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A population-based case–control teratologic study of nitrazepam, medazepam, tofisopam, alprazolum and clonazepam treatment during pregnancy

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Abstract

Objective: To study the association between nitrazepam, medazepam, tofisopam, alprazolum and clonazepam treatments during pregnancy and prevalence of different congenital abnormalities (CAs). *Materials and methods*: A matched case–control study using cases with CAs and population controls from the dataset of the nationwide Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA), 1980–1996. *Results*: Of 38,151 pregnant women who had babies without any defects (population control group), 75 (0.20%) were treated with these five benzodiazepines during pregnancy. Of 22,865 pregnant women who delivered offspring with CAs, 57 (0.25%) had benzodiazepine treatment. The occurrence of five benzodiazepine treatments during the second and third months of gestation, i.e. in the critical period for most major CAs did not show significant differences in matched case–control pairs. *Conclusion*: Treatment with five benzodiazepines studied during pregnancy did not present detectable teratogenic risk to the fetus in humans but the amount of information was limited for different CAs. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Benzodiazepines; Nitrazepam; Medazepam; Tofisopam; Alprazolum and clonazepam; Human teratogenic potential; Congenital abnormalities; Case–control analysis

1. Introduction

Benzodiazepines with tranquilizer, sedative, anticonvulsant, and muscle relaxant properties are among the frequently used drugs during pregnancy in Hungary [1]. Benzodiazepines cross the placenta and pharmacologically the fetus acts as a 'deep compartment' for benzodiazepines in which these chemicals are slow to accumulate but are also, with their active metabolites, eliminated slowly [2]. Benzodiazepines can accumulate therefore in fetal tissues after regular maternal ingestion and the teratogenic risk for children exposed to benzodiazepines particularly diazepam in utero has been reported [3–6]. Previously, we evaluated the teratogenic potential of diazepam [7,8] and chlordiazepoxide [9]. However, no epidemiologic studies of congenital abnormalities (CAs) in infants born to women who took nitrazepam, medazepam, tofisopam during pregnancy have been published [10]. We therefore examined the teratogenic effect of the above three benzodiazepines in the large population-based Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA) [11] between 1980 and 1996. In addition, the data of teratogenic analysis of two other benzodiazepines as alprazolam and clonazepam are also presented here.

2. Materials and methods

The Hungarian Congenital Abnormality Registry (HCAR) is a national-based registry of cases with CA [12]. Notification of CAs is compulsory for physicians, mainly obstetricians (practically all deliveries take place in inpatient obstetric clinics) and pediatricians (who are working in the neonatal units of inpatient obstetric clinics and various inpatient and outpatient pediatric clinics). Autopsy was obligatory for all infant deaths and usual in stillborn fetuses during the study period and pathologists sent a copy of the detailed autopsy report to the HCAR in lethal cases due to CA. The recorded total (birth + fetal) prevalences of cases with CA was 35 per 1000 informative offspring (liveborn

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infants, stillborn and selectively terminated malformed fetuses) and about 90% of major CAs were notified to the HCAR during 17 years of the study period.

The procedure of the HCCSCA included the following steps [11].

The first step was the identification of cases from the HCAR who were notified on the first three months after birth or termination of pregnancy (77% of the total dataset). Cases with isolated CAs and multiple CAs were included into the dataset of the HCCSCA. Three mild CAs (such as congenital dislocation of hip based on Ortolani click, congenital inguinal hernia, and hemangiomas), minor anomalies, (e.g. umbilical hernia), and CA-syndromes of Mendelian or chromosomal origin (such as Down syndrome) were excluded.

The second step was to ascertain appropriate controls. Two (or three between 1986 and 1992) newborn infants without CAs were matched to every case according to sex, birth week, and district of parents' residence from the National Birth Registry of the Central Statistical Office. The type of informative offspring was not matched, however, the proportion of stillborn and selectively terminated malformed fetuses was 1.7 and 0.3% in the group of cases, respectively [11], and the comparison of only liveborn infants did not change our results considerable.

The third step was to obtain exposure data from three sources. First, mothers were asked in the explanatory letter to send us the antenatal care logbook and all medical records concerning their diseases during pregnancy and the child's CA. Among respondents, antenatal care logbooks were available in 88.4% of cases and 91.8% of controls. Second, a structured questionnaire with a list of drugs and diseases were mailed immediately after the selection of cases and controls to their parents. The questionnaire requested information on, among others, drugs taken, pregnancy complications and maternal diseases during pregnancy according to gestational months. To standardize the answers, mothers were asked to read the enclosed lists of drugs and diseases as memory aid before they replied. Third, regional nurses were asked to visit and to question nonrespondent case families. Thus, information was available on 85.5% (73.6% from reply, 11.9% from house visit) of cases. The diagnosis of CAs was checked due to the recent medical records in the case group and 15.8% of cases were excluded. The response rate for controls was 68.9%, but regional nurses did not visit nonrespondent control families because the ethical committee considered this follow-up to be disturbing to the parents of healthy children. Only 200 (0.4%) nonrespondent control families were visited and questioned at home in the frame of a validation study.

The fourth step was the evaluation of benzodiazepine intakes in seven different aspects.

1. The *source of information:* Three sources were differentiated: (i) only data from the antenatal care logbook (antenatal care obstetricians are obliged to record all prescribed drugs for women concerning complications and diseases in the logbook) or other medical records; (ii) only maternal self-reported data from questionnaire (for drugs used for treatment of diseases unrelated to pregnancy are prescribed by general practitioners or other physicians, in addition drugs taken by the personal choice of pregnant women); (iii) data concordant from both medical records and questionnaire.
2. The *type of treatment:* Five benzodiazepines studied alone or in combination with other drugs. However, pregnancy supplements (vitamins, iron, calcium) were excluded from the analysis.
3. The *route of administration:* All benzodiazepines studied were only used orally in Hungary during the study period (Table 1).
4. *Dose:* The recommended daily doses (Table 1) were followed by most pregnant women.
5. *Duration of treatment:* The usual duration depended on the type of benzodiazepines, the usual duration is shown in Table 1.
6. *Gestational time:* This was calculated from the first day of the last menstrual period and three time intervals were considered. (i) First month of pregnancy, it is before the organ-forming period. The first two weeks are before conception, while the second 2 weeks comprise the pre- and implantation period with ''all-or-nothing'' rule, i.e. CAs cannot induce. (ii) The second–third months of gestation as the most sensitive, the so-called critical period for major CAs. (iii) The fourth–ninth

Table 1
Number of pregnant women with five oral benzodiazepine treatments in the HCCSCA, 1980–1996

Benzodiazepines	Dose of tablet (mg)	Recommended daily treatment (mg)	Duration of treatment	Cases ($N = 22865$)		Controls ($N = 38151$)		Crude OR 95% CI
				No.	%	No.	%	
Nitrazepam	10	10	3 Months	18	0.08	19	0.05	1.6 (0.8–3.0)
Medazepam	10	20–60	2 Months	18	0.08	17	0.04	1.8 (0.9–3.4)
Tofisopam	50	50–300	3 Weeks	13	0.06	34	0.09	0.6 (0.3–1.2)
Alprazolam	0.25, 0.5, 1.0	0.75–4.0	Continuous	10	0.04	3	0.01	5.6 (0.7–18.7)
Clonazepam	0.5, 1.0, 2.0	1–8	Continuous	4	0.02	4	0.01	1.7 (0.5–6.2)
Total treatments persons	–	–	–	63	0.28	77	0.20	1.4 (1.0–1.9)
	–	–	–	57	0.25	75	0.20	1.3 (0.9–1.8)

months of gestation. The time of benzodiazepine treatment was not known in two cases.

7. *Potential confounding factors:* Maternal age, birth order, acute and chronic maternal disorders and other drug uses were evaluated.

The fifth step was the *statistical analysis of data* using the STATA statistical software package [13]. First, the occurrence of benzodiazepine treatments was compared between the case and control group and crude odds ratios (OR) with 95% confidence interval (95% CI) were calculated. Second, source of information was compared using chi^2 test among study groups. Third, the differences of potential confounders were evaluated in the case and control groups. Fourth, the distribution of gestational months according to the start of benzodiazepine treatments was evaluated using chi^2 test. Fifth, the observed numbers of different CAs were compared with their expected numbers based on the dataset of the HCCSCA and OR with 95% CI were calculated. Sixth, the frequency of combined benzodiazepine treatments 24 CA groups by gestational time periods was evaluated in matched one case–one control pairs in the McNemar test and conditional logistic regression model was used for the calculation of adjusted OR with 95% CI for potential confounders. 1121 cases (4.9%) had no controls, they were replaced by extra controls chosen from the existing rest of 38,151 controls and they were also matched to each relevant case. A total of 4211 cases had two or three controls, only one was selected randomly.

3. Results

The evaluated dataset included 22,865 cases (69.7% of all reported informative offspring) and 38,151 (68.8%) controls. The study period covered 2,146,574 births in Hungary, hence 38,151 controls represent 1.8% of the Hungarian births. In the group of controls, 75 mothers (0.20%) were treated with one or two of benzodiazepines studied during pregnancy (Table 1). Of 22,865 malformed newborn infants or fetuses, 57 (0.25%) had mothers who received these benzodiazepine treatments. Two control and six pregnant women had two different benzodiazepine treatments, thus the number of treatments and persons was different. Of five

Table 2
More frequent potential confounders in the total case and control groups, in addition in treated case and control subgroups

Confounding factors	Cases				Controls				Statistical analysis for treated subgroups	
	Total dataset ($N = 22865$)		Treated ($N = 57$)		Total dataset ($N = 38151$)		Treated ($N = 75$)		t	P
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.		
Maternal age (year)	25.5	5.3	27.5	5.0	25.5	4.9	27.7	5.0	0.58	0.57
Birth order	1.9	1.1	2.0	1.0	1.7	0.9	2.2	1.2	0.71	0.48
	No.	%	No.	%	No.	%	No.	%	OR	95% CI
Acute maternal disorders (≥5)										
Influenza–common cold	4965	21.7	13	22.8	7054	18.5	17	22.7	1.0	0.4–3.1
Respiratory system	2092	9.1	9	15.3	3442	9.0	11	14.7	1.1	0.3–3.9
Urinary system	1574	6.9	3	5.3	2285	6.0	8	10.7	0.5	0.1–1.7
Genital organs	1540	6.7	3	5.3	2699	7.1	7	6.7	0.8	0.3–2.3
Others	1184	5.2	4	7.0	1577	4.1	4	5.3	1.2	0.4–4.2
Chronic maternal disorders (≥5)										
Psychiatric diseases	196	0.9	21	36.8	176	0.5	18	24.0	1.8	0.9–3.9
Epilepsy	152	0.7	5	8.8	194	0.5	4	5.3	1.5	0.8–1.6
Others	2.292	13.1	8	14.0	5857	15.4	11	14.6	0.9	0.5–1.6
Other drugs (≥5 in the treated subgroups)										
Acetylsalicylic acid	1001	4.4	4	7.0	1398	3.7	5	6.7	1.1	0.3–3.9
Allylestrenol	3486	15.2	9	15.8	5368	14.1	8	10.7	1.6	0.6–4.4
Aminophenazone	494	2.2	3	5.3	731	1.9	5	6.7	0.8	0.2–3.1
Aminophylline	1374	6.0	3	5.3	2286	6.0	9	12.0	0.4	0.1–1.5
Ampicillin	1624	7.1	6	10.5	2598	6.8	12	16.0	0.6	0.2–1.8
Clotrimazole	1641	7.2	3	5.3	3077	8.1	9	12.0	0.4	0.1–1.5
Diazepam	2746	12.0	20	35.1	4130	10.8	15	20.0	2.2	1.0–4.7
Drotaverine	2055	9.0	11	19.3	3484	9.1	9	12.0	1.8	0.7–4.6
Metronidazole	966	4.2	3	5.3	1415	3.7	7	9.3	0.5	0.1–2.0
Noraminophenazone	1383	6.0	5	8.8	1912	5.0	12	16.0	0.5	0.2–1.5
Penamecillin	1597	7.0	3	5.3	2246	5.9	6	8.0	0.6	0.3–1.9
Promethazine	3653	16.0	15	26.3	6033	15.8	21	28.0	0.9	0.4–2.0
Terbutaline	2351	10.3	11	19.3	4000	10.5	5	6.7	3.3	1.1–10.3

Table 3
Distribution of observed (Obs) number of different CAs after the use of five benzodiazepines and the comparison with their expected (Exp) numbers based on the HCCSCA; crude ORs with 95% confidence interval (95% CI) were calculated

Congenital abnormality (CA) groups	Exp. (%)	Nitrazepam				Medazepam				Tofisopam				Alprazolam				Clonazepam			
		Exp. no.	Obs. no.	OR	95% CI	Exp. no.	Obs. no.	OR	95% CI	Exp. no.	Obs. no.	OR	95% CI	Exp. no.	Obs. no.	OR	95% CI	Exp. no.	Obs. no.	OR	95% CI
Isolated Cas																					
Neural-tube defects	5.2	0.9	2	0.5	0.1–4.0	0.9	0	3.2	0.1–83.2	0.7	2	0.5	0.1–4.0	0.5	0	3.3	0.1–91.6	0.2	0	1.0	0.0–62.3
Cleft lip ± palate	6.0	1.1	1	1.0	0.1–10.6	1.1	1	1.0	0.1–10.6	0.8	0	3.2	0.1–87.1	0.6	0	3.3	0.1–91.6	0.2	1	0.3	0.0–8.5
Cleft palate	2.6	0.5	0	1.0	0.0–53.1	0.5	0	1.0	0.0–53.1	0.3	0	1.0	0.0–54.2	0.3	0	1.0	0.0–55.3	0.1	0	1.0	0.0–62.3
Limb deficiencies	2.4	0.4	2	0.2	0.0–4.0	0.4	0	1.0	0.0–53.1	0.3	1	0.3	0.0–8.3	0.2	0	1.0	0.0–55.3	0.1	0	1.0	0.0–62.3
Poly/syndactyly	7.6	1.4	1	1.0	0.1–10.6	1.4	1	1.0	0.1–10.6	1.0	2	0.5	0.1–4.0	0.8	1	1.0	0.1–11.4	0.3	0	1.0	0.0–62.3
Exomphalos/gastroschisis	1.0	0.2	0	1.0	0.0–53.1	0.2	0	1.0	0.0–53.1	0.1	0	1.0	0.0–54.2	0.1	1	0.3	0.0–8.3	0.0	0	1.0	0.0–62.3
Esophageal atresia/stenosis	0.9	0.2	0	1.0	0.0–53.1	0.2	0	1.0	0.0–53.1	0.1	0	1.0	0.0–54.2	0.1	0	1.0	0.0–55.3	0.0	0	1.0	0.0–62.3
Rectal–anal atresia/stenosis	1.0	0.2	1	0.3	0.0–8.3	0.2	1	0.3	0.0–8.3	0.1	0	1.0	0.0–54.2	0.1	0	1.0	0.0–55.3	0.0	0	1.0	0.0–62.3
Microcephaly	0.5	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–54.2	0.1	0	1.0	0.0–55.3	0.0	0	1.0	0.0–62.3
Hydrocephaly	1.4	0.3	0	1.0	0.0–53.1	0.3	0	1.0	0.0–53.1	0.2	0	1.0	0.0–54.2	0.1	0	1.0	0.0–55.3	0.1	0	1.0	0.0–62.3
CAs of eye	0.4	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–54.2	0.0	0	1.0	0.0–55.3	0.0	0	1.0	0.0–62.3
CAs of ear	1.5	0.3	0	1.0	0.0–53.1	0.3	0	1.0	0.0–53.1	0.2	0	1.0	0.0–54.2	0.2	0	1.0	0.0–55.3	0.1	0	1.0	0.0–62.3
Cardiovascular CAs	19.5	3.5	6	0.6	0.1–2.4	3.5	4	1.0	0.2–4.5	2.5	4	0.7	0.1–3.5	2.0	2	1.0	0.1–7.3	0.8	1	1.0	0.1–15.0
CAs of diaphagm	1.0	0.2	0	1.0	0.0–53.1	0.2	0	1.0	0.0–53.1	0.1	0	1.0	0.0–54.2	0.1	0	1.0	0.0–55.3	0.0	0	1.0	0.0–62.3
Pyloric stenosis	1.0	0.2	0	1.0	0.0–53.1	0.2	0	1.0	0.0–53.1	0.1	0	1.0	0.0–54.2	0.1	0	1.0	0.0–55.3	0.0	0	1.0	0.0–62.3
Intestinal atresia/stenosis	0.7	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–54.2	0.1	0	1.0	0.0–55.3	0.0	0	1.0	0.0–62.3
Renal agenesis	0.4	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–54.2	0.0	0	1.0	0.0–55.3	0.0	0	1.0	0.0–62.3
Cystic kidney	0.6	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–54.2	0.1	0	1.0	0.0–55.3	0.0	0	1.0	0.0–62.3
CAs of genital organs	0.5	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–53.1	0.1	0	1.0	0.0–54.2	0.1	0	1.0	0.0–55.3	0.0	0	1.0	0.0–62.3
Hypospadias	13.3	2.4	2	1.0	0.2–6.6	2.4	2	1.0	0.2–6.6	1.7	1	2.2	0.2–19.2	1.3	1	1.0	0.1–11.4	0.5	1	1.0	0.1–15.0
Undescended testis	9.0	1.6	0	5.6	0.3–125.4	1.6	2	1.0	0.2–6.6	1.2	1	1.0	0.1–11.0	0.9	1	1.0	0.1–11.4	0.4	0	1.0	0.0–62.3
Clubfoot	10.6	1.9	0	5.6	0.3–125.4	1.9	3	0.6	0.1–3.7	1.4	0	3.2	0.1–87.1	1.1	3	0.3	0.0–2.2	0.4	0	1.0	0.0–62.3
Other isolated CAs	6.0	1.1	2	0.5	0.1–4.0	1.1	2	0.5	0.1–4.0	0.8	1	1.0	0.1–11.0	0.6	1	1.0	0.1–11.4	0.2	0	1.0	0.0–62.3
Multiple CAs	6.9	1.2	1	1.0	0.1–10.6	1.2	2	0.5	0.1–4.0	0.9	1	1.0	0.1–11.0	0.7	0	3.3	0.1–91.6	0.3	1	0.3	0.0–8.5
Total	100.0	18.0	18	–	–	18.0	18	–	–	13.0	13	–	–	10.0	10	–	–	4.0	4	–	–

Table 4
Occurrence and adjusted odds ratio (OR) with 95% confidence interval (95% CI) of five combined benzodiazepine treatments studied by gestational time periods in the two study groups

Controls	First month				Second–third months				Fourth–ninth months				Entire pregnancy				Grand total No.
	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI	
	33	0.1	Referent		15	0.0	Referent		27	0.1	Referent		75	0.2	Referent		38151
Isolated CAs																	
Neural-tube defects	1	0.1	1.0	0.1–7.0	0	0.0	–	–	3	0.3	3.9	1.2–13.0	4	0.3	1.8	0.6–4.8	1192
Cleft lip ± palate	1	0.1	0.7	0.1–5.4	0	0.0	–	–	2	0.1	2.1	0.5–8.7	3	0.2	1.0	0.3–3.3	1369
Limb deficiencies	1	0.2	1.9	0.3–14.0	0	0.0	–	–	1	0.2	2.6	0.3–19.0	2	0.4	1.7	0.4–7.1	
Poly/syndactyly	2	0.1	1.3	0.3–5.5	1	0.1	1.5	0.2–11.1	1	0.1	0.9	0.1–6.5	4	0.2	1.2	0.4–3.3	545
Rectal-anal atresia/stenosis	1	0.5	4.4	0.6–32.3	1	0.5	10.4	1.3–79.8	0	0.0	–	–	2	0.9	4.1	1.0–17.1	220
Cardiovascular CAs	7	0.2	1.6	0.7–3.7	2	0.0	1.0	0.2–4.6	6	0.1	1.9	0.8–4.6	15	0.3	1.6	0.9–2.8	4467
Hypospadias	6	0.2	2.3	1.0–5.6	0	0.0	–	–	0	0.0	–	–	6	0.2	1.0	0.4–2.4	3033
Undescended testis	1	0.0	0.6	0.1–4.1	1	0.0	1.2	0.2–9.3	2	0.1	1.5	0.4–6.3	4	0.2	1.0	0.4–2.8	2048
Clubfoot	3	0.1	1.4	0.4–4.7	1	0.0	1.0	0.1–7.7	1	0.0	0.6	0.1–4.6	5	0.2	1.1	0.4–2.7	2420
Other isolated CAs	3	0.1	0.8	0.2–2.5	4	0.1	2.3	0.8–7.0	0	0.0	–	–	7	0.2	0.8	0.4–1.8	4277
Multiple CAs	3	0.1	1.9	0.6–6.4	1	0.1	1.4	0.2–10.9	1	0.1	0.9	0.1–6.4	5	0.3	1.5	0.6–3.7	1564
Total	27	0.1	1.4	0.9–2.3	11	0.0	1.2	0.6–2.7	17	0.1	1.1	0.6–2.0	57[a]	0.2	1.3	0.9–1.8	22865

[a] The time of benzodiazepine use was unknown in two cases.

benzodiazepines studied, two needs some comments. The OR was less than one after the use tofisopam, while OR was 5.6 in the alprazolam group because this drug had a four-time higher use in the case group than in the control group. However, these differences did not reach the level of significance.

Of 132 pregnant women who were treated with benzodiazepine, only 3 (2.3%) were treated with benzodiazepines alone (all of them belonged to cases), therefore benzodiazepines alone and benzodiazepines plus other drugs were grouped together.

The source of information concerning benzodiazepine treatment during pregnancy showed a same pattern in the study groups. Of 63 benzodiazepine treatments in the case group, 18 (28.6%), while of 77 benzodiazepine uses in the control group, 22 (28.6%) had medically recorded benzodiazepine treatments during pregnancy. The rest of benzodiazepine treatment was based on maternal self-reported information.

Pregnancy complications (as threatened abortion or preterm delivery) had similar occurrences in the case and control groups.

Potential confounders in the total datasets of cases and controls, in addition in cases and controls with benzodiazepine treatments are shown in Table 2. There was no difference in maternal age and birth order, in addition acute maternal diseases between the treated case and control subgroups. The much higher rates of psychiatric diseases and epilepsy in the treated subgroups compared with the total groups are explained by the major reasons of benzodiazepine treatment. However, there was no significant difference between the two treated groups though their proportion was somewhat higher among treated case pregnant. Among other drugs all but one had similar use in the treated case group than in the treated control group. This exception was terbutaline because it was used more frequently in the treated case group. It is worth mentioning the frequent combination of diazepam and benzodiazepines studied. The rarely used human teratogenic drugs (eg. valproic acid) had also a similar occurrence in the two study groups.

At the preliminary analysis of teratogenic potencial of nitrazepam, medazepam, tofisopam, alprazolam and clonazepam, the observed number of different CAs after benzodiazepine treatments studied during pregnancy and their expected numbers based on the dataset of the HCCSCA were compared (Table 3). Difference was not found.

The start of benzodiazepine use according to the month of gestation in the case and control groups showed no difference ($\chi^2_8 = 9.0$; $P = 0.34$) with a maximum in the first month, i.e. these treatments were started before the pregnancy studied. In general, this treatment was continued for a longer period of pregnancy. The occurrence of benzodiazepine treatments was also similar during the second and third months of gestation in the study groups ($\chi^2_1 = 0.01$; $P = 0.92$).

First, the occurrence of combined benzodiazepine treatments in 10 isolated and one multiple CA groups including two or more cases was compared to the figure of total control group as referent by gestational time periods and adjusted OR with 95% CI for confounding factors (maternal age, birth order, maternal disorders and other drug uses) were estimated (Table 4). Of 11 CA groups, no CA group had higher OR for benzodiazepine treatment studied during the entire pregnancy. After restricting the exposure time period to second and third months of gestation months, no CA group (including two or more cases) showed higher adjusted OR for benzodiazepine treatment.

Second, the occurrence of combined benzodiazepine treatments in 10 isolated and one multiple CA groups including two or more cases was compared in matched one case–one control pairs by gestational time periods and adjusted OR with 95% CI for confounding factors (maternal age, birth order, maternal disorders and other

Table 5
Results of McNemar analysis of case–control pairs and adjusted ORs with 95% confidence interval (95% CI) of five benzodiazepines studied during pregnancy[a]

Congenital abnormality (CA) groups	Case–control pairs									Entire pregnancy		Second–third months	
	No	No	Yes	No	No	Yes	Yes	Yes	Total	OR	95% CI	OR	95% CI
Isolated CAs													
Neural-tube defects	1184	(1191)	4	(0)	4	(1)	0	(0)	1192	1.0	0.3–3.9	0.3	0.0–8.2
Cleft lip ± palate	1366	(1369)	2	(0)	1	(0)	0	(0)	1369	2.0	0.2–22.1	–	–
Limb deficiencies	543	(545)	2	(0)	0	(0)	0	(0)	545	5.0	0.2–104.2	–	–
Poly/syndactyly	1722	(1729)	4	(1)	4	(0)	0	(0)	1730	1.1	0.3–4.7	3.0	0.1–73.7
Rectal-anal atresia/stenosis	218	(219)	2	(1)	0	(0)	0	(0)	220	5.0	0.2–104.2	3.0	0.1–73.7
Cardiovascular CAs	4444	(4465)	14	(2)	9	(0)	0	(0)	4467	1.6	0.7–3.6	5.0	0.2–104.2
Hypospadias	3019	(3031)	6	(0)	8	(2)	0	(0)	3033	0.8	0.3–2.2	0.2	0.0–4.2
Undescended testis	2036	(2045)	5	(1)	7	(2)	0	(0)	2048	0.7	0.2–2.3	0.5	0.1–5.1
Clubfoot	2409	(2416)	5	(1)	6	(3)	0	(0)	2420	0.8	0.3–2.7	0.3	0.0–3.2
Other isolated CAs	3915	(3920)	8	(5)	3	(1)	0	(0)	3926	2.7	0.7–10.1	5.0	0.6–42.8
Multiple CAs	1555	(1563)	5	(1)	4	(0)	0	(0)	1564	1.3	0.3–4.7	3.0	0.1–73.7

[a] The data of the second–third month of gestation are shown in brackets.

drug uses) were estimated (Table 5). Of 11 CA groups, no CA group had higher OR for benzodiazepine treatment studied during the entire pregnancy or in the second and third month of gestation. At the evaluation of only medically recorded drug exposure data, there was no CA group with a higher use of combined benzodiazepines during the entire pregnancy and in the second–third months of gestation.

4. Discussion

Our analyses did not indicate a teratogenic effect of five benzodiazepines studied during the second–third month of gestation, i.e. in the critical period for most major CA.

The strengths of the HCCSCA are (i) the large and (ii) population-based dataset, including 132 pregnant women with five benzodiazepine treatments studied, (iii) in a racially homogeneous (European–Caucasian) population. In addition (iv) the matching of cases and their controls, the knowledge (v) of exposure time of drugs studied and (vi) of potential confounders, (vii) the prospective and medically recorded benzodiazepine treatments in a certain part of women were important. (viii) Finally the diagnosis of reported CAs was checked and modified (if it was necessary) on the basis of recent medical records, thus the validity of CA-diagnosis is good. However, this dataset has also drawbacks. (i) The response rate for controls was 69% compared with the data in 74% of cases. (ii) In addition, there was an active follow-up for all nonrespondent case families while only 200 nonrespondent control families were visited at home and these two differences may result in a selection bias due to some asymmetry between datasets of cases and controls. However, families with or without response did not show a significant difference in the more frequently used drugs as benzodiazepines. (iii) In general, these benzodiazepines were prescribed for psychiatric diseases unrelated to pregnancy by general practitioners, therefore the data of antenatal logbooks had limited value. (iv) Most women with benzodiazepine use were treated with other drugs as well, though in general this proportion was similar in the case and control groups studied. (iv) The number of cases and controls was small in different benzodiazepine groups, thus it was necessary to combine these drugs.

The main rules of drug teratogenicity helped us to exclude the possible teratogenic potential of five benzodiazepines studied. (i) A higher drug use only in the ciritical period of CAs may indicate a causal association while its higher use before and/or after the critical period are against it. There was no higher use of benzodiazepines studied during the second and third months of pregnancy. (ii) Noxa specificity means that different drugs cause different CAs. Thus, a general increase of drug use in all or many CAs may reveal technical biases. There was no higher rate of one or more specific CAs after the use of benzodiazepines studied. (iii) Nearly all teratogens cause multiple CAs [14] as fetal alcohol, radiation, hydantoin, rubella, etc. syndrome. There was no higher rate of multiple CAs after maternal benzodiazepine treatments during pregnancy and five multimalformed infants had no specific pattern of CAs. (iv) Finally a somewhat higher OR for drug use during pregnancy may be the consequence of biologic interactions e.g. underlying maternal diseases [15].

Only *alprazolam* has become suspected teratogenicity on the basis of our data. However, the rate of CAs did not appear to be higher in two series of infants born to women treated with alprazolum during the first trimester of pregnancy. The study of St. Clair and Schirmer [16] included 276 liveborn infants of women reported to the manufacturer and 4.7% had CAs. Two additional exposed pregnancies were terminated due to antenatally diagnosed fetal defects. The study of Schick-Boschetto and Zuber [17] consisted of 128 liveborn infants of women with alprazolam treatment who consulted a teratogen information service and 3.9% of the infants had CAs. No characteristic pattern of CAs was seen in either series. A higher rate of CAs was not found among the offspring of rats and rabbits treated with *alprazolam* at a dose eight times that used in humans [18,19]. However, an increased frequency of minor skeletal anomalies and fetal mortality was observed in the rabbits after a higher doses of *alprazolam* which produced maternal toxicity [19]. *Clonazepam* is used as an anticonvulsant. Maternal use of *clonazepam* during pregnancy was not significantly increased in our previous study of 10,698 infants with CA [20]. CAs have been reported among the children of epileptic women who took *clonazepam* during pregnancy but the pattern of CAs did not represent a CA-syndrome similar to that seen with other anticonvulsants [21,22]. Studies in rats, mice, and rabbits suggest that treatment of pregnant women with *clonazepam* in usual therapeutic doses is unlikely to increase the children's risk of CAs greatly [23–27].

In conclusion, treatments with five benzodiazepines studied during second and third months of gestation, i.e. in the critical period for most major CAs did not cause detectable teratogenic risk in humans, but the amounts of information was limited for different CAs and it explains the very large confidence limits. Thus, it is a hypothesis generating, rather than hypothesis confirmation report.

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