect of folic acid supplementation (i) during the second and/or third gestational month (nearly all pregnant women continued folic acid use later in their pregnancy) (ii) in 4–9 months (late onset) and (iii) no use of folic acid. Unfortunately, the numbers are too low for appropriate statistical analysis. Neural-tube defects and renal a/dysplasia did not occur in the offspring of mothers with folic acid supplementation; therefore, there was no chance to differentiate these cases according to the time of folic acid use. Of 2 cases with cleft palate and of 4 cases with obstructive CA of urinary tract, none occurred in the children of diabetic pregnant women with folic acid supplementation during the second and/or third gestational months. However, of 4 cases with cleft lip with or without cleft palate, 1 was recorded in the child of diabetic pregnant woman with folic acid supplementation in the critical period of this CA. Obviously folic acid had no preventive effect for isolated cardiovascular and other isolated CAs, as well as multiple CAs during its use in the second and/or third gestational months.

DISCUSSION

The maternal teratogenic effect of DM, particularly DM-1 is well-known due to the poor metabolic control in pregnant women because there is no higher risk of CA in the children of diabetic fathers and normoglycemic pregnant women. In our previous study a higher rate of isolated renal a/dysgenesis, obstructive CAs of urinary tract and cardiovascular CAs, particularly transposition of great vessels and the specific multiple CA (caudal dysplasia sequence) was found in the offspring of pregnant women with DM-1 (Bánhidy *et al*. 2010). However, the lower risk (1.5-fold) found in this study than was expected (4 fold) based on previous reports suggested the beneficial effect of the recent progress in the special medical care of diabetic pregnant women.

The periconceptional low (0.4 mg) doses of folic acid (Berry *et al*. 1999) or folic acid (0.8 mg) containing multivitamin (Czeizel and Dudas 1992) supplementation can reduce significantly the first occurrence of neural-tube defects. In addition, as the Hungarian randomized controlled and cohort controlled trials showed, periconceptional folic acid-containing multivitamin supplementation was able to reduce the occurrence of cardiovascular (particularly conotruncal) and urinary tract (mainly obstructive) CAs (Czeizel 1996; Czeizel *et al*. 2004). However, this primary preventive method was not able to reduce the occurrence of multiple CAs (Czeizel and Medvecki 2003). The evaluation of population-based case-control dataset in Hungary showed that high doses of folic acid reduced the birth prevalence of isolated cardiovascular CAs (Czeizel *et al*. 1996) and orofacial clefts (Czeizel *et al*. 1999), but not the occurrence of multiple CAs (Czeizel *et al*. 2006). Thus, the objective of this study was whether the recent use of folic acid in diabetic pregnant women has contributed to the diminished risk of CAs in pregnant women with DM-1.

However, the reply to question expressed in the title of this paper is controversial. On the one hand the first approach of the study showed the beneficial effect of folic acid for the reduction of DM-related CAs in the offspring of pregnant women with DM-1. There was a lower risk of total CAs, in addition neural-tube defects and renal a/dysgenesis did not occur in the offspring of diabetic pregnant women after folic acid use during pregnancy. However, when the effect of folic acid supplementation was evaluated during the second and/or third gestational months (i.e. the critical period of major CAs), our hypothesis was not confirmed, partly due to the limited number of cases with CA. Obviously there was no reduction in the rate of caudal dysplasia sequence in agreement with the lack of reduction of multiple CAs after the periconceptional use of folic acid-containing multivitamin or high dose of folic acid (Czeizel and Medvecki 2003, Czeizel *et al*. 2006).

Table 1 Characteristics of case and control mothers with diabetes mellitus type 1 (DM-1), in addition to diabetic pregnant women with or without folic acid (FA) use during pregnancy

	Case mothers				Case mothers with DM-1				Control mothers				Control mothers with DM-1			
	No DM		DM-1		without FA		with FA		No DM		DM-1		without FA		with FA	
Variable	(N = 22 567)		(N = 79)		(N = 39)		(N = 40)		(N = 37 693)		(N = 88)		(N = 34)		(N = 54)	
Quantitative	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Maternal age (years)																
19 or less	2 484	11.0	3	3.8	1	2.6	2	5.0	3 256	8.6	2	2.3	1	2.9	1	1.9
20–29	15 430	68.4	55	69.6	26	66.7	29	72.5	27 308	72.4	50	56.8	19	55.9	31	57.4
30–34	3 222	14.3	15	19.0	7	17.9	8	20.0	5 209	13.8	20	22.7	8	23.5	12	22.2
35 or more	1 431	6.3	6	7.6	5	12.8	1	2.5	1 920	5.1	16	18.2	6	17.6	10	18.5
Mean (SD)	25.4	5.3	27.8	5.3	28.6	6.1	27.1	4.3	25.4	4.9	28.7	5.4	29.0	5.8	28.6	5.2
Birth order																
1	10 586	46.9	33	41.8	15	38.5	18	45.0	17 987	47.7	41	46.6	18	52.9	23	42.6
2	7 641	33.9	31	39.2	16	41.0	15	37.5	14 125	37.5	29	33.0	9	26.5	20	37.0
3 or more	4 340	19.2	15	19.0	8	20.5	7	17.5	5 581	14.8	18	20.5	7	20.6	11	20.4
Mean (SD)	1.9	1.1	2.0	1.2	2.0	1.3	1.9	1.2	1.7	0.9	1.8	1.0	1.9	1.9	1.8	0.9
Categorical	No.	%	No.	%					No.	%	No.	%				
Unmarried	1 254	5.6	6	7.6	3	7.7	3	7.5	1 454	3.9	3	3.4	2	5.9	1	1.9
Employment status																
Professional	1 953	8.7	8	10.1	2	5.1	6	15.0	4 362	11.6	14	15.9	6	17.6	8	14.8
Managerial	5 023	22.3	23	29.1	11	28.2	12	30.0	10 131	26.9	25	28.4	10	29.4	15	27.8
Skilled worker	6 440	28.5	13	16.5	6	15.4	7	17.5	11 766	31.2	31	35.2	12	35.3	19	35.2
Semiskilled worker	4 152	18.4	12	15.2	9	23.1	3	7.5	6 103	16.2	5	5.7	1	2.9	4	7.4
Unskilled worker	1 753	7.8	7	8.9	3	7.7	4	10.0	2 167	5.7	6	6.8	3	8.8	3	5.6
Housewife	2 380	10.5	9	11.4	3	7.7	6	15.0	2 329	6.2	4	4.5	1	2.9	3	5.6
Other	866	3.8	7	8.9	5	12.8	2	5.0	835	2.2	3	3.4	1	2.9	2	3.7

Table 2 Estimation of risk for different congenital abnormalities (CAs) in the offspring of pregnant women with or without diabetes mellitus type 1 (DM-1) (as reference) and diabetic pregnant women with or without folic acid supplementation

		DM-1			Folic acid supplementation					No folic acid supplementation				
					No. diabetic		DM-1			No. diabetic		DM-1		
Study group	Grand total No.	No.	%	OR (95% CI)‡	No.	%	No.	%	OR (95% CI)	No.	%	No.	%	OR (95% CI)
Controls	38 151	88	0.23	Reference	20 518	54.4	54	0.14	Reference	17 175	45.5	34	0.09	Reference
Isolated CAs														
Neural-tube defects	1202	3	0.25	1.1 (0.3–3.4)	528	43.9	0	0.00	– (–)	674	56.1	3	0.25	2.3 (0.7–7.4)
Cleft lip with or without cleft palate	1375	5	0.36	1.6 (0.6–3.9)	679	49.4	1	0.07	0.5 (0.1–3.3)	696	50.6	4	0.29	**2.9 (1.0–8.3)**
Cleft palate	601	3	0.50	2.2 0.7–6.8	286	47.6	1	0.17	1.0 (0.1–7.4)	315	52.4	2	0.33	3.3 (0.8–13.6)
Renal a/dysgenesis	126	3	2.38	**10.4 (3.3–33.5)**	61	48.4	0	0.00	– (–)	65	51.6	3	2.38	**24.7 (7.4–82.5)**
Obstructive CAs of urinary tract	343	4	1.17	**5.2 (1.9–14.3)**	161	46.9	1	0.29	1.8 (0.2–13.0)	182	53.1	3	0.87	**8.5 (2.6–28.1)**
Hypospadias	3038	7	0.23	1.0 (0.5–2.2)	1474	48.5	5	0.16	1.0 (0.4–2.6)	1564	51.5	2	0.07	0.7 (0.2–2.7)
Undescended testis	2052	3	0.15	0.6 0.2–2.0	1062	51.8	2	0.10	0.6 (0.2–2.7)	990	48.2	1	0.05	0.5 (0.1–3.8)
Ear CAs	354	2	0.56	2.4 (0.6–10.0)	190	53.7	2	0.56	3.9 (0.9–6.2)	164	46.3	0	0.00	– (–)
Cardiovascular CAs	4480	26	0.58	**2.5 (1.6–3.9)**	2175	48.6	14	0.31	**2.1 (1.1–3.5)**	2305	51.4	12	0.27	**2.7 (1.4–5.2)**
Clubfoot	2425	5	0.21	0.9 (0.4–2.2)	1216	50.1	3	0.12	0.8 (0.2–2.5)	1209	49.9	2	0.08	0.8 (0.2–3.5)
Poly/syndactyly	1744	3	0.17	0.7 (0.2–2.4)	909	52.1	1	0.06	0.4 (0.1–2.8)	835	47.9	2	0.11	1.2 (0.3–5.1)
Other isolated CAs	3754	6†	0.16	0.7 (0.2–2.9)	1907	50.8	3	0.08	0.5 (0.2–1.7)	1847	49.2	3	0.08	0.8 (0.3–2.7)
Multiple CAs	1349	9	0.67	**2.9 (1.5–5.8)**	631	46.8	7	0.52	**3.1 (1.4–6.9)**	718	53.2	2	0.15	1.4 (0.3–5.9)
Total	22 843	79	0.35	**1.5 (1.1–2.0)**	11 124	49.3	40	0.18	1.1 (0.7–1.7)	11 443	50.6	39	0.17	**1.7 (1.1–2.7)**

†Congenital stenosis of trachea 1, oesophageal atresia 1, rectal stenosis 1, limb deficiency 1, congenital hiatus hernia 1, torticollis 1; ‡Adjusted for maternal age and employment status, birth order and hypertension-related drugs.

Bold numbers show significant associations.

Table 3 Number (%) of cases born to pregnant women with diabetes mellitus type 1 (DM-1) according to the time-window of folic acid supplementation during the study pregnancy

	Folic acid use						
	No folic acid		1–3 months		4–9 months		Total
Study group	No.	%	No.	%	No.	%	No.
Controls	34	38.6	36	40.9	18	20.5	88
Isolated CAs							
Neural-tube defects	3	100.0	0	0.0	0	0.0	3
Cleft lip with or without cleft palate	4	80.0	1	20.0	0	0.0	5
Cleft palate	2	67.7	0	0.0	1	33.3	3
Renal a/dysgenesis	3	100.0	0	0.0	0	0.0	3
Obstructive CAs of urinary tract	3	75.5	0	0.0	1	25.0	4
Cardiovascular CAs	12	46.2	9	34.6	5	19.2	26
Other isolated CAs	10	38.5	10	38.5	6	23.1	26
Multiple CAs	2	22.2	3	33.3	4	44.4	9
Total	39	49.4	23	29.1	17	21.5	79

Thus the seemingly beneficial effect of folic acid for the reduction of CAs in the offspring of pregnant women with DM-1 may be an indirect association explained by the higher socioeconomic status and better medical care of these pregnant women. The latter was indicated by their higher use of folic acid supplementation (particularly in diabetic control women) compared to non-diabetic pregnant women. Obviously other unevaluated confounders should also be considered. Nevertheless it is worth checking the possible preventive effect of folic acid for DM-related CAs in other larger observational studies or intervention trials because folic acid may be a promising contributor to the further progress of the medical care of diabetic pregnant women to reduce the risk of adverse birth outcomes

An important purpose of the Hungarian pre/periconceptional service including folic acid containing multivitamin supplementation introduced in 1984 was to provide a special care for diabetic pregnant women (Czeizel 1999). Of about 25 thousand women between 1984 and 2009, 144 pregnant women were diabetic and a folic acid-containing multivitamin (Elevit Prenatal; Roche/Bayer, Leverkusen, Germany) during the periconceptional period was able to reduce the maternal teratogenic effect of diabetes mellitus.

The strengths of the HCCSCA are the population-based and large dataset, including 167 pregnant women with prospectively and medically recorded DM-1 in an ethnically homogeneous Hungarian (Caucasian) population. Additional strengths are the differentiation of DM-1 from other types of DM, the matching of cases to controls without CAs; the knowledge of potential confounders. Finally the diagnosis of medically notified CAs was checked in the HCAR and later modified, if necessary, on the basis of recent medical examination within the HCCSCA.

The weakness of our study is the 0.23% prevalence of DM-1 that indicates an underascertainment of pregnant women with DM-1 in our study sample and the limited number of cases with different CAs avoided the more detailed analysis of the putative preventive effect of folic acid during the critical period of major CAs.

In conclusion our study was not able to prove that the lower risk of DM-related CAs in the offspring of diabetic pregnant women with folic acid supplementation can be explained by the direct CA preventive effect of folic acid, but this possible beneficial effect needs studies to improve further the medical care of diabetic pregnant women.

REFERENCES

Bánhidy F, Ács N, Puho HE, Czeizel AE (2010) Congenital abnormalities in the offspring of pregnant women with type 1, type 2 and gestational diabetes mellitus – a population-based case-control study. *Congenit Anom* **50**: 115–121.

Becerra JE, Khoury MJ, Cordero JF, Erickson JD (1990) Diabetes mellitus during pregnancy and the risk for specific birth defects: a population-based case-control study. *Pediatrics* **85**: 1–9.

Berry RJ, Li Z, Erickson JD, Wang H *et al*. (1999) Prevention of neural tube defects with folic acid in China-US Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med* **341**: 1485–1490.

Czeizel AE (1996) Reduction of urinary tract and cardiovascular defects by periconceptional multivitamin supplementation. *Am J Med Genet* **62**: 179–183.

Czeizel AE (1997) The first 25 years of the Hungarian Congenital Abnormality Registry. *Teratology* **55**: 299–305.

Czeizel AE (1999) Ten years experience in periconceptional care. *Eur J Obstet Gynecol Reprod Biol* **84**: 43–49.

Czeizel AE (2008) Specified critical period of different congenital abnormalities: a new approach for human teratological studies. *Congenit Anom* **48**: 103–109.

Czeizel AE (2009a) Periconceptional folic acid and multivitamin supplementation for the prevention of neural tube defects and other congenital abnormalities. *Birth Defects Res (Part A)* **85**: 260–268.

Czeizel AE (2009b) The estimation of human teratogenic/fetotoxic risk of exposures to drugs on the basis of Hungarian experience: a critical evaluation of clinical and epidemiological models of human teratology. *Expert Opin Drug Saf INFORMA Pharm Sci* **8**: 283–303.

Czeizel AE, Dudas I (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* **327**: 1832–1835.

Czeizel AE, Medvecki E (2003) No difference in the occurrence of multimalformed offspring after periconceptional multivitamin supplementation. *Obstet Gynecol* **102**: 1255–1261.

Czeizel AE, Vargha P (2004) Periconceptional folic acid/multivitamin supplementation and twin pregnancy. *Am J Obstet Gynecol* **191**: 790–794.

Czeizel AE, Dobo M, Vargha P (2004) Hungarian cohort-controlled trial of periconceptional multivitamin supplementation shows reduction in certain congenital abnormalities. *Birth Defects Res (Part A)* **70**: 853–861.

Czeizel AE, Intődy Z, Modell B (1993) What proportion of congenital abnormalities can be prevented? *Br Med J* **306**: 499–503.

Czeizel AE, Petik D, Vargha P (2003) Validation studies of drug exposures in pregnant women. *Pharm Drug Saf* **12**: 409–416.

Czeizel AE, Puho HE, Ács N, Bánhidy F (2008) Use of specified critical periods of different congenital abnormalities instead of the first trimester concept. *Birth Defects Res (Part A)* **82**: 139–146.

Czeizel AE, Puhó E, Bánhidy F (2006) No association between periconceptional multivitamin supplementation and risk of multiple congenital abnormalities. A population-based case-control study. *Am J Med Genet Part A* **140A**: 2469–2477.

Czeizel AE, Rockenbauer M, Siffel C, Varga E (2001) Description and mission evaluation of the Hungarian Case-Control Surveillance of Congenital Abnormalities, 1980–1996. *Teratology* **63**: 176–185.

Czeizel AE, Timás L, Sárközi A (1999) Dose-dependent effect of folic acid on the prevention of orofacial clefts. *Pediatrics* **104**: e66.

Czeizel AE, Tóth M, Rockenbauer M (1996) Population-based case-control study of folic acid supplementation during pregnancy. *Teratology* **53**: 345–351.

Ferencz C, Rubin JN, McCarter RJ, Clark EB (1990) Maternal diabetes and cardiovascular malformations: predominance of double outlet right ventricle and truncus arteriosus. *Teratology* **41**: 319–326.

Gareskog M, Ericksson UJ, Wentzel P (2006) Combined supplementation of folic acid and vitamin E diminishes diabetes-induced embryotoxicity in rats. *Birth Defects Res (Part A)* **76**: 483–490.

Greene MF (1999) Spontaneous abortions and major malformations in women with diabetes mellitus. *Semin Reprod Endocrinol* **17**: 127–136.

Kucera J, Lenz W, Maier W (1965) Missbildungen der Beine und der kaudalen Wirbelsaule bei Kindern diabetischer Mütter. *Dtsch Med Wochenschr* **90**: 901–905.

Loffredo CA, Wilson PD, Ferencz C (2001) Maternal diabetes: an independent risk factor for major cardiovascular malformations with increased mortality of affected infants. *Teratology* **64**: 98–106.

Martinez-Frias NL, Bemejo E, Rodriguez-Pinilla E *et al*. (1998) Epidemiological analysis of outcomes of pregnancy in gestational diabetic mothers. *Am J Med Genet* **78**: 140–145.

Milunsky A, Alpert E, Kitzmiller JL *et al*. (1982) Prenatal diagnosis of neural tube defects. The importance of serum alfa-fetoprotein in diabetic pregnant women. *Am J Obstet Gynecol* **142**: 1030–1032.

Molsted-Pedersen L, Tygstrup I, Pedersen J (1964) Congenital malformations in newborn infants of diabetic women. Correlation with maternal diabetes vascular complications. *Lancet* **1**: 1124–1126.

Moore TR (2004) Diabetes in pregnancy. In: Creasy RK, Resnik R, Iams JS, eds. *Maternal-Fetal Medicine*, 5th edn. Saunders, Philadelphia, pp. 1023–1061.

Nielsen GL, Norgard B, Puho E *et al*. (2005) Risk of specific congenital abnormalities in offspring of women with diabetes. *Diabet Med* **22**: 693–696.

Passarge E, Lenz W (1966) Syndrome of caudal regression in infants of diabetic mothers: observations of further cases. *Pediatrics* **37**: 672–675.

Puho HE, Métneki J, Czeizel AE (2004) Maternal employment status and isolated orofacial clefts in Hungary. *Cent Eur J Publ Health* **13**: 144–148.

Rusnak SL, Driscoll SG (1965) Congenital spinal anomalies in infants of diabetic mothers. *Pediatrics* **35**: 989–995.

Sheffield JS, Butler-Koster EL, Casey BM *et al*. (2002) Maternal diabetes mellitus and infants malformations. *Obstet Gynecol* **100**: 925–930.