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No association between vulvovaginitis-bacterial vaginosis, related drug treatments of pregnant women, and congenital abnormalities in their offspring - A population-based case-control study

Research Article

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Abstract: The possible association between prospectively and medically recorded vulvovaginitis-bacterial vaginosis (VV-BV) and different congenital abnormalities (CA) has not been studied. The data set of the population-based Hungarian Case-Control Surveillance of Congenital Abnormalities between 1980 and 1996 were evaluated, *i.e.* 22,843 pregnant women who had newborns or fetuses with congenital abnormality (cases) and 38,151 pregnant women who delivered newborn babies without any congenital abnormality (controls). The main outcome measures were different congenital abnormalities. Of 22,843 cases with CA, 1,536 (6.7%) had mothers with VV-BV, while of 38,151 matched controls without CA, 2,698 (7.1%) had mothers with VV-BV in the second and/or third gestational month of pregnancy. Nearly all pregnant women with VV-BV were treated during pregnancy, but a higher risk for the total group of CAs (adjusted POR with 95% CI: 0.95, 0.89-1.02) or any CA group was not found. In addition, the risk for total CAs was significantly lower in cases born to mothers with VV-BV and appropriate treatment than born to mothers with VV-BV but without treatment. Thus maternal VV-BV needs treatment during pregnancy as well, because it helps reduce the rate of preterm birth without a risk for CAs.

Keywords: *Vulvovaginitis-bacterial vaginosis • Congenital abnormalities • Population-based case-control study*

1. Introduction

Vulvovaginal infections are among the most common reason why women seek help of medical doctors [1]. The association between antenatal infection/inflammation and fetal tissue injuries particularly in the origin of preterm premature rupture of membranes, cerebral palsy, bronchopulmonary dysplasia is well-known [2]. Vulvovaginal infections/diseases are believed to account for 40% of preterm births [3-5]. The association of some sexually transmitted diseases such as herpes genitalis [6,7] or syphilis [8,9] with structural birth defects, *i.e.* congenital abnormalities (CA) was shown. However, as far as we know the possible association between vulvovaginitis-bacterial vaginosis (VV-BV) during pregnancy and CAs has not been analysed in controlled epidemiological studies [10]. On the other hand, the potential teratogenic effect of drugs used for the treatment of maternal VV-BV during pregnancy was frequently evaluated. For example the high dose of fluconazole [11,12] was found to be teratogenic in human beings.

Thus we decided to evaluate the large population-based data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities [13] regarding different CAs in the offspring of pregnant women affected with VV-BV with or without related treatment.

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2. Material and Methods

The protocol of the HCCSCA includes five steps.

2.1. The selection of cases with CAs

Cases with CA were selected from the Hungarian Congenital Abnormality Registry (HCAR) [14] for the HCCSCA. Notification of CAs is mandatory for physicians in cases from the birth until the first birthday to the HCAR; most CAs are reported by obstetricians (in Hungary practically all deliveries take place in inpatient obstetric clinics and birth attendants are obstetricians) or pediatricians (working at neonatal units of inpatient obstetric clinics as well as of various general and special, such as surgical, cardiologic, orthopaedic, etc. inpatient and outpatient pediatric 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 HCAR 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 HCAR since 1984. Thus malformed fetuses diagnosed during the second and third trimester of pregnancy are included in the data set of the HCAR. Isolated minor anomalies (e.g. hydrocele, umbilical hernia, minor hemangioma) were recorded in the HCAR, but they were not considered at the calculation of rates of cases with different CAs. The recorded total (birth and fetal) prevalence of cases with CA 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) and about 90% of major CAs were notified to the HCAR during the study period [15].

However, there were three exclusion criteria at the selection of cases from the HCAR for the HCCSCA. First, cases notified after three months of birth or pregnancy termination to the HCAR were not selected for the HCCSCA. These cases comprised 33% of all cases with CAs in the HCAR; however. the shorter time between birth or pregnancy termination and data collection increased the accuracy of information regarding the exposure data of the study pregnancy without undue loss of power. Second, three mild isolated CAs such as congenital dislocation/dysplasia of the hip based on Ortolani click, congenital inguinal hernia, and major hemangioma were excluded from the HCCSCA. Third, CA-syndromes caused by major gene mutations or chromosomal aberrations (*i.e.* preconceptional and not teratogenic) origins were also not included in the HCCSCA.

2.2. Selection of controls

Controls were defined as newborn infants without CA selected from the National Birth Registry of the Central Statistical Office for the HCCSCA. In general two controls 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. We increased the number of controls from 1986 to three, but unfortunately we did not have financial support for the third control after 1992.

2.3. Collection of exposure and confounder data

The *necessary exposure and confounder data* such as pregnancy complications, maternal disorders and related drug treatments were obtained from *three sources of information.*

2.3.1. Medically recorded prospective data

Mothers were informed about the objectives and benefits of the HCCSCA in a mailed explanatory letter. They were asked to send us the prenatal care logbook and other medical records (mainly discharge summaries) related to diseases and their treatment during the study pregnancy and their child's CA. 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 6 and 12 gestational weeks. The task of licensed obstetricians is to record all pregnancy complications, maternal diseases (e.g. VV-BV) and related drug prescriptions in the prenatal care logbook. If pregnant women were hospitalised, in general the discharge summary was also available.

2.3.2. Retrospective maternal information

A structured questionnaire together with a list of diseases and medicaments, and a printed informed consent were also 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 standardise the answers, mothers were asked to read the enclosed list of diseases including VV-BV, in addition medications to refresh their memory before replying to achieve as complete as possible collection of necessary data. Mothers were asked to give a signature for informed consent which authorized us to record the name and address of their children in the HCCSCA.

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.

2.3.3. 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 part of a validation study, as the ethics committee considered that this follow-up would be disturbing to the parents of healthy children [16]. Regional nurses helped mothers to fill in the same questionnaire and they evaluated available medical documents.

Finally 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 children without signed informed consent were deleted in the HCCSCA.

The procedure of data collection in the HCCSCA was changed in 1997 because since regional nurses visited and questioned all cases and controls, however, unfortunately these data have not been validated until now. It explains why the data set of 17 years between 1980 and 1996 is evaluated here.

2.4. The definition of maternal diseases studied

This step in the protocol of the HCCSCA is connected with maternal diseases and/or drug treatments studied because it is necessary to define these pathological conditions, diagnostic criteria, etc. The group of VV-BV included two main categories of acute infections/diseases of external genital organ of pregnant women:

Vulvovaginitis (*i.e.* vaginitis, vulvitis, inflammatory diseases of vulva and/or vagina) as a clinical syndrome comprises not only discrete vulvar and vaginal lesions, but also abnormal vaginal secretions [17]. Most cases of vulvovaginitis are due to specific microbiological agents.

Bacterial vaginosis, its diagnosis is based in Hungary on the presence of 3 of 4 Amsel's criteria [18]: (i) a homogeneous white adherent ("watery") vaginal discharge, (ii) vaginal fluid pH less than 4.5, (iii) release of a fishy odor on mixing 5-10% potassium hydroxide with vaginal secretion, *i.e.* amine test [19], and (iv) presence of vaginal epithelium cells covered with the so-called clue cells, *i.e.* obscured by bacteria on fresh wet mount [20]. Vaginosis is caused by bacteria or bacterial vaginosis-related organisms such as *Gardenella vaginalis, Mobiluncus, Atrobium vaginalis, anaerobes, Mycoplasma hominis.*

Pregnant women with vulvovaginitis and bacterial vaginosis were planned to include to the group of VV-BV in our data set. The diagnosis of VV-BV was based partly on retrospective maternal information, partly prospective medically recorded data. However, it is difficult to differentiate normal and intermediate or abnormal vaginal flora on Gram stain [21], therefore only prospectively and medically recorded VV-BV in the prenatal care logbook were evaluated in the study.

VV-BV with pelvic inflammatory diseases, abscess of Bartholin's gland and vulva, in addition genital herpes were excluded from the study. Gonococcal and syphilitic infections were not recorded in our case and control mothers.

VV-BV was evaluated according to six aspects.

a) With or without *identification of microbial causes.*

b-c) *Onset* and *duration* of VV-BV according to gestational month during the study pregnancy.

d) 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 CAs 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, as the most sensitive, the so-called critical period for most major CAs. (iii) The fourth through ninth months of gestation, *i.e.* pregnancy after the organforming period.

e) *Medication* used during pregnancy including administrative route (oral, parenteral, topical), dose, and duration of treatment.

f) *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.

2.5. The statistical analyses of data

Statistical analyses were performed using the software package SAS version 8.02 (SAS Institute Ins., Cary, North Caroline, USA). First, the occurrence of VV-BV during the study pregnancy was compared between case and control mothers 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 total and affected study groups. Third, the prevalence

Table 1. Maternal characteristics.

Variables	Vulvovaginitis-bacterial vaginosis (VV-BV)								
	Case mothers				Control mothers				Comparison of
	without VV-BV (N = 21,307)		with VV-BV (N = 1,536)		without VV-BV (N = 35,453)		with VV-BV (N = 2,698)		case and control mothers with VV-BV
Maternal age	No.	%	No.	%	No.	%	No.	%	
19 or less	2,329	10.9	177	11.5	3,030	8.6	247	9.2	
20 – 29	14,486	68.0	1,107	72.1	25,573	72.1	2,029	75.2	$X^2_2 = 7.1$, p = 0.03
30 or more	4,492	21.1	252	16.4	6,850	19.3	422	15.6	
Mean, S.D.	25.5	5.3	24.9	4.8	25.5	4.9	25.0	4.6	t = 0.6, p = 0.54
Birth order									
1	9,957	46.7	751	48.9	16,837	47.5	1,372	50.9	$X^2_1 = 1.5$, p = 0.22
2 or more	11,350	53.3	785	51.1	18,616	52.5	1,326	49.1	
Mean, S.D.	1.9	1.1	1.8	0.9	1.7	0.9	1.7	0.9	t = 3.3, p = 0.0009
Unmarried	1,194	5.6	75	4.9	1,356	3.8	115	4.3	$X^2_1 = 0.9$, p = 0.35
Employment status									
Professional	1,740	8.2	161	10.5	3,988	11.3	365	13.5	
Managerial	4,631	21.7	337	21.9	9,395	26.5	739	27.4	
Skilled worker	5,931	27.8	398	25.9	10,900	30.7	790	29.3	
Semiskilled worker	3,605	16.9	264	17.2	5,418	15.3	365	13.5	$X^2_6 = 67.5$, p<0.0001
Unskilled worker	1,404	6.6	99	6.5	1,738	4.9	121	4.5	
Housewife	2,011	9.4	117	7.6	1,919	5.4	119	4.4	
Others	1,985	9.3	160	10.4	2,095	5.9	199	7.4	

of other acute and chronic maternal diseases, drugs, and pregnancy supplements used during pregnancy were compared between the groups of case and control groups. Crude POR with 95% CI were evaluated. Fourth, the prevalence of VV-BV in different CA groups was compared with the frequency of these diseases in the mother of all matched controls, and adjusted PORs with 95% CI were evaluated in a conditional logistic regression model. The latter PORs were adjusted for maternal age (<25yr 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 other acute maternal diseases (as a dichotomous variable).

3. Results

CAs were evaluated in 22,843 cases and 1,536 (6.7%) had mothers affected with prospectively and medically recorded VV-BV. Of 1,536 case mothers, 215 (14.0%) were recorded specified vulvovaginal candidiasis, 190 (12.4%) trichomonal infection, 230 (15.0%) bacterial vaginosis, while the rest of VV-BV were unspecified.

The total number of births in Hungary was 2,146,574 during the study period. Thus 38,151 controls represented 1.8% of all Hungarian births. There were 2,698 (7.1%) mothers with prospectively and medically recorded VV-BV in the control group. The proportion of candidiasis (N: 723; 26.8%), trichomoniasis (N: 418; 15.5%) and bacterial vaginosis (N: 448; 16.6%) in control mothers showed some difference from case mothers with VV-BV.

There was no significant difference in the occurrence of VV-BV (crude POR with 95% CI: 0.94, 0.88-1.01) between case and control mothers.

VV-BV were recorded in pregnant women at their first visit in prenatal care clinics between the 6th and 12th gestational week.Thus the origin and the onset of VV-BV were likely before the conception or at least in early pregnancy.

Characteristic data of case and control mothers with VV-BV, in addition to mothers without these diseases as referent, are shown in Table 1. There was no difference in the mean maternal age between the study groups, though the distribution of age groups was different due

Table 2. Occurrence of frequently used drugs (more than 1% in case or control mothers) during the study pregnancy.

Drugs	Vulvovaginitis-bacterial vaginosis (VV-BV)								
	Case mothers				Control mothers				
	without VV-BV (N = 21,307)		with VV-BV (N = 1,536)		without VV-BV (N = 35,453)		with VV-BV (N = 2,698)		Comparison of case and control mothers with VV-BV
	No	%	No	%	No	%	No	%	POR (95% CI)
Allylestrenol	3,251	15.3	230	15.0	5,010	14.1	347	12.9	1.2 (0.9 – 1.4)
Aminophylline	1,264	5.9	110	7.2	2,101	5.9	183	6.8	1.1 (0.8 – 1.3)
Ampicillin	1,494	7.0	150	9.8	2,361	6.7	263	9.8	1.0 (0.8 – 1.2)
Clotrimazole	811	3.8	830	54.0	1,420	4.0	1,657	61.4	**0.7 (0.6 – 0.8)**
Diazepam	2,539	11.9	207	13.5	3,803	10.7	327	12.1	1.1 (0.9 – 1.4)
Drotaverine	1,894	8.9	160	10.4	3,204	9.0	284	10.5	1.0 (0.8 – 1.2)
Magnesiums	1,178	5.5	111	7.2	1,994	5.6	203	7.5	1.0 (0.8 – 1.2)
Metronidazole	399	1.9	565	36.8	582	1.6	834	30.9	**1.3 (1.1 – 1.5)**
Metamizole (dipyrone)	1,298	6.1	84	5.5	1,776	5.0	135	5.0	1.1 (0.8 – 1.4)
Nitrofurantoin	686	3.2	88	5.7	977	2.8	102	3.8	**1.5 (1.2 – 2.1)**
Penamecillin	1,492	7.0	104	6.8	2,074	5.9	172	6.4	1.1 (0.8 – 1.4)
Pholedrin	698	3.3	70	4.6	1,372	3.9	137	5.1	0.9 (0.7 – 1.2)
Promethazine	3,389	15.9	276	18.0	5,672	16.0	404	15.0	**1.2 (1.1 – 1.5)**
Sulfamethoxazole+ trimethoprim	322	1.5	29	1.9	396	1.1	47	1.7	1.1 (0.7 – 1.7)
Terbutaline	2,170	10.2	180	11.7	3,712	10.5	282	10.5	1.1 (0.9 – 1.4)

Bold numbers indicate significant associations

to mainly the lower proportion of women over 30 years in the groups of VV-BV. The mean birth order was lower in control mothers. The marital status did not show any difference between case and control mothers with VV-BV, but the distribution of their employment status was different due to the lower proportion of professionals and higher proportion of housewives (in Hungary, most housewives belong to the lowest socioeconomic status) among case mothers with VV-BV.

At the evaluation of pregnancy complications, only anemia was more frequent in pregnant women with VV-BV, if we compare case and control mothers with or without VV-BV together, the difference was significant (POR with 95% CI: 1.2, 1.1-1.3).

Among other acute maternal diseases, only the infectious diseases of urinary tract showed a higher rate in case mothers (N: 150; 9.8%) than in control mothers (N: 197; 7.3%) with VV-BV (POR with 95% CI: 1.4, 1.1-1.7). There was no difference in the prevalence of chronic maternal diseases such as diabetes mellitus and epilepsy among the study groups.

The occurrence of most frequently used drugs was evaluated in the next step (Table 2). The use of clotrimazole and metronidazole was much more frequent in case and control mothers with VV-BV than in mothers without VV-BV, in addition the treatment of ampicillin, nitrofurantoin and sulphamethoxazole + trimethoprim (co-trimoxazole) was more frequent in mothers with VV-BV. At the comparison of case and control mothers with VV-BV, the occurrence of metronidazole, nitrofurantoin and promethazine treatment was higher in case mothers.

There was no difference in the use of folic acid between case mothers (51.6% vs. 49.2%) or control mothers (55.0% vs. 54.4%) with and without VV-BV, but case mothers used folic acid less frequently than control mothers with VV-BV (POR with 95% CI: 0.8, 0.7-0.9). The use of folic acid-containing multivitamins did not show any difference among the study groups.

The objective of the study was to evaluate the possible association between VV-BV and different CAs. All case and control mothers were affected with VV-BV in the second and/or third month of pregnancy, *i.e.* the most sensitive, the so-called critical period for most major CAs. Table 3 summarizes the prevalence of maternal VV-BV in cases with 25 different CA groups, including 4 or more cases with specified CAs compared with the prevalence of VV-BV in the mothers of all (1-3) matched controls. A higher prevalence of maternal VV-BV was not found in any CA group at the calculation of adjusted POR with 95% CI. However, there were two CA groups: limb deficiencies and CAs of musculoskeletal system with a lower risk in babies of mothers with VV-BV during pregnancy.

Table 3. Results of multivariate analysis for each case group with different CAs and its all (1-3) matched controls using conditional logistic regression model to estimate adjusted prevalence odds ratio (POR) with 95% confidence interval (CI) of maternal VV-BV (vulvovaginitis-bacterial vaginosis) during the study pregnancy.

Study groups	Grand total	VV-BV		
	No.	No.	%	Adjusted POR* with 95% CI
Isolated CAs				
Neural-tube defects	1,202	86	7.2	1.2 (0.9 – 1.7)
Cleft lip± palate	1,374	113	8.2	1.1 (0.8 – 1.4)
Posterior cleft palate	582	31	5.3	0.8 (0.5 – 1.2)
Esophageal atresia/stenosis	217	17	7.8	1.3 (0.7 – 2.6)
Congenital pyloric stenosis	241	16	6.6	1.0 (0.5 – 2.0)
Intestinal atresia/stenosis	153	14	9.2	1.6 (0.7 – 3.6)
Rectal/anal atresia/stenosis	220	18	8.2	1.6 (0.7 – 3.6)
Renal a/dysgenesis	104	8	7.7	0.7 (0.3 – 1.8)
Obstructive urinary CAs	271	25	9.2	0.9 (0.5 – 1.6)
Hypospadias	3,038	193	6.4	0.9 (0.7 – 1.0)
Undescended testis	2,051	124	6.1	0.8 (0.7 – 1.1)
Exomphalos/gastroschisis	238	14	5.9	1.0 (0.5 – 2.0)
Microcephaly, primary	109	4	3.7	0.5 (0.2 – 1.8)
Congenital hydrocephaly	314	22	7.0	0.9 (0.5 – 1.7)
Eye CAs	99	6	6.1	0.6 (0.2 – 1.9)
Ear CAs	354	19	5.4	0.6 (0.3 – 1.1)
Cardiovascular CAs	4,479	278	6.2	0.9 (0.8 – 1.0)
CAs of genital organs	123	6	4.9	0.5 (0.2 – 1.4)
Clubfoot	2,424	152	6.3	0.9 (0.7 – 1.1)
Limb deficiencies	548	35	6.4	**0.6 (0.4 – 0.9)**
Poly/syndactyly	1,744	142	8.1	1.2 (0.9 – 1.6)
CAs of musculo-skeletal system	211	8	3.8	**0.4 (0.2 – 0.9)**
Diaphragmatic CAs	243	24	9.9	1.9 (0.9 – 3.7)
Other isolated CAs	1,155	86	7.5	1.2 (0.9 – 1.7)
Multiple CAs	1,349	95	7.0	1.1 (0.8 – 1.4)
Total cases	22,843	1,536	6.7	0.95 (0.89 – 1.02)
Total controls	38,151	2,698	7.1	reference

** Matched POR adjusted for maternal age (<25 yr vs. 25-29 yr vs. 30 yr or more) and employment status (professional-managerial-skilled worker vs. semiskilled worker-unskilled worker-housewife vs. others), birth order (first delivery vs. one or more previous deliveries) and drug uses in conditional logistic regression model;*
Bold numbers show significant associations.

Finally we evaluated the maternal VV-BV with or without appropriate treatment. Six drugs such as clotrimazole, metrodinazole, ampicillin, penamecillin, nitrofurantoin and co-trimoxazole (sulphamethoxazole + trimethoprim) were used frequently for the treatment of VV-BV in case and control mothers; the data from patients who used these drugs were evaluated separately and compared with the data of mothers with VV-BV who did not use treatment of these drugs. The risk for total CAs was significantly lower in the group of maternal VV-BV with appropriate treatment (OR with 95% CI: 0.85, 0.78-0.91) than in the group of maternal VV-BV without treatment (1.11, 0.90-1.32). In addition there were 4 CA groups with lower risk in cases born to mothers with VV-BV and treatment. Of these 4 groups, one: limb deficiencies (0.6, 0.4-0.9) was also found in the previous analysis without differentiation of treatment. However, 3 "new" CA groups: hypospadias (0.8, 0.7-0.9), cardiovascular CAs (0.8, 0.7-0.9), and multiple CAs (0.7, 0.6-0.9) also showed a lower risk in the babies of treated mothers. The previously found lower risk for CAs of musculoskeletal system (0.6, 0.3-1.2) disappeared. On the other hand there was no CA group with lower risk in cases born to mothers with VV-VB and without appropriate treatment

4. Discussion

As far as we know, this is the first controlled epidemiological study to evaluate the possible association between maternal VV-BV and different CAs in their offspring. Our population-based case-control study did not show a higher risk for total CAs or any CA in the newborns of pregnant women with VV-BV.

The strengths of the study are population-based large data set of the HCCSCA including 4,234 pregnant women with VV-BV, based on ethnically homogenous European (Caucasian) people. In addition the matching of cases and controls with prospectively and medically recorded VV-BV, the knowledge of onset of these diseases and confounders, and finally good validity of CA diagnoses due to the medically reported cases to the HCAR which were checked by the experts of CAs in the HCAR. New information from recent medical examinations and the questionnaire was also helpful to exclude cases with misdiagnosed CA or to correct the CA diagnosis in the HCCSCA [13,14].

However, our data set also has weaknesses:

(a) The diagnosis of VV-BV was based on the clinical symptoms without the identification of microbial agents in 59% of case and 41% of control pregnant women. In the recent years, significant progress has been made in developing specific diagnostic assays, e.g. monoclonal antibodies in the etiological diagnosis of VV-BV [21], however, these tests were not used in our pregnant women during the study period. In addition, some laboratory tests are time consuming and the results of these assays were not available at the collection of data in the HCCSCA.

(b) We were not able to evaluate the recently recognized pathological manifestation of vaginal infections such as aerobic vaginitis [22] and cytolytic vaginosis [23].

(c) About 7% of the pregnant women in our study had medically recorded VV-BV. This is lower than the average of our previous study based on the preconceptional screening of women in our periconceptional care clinic [24]. The explanation may be that only severe VV-BV were recorded in the prenatal care logbooks and/or some women with VV-BV were screened, diagnosed and treated before the first visit in the prenatal care clinics.

(d) Response rate was similar in controls (83%), and cases (82%) but all non-respondent case mothers were visited at home, while only 200 non-respondent control mothers were visited at home.

(e) The time between the end of pregnancy and return of the information package was longer in the group of controls than in the group of cases ($t=84.4$; $p<0.001$). The above two asymmetries may have resulted in a certain selection bias, however, all VV-BV were prospectively and medically recorded. Some part of drug treatments and other maternal diseases was based on maternal information, but there was no difference in the distribution and occurrence of frequently used drugs and diseases between respondent and non-respondent mothers in our validation study [16].

(f) Our study period covered 17 years between 1980 and 1996, thus we were not able to evaluate the recently introduced drugs in the treatment of maternal VV-BV.

Our study confirmed the well-known association between VV-BV and infectious diseases of urinary tract (cystitis, pyelitis, pyelonephritis) [25]. We have found an association of VV-BV with maternal anemia which may be connected with the lower socioeconomic status and the lower intake of folic acid (and iron) of mothers with VV-BV.

The main objective of our study was to check the possible association between VV-BV and CAs, and we did not find a higher risk for any CA. Thus localised infections and infectious diseases in the vagina during early pregnancy cannot disturb the organogenesis of the human embryo. In fact two CA groups had a lower risk, which means some “protective effect” of maternal VV-BV for limb deficiencies and CAs of musculoskeletal system. In the latter group, the lower rate of torticollis explains this beneficial effect. Torticollis occurs more frequently in preterm babies [26], and the appropriate treatment of maternal VV-BV can reduce the preterm births, thus preterm related CAs such a torticollis (*i.e.* deformity of sternocleidomastoid muscle as well). We have no reasonable hypothesis for the reduction of cases with limb deficiencies in the children of mothers with VV-BV. However, it is necessary to mention the chance effect because multiple comparisons may produce a non-causal association in every 20th estimation as a result of chance.

An important finding of the study is that there was a significantly lower risk for the total group of CAs and 4 CA groups in cases born to pregnant women with VV-BV and related treatment. Thus the treatment of pregnant women with most frequently used drugs for VV-BV during the study pregnancy did also not associate with a higher risk for any CA. This finding is an important argument against the teratogenic effect of metronidazole [27], clotrimazole [28], ampicillin [29], penamecillin [30] and nitrofurantoin [31]. On the other hand these drugs can reduce the maternal VV-BV related preterm births in their babies;therefore their use is important during early pregnancy as well.

In conclusion, our population-based case-control study did not indicate a teratogenic risk for any CA of maternal VV-BV and related drug treatments during pregnancy.

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