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Prenatal Exposure to Misoprostol and Vascular Disruption Defects: A Case-Control Study

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Prenatal exposure to misoprostol has been associated with Moebius and limb defects. Vascular disruption has been proposed as the mechanism for these teratogenic effects. The present study is a multicenter, case-control study that was designed to compare the frequency of prenatal misoprostol use between mothers of Brazilian children diagnosed with vascular disruption defects and matched control mothers of children diagnosed with other types of defects. A total of 93 cases and 279 controls were recruited in eight participating centers. Prenatal exposure was identified in 32 infants diagnosed with vascular disruption defects (34.4%) compared with only 12 (4.3%) in the control group ($P < 0.0000001$). Our data suggest that prenatal exposure to misoprostol is associated to the occurrence of vascular disruption defects in the newborns. Am. J. Med. Genet. 95:302–306, 2000. 2000 Wiley-Liss, Inc.

KEY WORDS: misoprostol; vascular disruption; Moebius sequence; limb defect

INTRODUCTION

Misoprostol is an orally active prostaglandin, which was originally marketed for the treatment of peptic ulcer. Although misoprostol can increase uterine contractility, it is not an effective abortifacient per se [Norman et al., 1991]. Still, in some countries like Brazil where abortions are illegal, misoprostol is sold over-the-counter and has been widely used as an abortifacient agent [Costa, 1998]. A spectrum of malformations ranging from scalp anomalies to Moebius sequence, arthrogryposis, abdominal wall defects, and limb reduction defects has been associated with prenatal exposure to misoprostol [Fonseca et al., 1991; Gonzalez et al., 1993, 1998; Castilla and Orioli, 1994; Genest et al., 1999]. One common pathogenetic mechanism that could theoretically explain the occurrence of such defects is vascular disruption during the first trimester of pregnancy. Such an event would be the product of either a vascular defect or rupture of amniotic membranes following exposure to misoprostol and failed abortion [Shepard, 1995; Orioli and Castilla, 2000].

Due to the clandestine character of termination of pregnancy in Brazil, the epidemiological analysis of such a problem is necessarily hampered by moral and legal issues that create a reporting bias of the use of misoprostol as an abortifacient. However, the impact of the publications of Moebius and limb deformity/reduction cases after maternal misoprostol use may have prompted physicians to investigate prenatal events more thoroughly when faced with such cases, as opposed to other types of congenital defects.

The main objective of this study was to compare the frequency of misprostol use during the first trimester of pregnancy between mothers of Brazilian children with congenital malformations belonging to the spectrum of

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vascular disruption defects and mothers of children with other kinds of congenital anomalies.

SUBJECTS AND METHODS

We considered eligible subjects for the study all children born in Brazil after 1992 (when misoprostol use as an abortifacient became widespread) with a structural congenital anomaly (isolated or multiple, syndromic or not), seen at one of eight participating clinical genetic centers during a 21-month study period. All children born before 1992 or with a diagnosis of a non-structural anomaly or disease were excluded. Cases were defined as all those children in which at least one of the malformations fit in the spectrum of vascular disruption defects (as defined below). To each case we randomly selected three controls from the sample of eligible patients but with congenital malformations classified as not to be caused by vascular disruption. Controls were matched to cases according to maternal age (± 2 year) and parity.

We developed a structured questionnaire that was applied to all eligible patients containing general demographic questions, obstetrical and genetic history, and detailed inquiry about exposures during the first trimester of pregnancy. Specifically, possible pregnancy termination attempts and misoprostol use were interrogated by open, semi-open, or closed questions.

Vascular disruption can be defined as structural anomalies resulting from damage to or interruption of normal embryonic or fetal development of the vasculature [van Allen, 1981]. For the purpose of this study, vascular disruption anomalies included the following: transverse terminal limb reductions; Moebius and/or Poland sequences; hypoglossia-hypodactyly sequence; arthrogryposis; intestinal atresia; hemifacial microsomia; microtia; and porencephalic cyst.

During the study period, 732 children were considered eligible, and the questionnaire was applied to their mothers. Of them, 93 patients fit the inclusion criteria for vascular disruption defects and were enrolled. From the 639 remaining we randomly selected the three matched controls for each case, totaling 279 controls.

The Mantel-Haentzel matched odds ratio was calculated using computer program EPI-Info 6.0. Initially the matched odds ratio was calculated considering all patients with vascular disruption defects as a whole. Subsequently we subdivided this group according to major diagnostic categories and calculated the matched odds ratio for each subgroup. Continuous data were compared using paired t-test, and categorical data were compared with χ^2 analysis or Fisher's exact test, whenever appropriate.

RESULTS

Our 93 cases of children with vascular disruption defects included seven different diagnostic categories (Table I). Moebius sequence (31.2%) and transverse limb reduction defects (29.0%) were the most common anomalies found. The control group was much more heterogeneous; minor malformations (21.5%), chromosomal anomalies (17.2%) and malformation syndromes (14.0%) were the most prevalent.

The mothers of cases and controls did not differ in age, parity, previous miscarriages, education, or consanguinity with the proband's father (Table II). The mothers of cases reported more previous attempted abortions. Conversely, mothers of controls lived more frequently with their partners and had significantly higher rates of recurrence of anomalies in their relatives, which was expected because the control group included many hereditary conditions.

The case and control group did not differ in maternal cigarette smoking, alcohol drinking, use of medications, or occurrence of fever during pregnancy. Mothers of cases admitted significantly more unplanned pregnancies and vaginal bleeding (Table III). Thirty-three mothers of the 93 (35.5%) in the vascular disruption group, and 28 of the 279 controls (10%) had tried to terminate their pregnancies ($P < 0.000001$). Most of these women tried the abortion using drugs or herbal teas. Almost one-half of them co-administered drugs with an herbal tea.

Misoprostol use was reported by 32 (34.4%) mothers in the study group but only 12 (4.3%) in the control group as the drug used for abortion attempt ($P < 0.0000001$) (Table IV). We did not have any case of use of misoprostol for peptic disease. The majority of the women in the study group were exposed for only 1 day. Approximately one-half of the women took the drug

TABLE I. Main Diagnostic Categories

Cases			Controls		
Diagnostic group	n	%	Diagnostic group	n	%
Moebius	29	31.2	Minor malformations	60	21.5
Transverse limb reduction	27	29.0	Chromosomal syndromes	48	17.2
Hemifacial microsomia	16	17.2	Malformation syndromes[a]	39	14.0
Arthrogryposis	9	9.7	Central nervous system defects[b]	34	12.2
Microtia	9	9.7	Cleft lip/palate	25	9.0
Porencephalic cyst	2	2.1	Combined malformations, unknown syndrome	19	6.8
Hypoglossia hypodactyly	1	1.1	Skeletal dysplasias	14	5.0
			Heart defects	12	4.3
			Other	28	10.0
Total	93	100.0	Total	279	100.0

[a]Excludes chromosomal disorders.
[b]Includes microcephaly.

TABLE II. Maternal Demographics

	Cases		Controls		
	n	%	*n*	%	*P*
Maternal age (mean ± SD)	25.7 ± 5.9		25.9 ± 5.9		NS
Gender of proband: masculine	51/93	55.9	138/279	49.5	NS
Parity					
1	51/87	58.7	157/274	57.3	NS
2	17/87	19.5	60/274	21.9	
3 or more	19/87	21.8	57/24	20.8	
Spontaneous abortions					
1 or more	12/88	13.7	40/260	15.4	NS
Attempted abortions					
1 or more	11/88	12.5	8/260	3.1	0.002
Maternal education					
Illiteracy	2/87	2.3	15/258	5.8	NS
Elementary	56/87	64.4	147/258	57.0	
High school	27/87	31.1	87/258	33.7	
University	2/87	2.2	9/258	3.5	
Lives with partner	58/89	65.2	212/261	81.3	0.002
Consanguinity	7/88	8.0	23/269	8.5	NS
Recurrence	4/88	4.5	37/261	14.2	0.015

orally, and another one-half combined oral and vaginal administration. Most of them used four pills or 800 μg (16/31), but one woman used only one pill and another one used 28 pills. The majority of the women (16/30) reported the use of misoprostol between the 5th to 8th week after the last menstrual period, and in two the exposure was beyond the first trimester.

Table V summarizes the odds ratio calculations for the entire group of vascular disruption as well as for specific groups of defects. The matched odds ratio (OR) for the entire group of vascular disruption was 22.0 with a 95% confidence interval (CI_{95}) of 7.3 to 81.3. The proportion of misoprostol exposure when we analyzed only the cases with Moebius sequence was 18/29 (62.1%) compared with only 6/87 (6.9%) in the controls, which gave a matched OR of 49.0 (CI_{95} = 7.07 to 1907). The same calculation for only the transverse limb reduction defect showed maternal exposure in 9/27 cases (33.3%) compared with 4/81 (4.9%) in the controls (matched OR = 24.0; CI_{95} = 3.0 to 99.1). The remaining subgroups were too small for separate calculations so we combined them together, including cases of arthrogryposis, microtia, hemifacial microsomia, porencephalic cyst, and hypoglossia-hypodactyly. The maternal use of misoprostol in this last group was 13.5% (5/37) compared with 1.8% in the control group (matched OR = 7.5; CI_{95} = 1.23 to 78.7).

DISCUSSION

In the present study we detected a very strong association between different kinds of congenital defects belonging to vascular disruption spectrum and first trimester use of misoprostol. A previous study based on hospital records detected a significantly greater frequency of first trimester misoprostol exposure in Moebius cases when compared with a control group of neural tube defects [Pastuszak et al., 1998]. There was no significant overlap between the Moebius cases studied by Pastuszak et al. [1998] and the ones included in our study, the exception being three Moebius cases (3/29) that came from the only one center common to both studies. Subsequently, Schuler et al. [1999] followed 86 pregnancies exposed to misoprostol and an equal number of matched controls and found no significant difference in the rates of major defects between both groups. Such results, however, might be due to the small sample size in this cohort. More recently Orioli and

TABLE III. Information About Pregnancy Events

	Cases		Controls		
	n	%	*n*	%	*P*
Fever	10/87	11.5	32/261	12.3	NS
Use of medications	83/91	91.2	241/264	91.3	NS
Cigarette smoking	25/91	27.5	60/259	23.2	NS
Alcohol	14/91	15.4	37/259	14.3	NS
Unplanned pregnancy	65/88	73.9	146/260	56.2	0.003
Vaginal bleeding	37/88	42.0	51/252	20.2	<0.0001
Tried to interrupt pregnancy	33/93	35.5	28/279	10.0	<0.000001
Interruption attempted by[a]					
Pharmacological	32/33	97.0	19/28	67.9	NS
Herbal teas	14/33	42.4	15/28	53.6	
Surgical method	0/33	0.0	0/28	0.0	

[a]Thirteen women used both pharmacological and herbal preparations in the case group and six in the control group.

TABLE IV. Misoprostol Use: Characterization

	Cases		Controls		
	n	%	*n*	%	*P*
Misoprostol use	32/93	34.4	12/279	4.3	<0.0000001
Route					
Oral	16/30	53.3	7/12	58.3	NS
Vaginal	0/30	0.0	0/12	0.0	
Oral + vaginal	14/30	46.7	5/12	41.7	
How many pills[a]					
1–4	23/31	74.2	9/11	81.8	NS
5–8	5/31	16.1	2/11	18.2	
>8	3/31	9.7	0/11	0.0	
Timing (weeks post LMP)[b]					
0–4	5/30	16.7	2/12	16.7	NS
5–8	16/30	53.3	6/12	50.0	
9–12	7/30	23.3	1/12	8.3	
>12	2/30	6.7	3/12	25.0	
Single dose	27/30	90.0	10/12	83.3	NS
Other substance for abortion attempt	13/31	41.9	2/12	16.7	NS

[a]Each pill: 200 mcg misoprostol.
[b]LMP, last menstrual period.

Castilla [2000] found an excess of four defects (constriction ring, terminal transverse limb defects, hydrocephalus, and arthrogryposis) among a sample of misoprostol-exposed newborns

Epidemiological studies designed to investigate the teratogenicity of misoprostol after abortion attempt have faced two major problems: the first was potential maternal recall bias due to the illegality of abortions in Brazil, and the second is the concern that case reports of limb defects ± Moebius sequence after maternal use of misoprostol might have prompted physicians to investigate prenatal events more thoroughly when faced with such patients. We tried to minimize both effects. By choosing the controls among malformed children, we tried to reduce maternal recall bias after the birth of a baby with malformations. Similarly, to minimize the effect of the way physicians investigate the exposure to misoprostol, we designed a structured questionnaire to be applied to every mother of any child with malformations.

All misoprostol exposures in our sample were related to abortion attempts. Other answers given to the questionnaire confirmed this observation, including a higher rate of unwanted pregnancies, previous attempted abortions, and lower rate of cohabitating with the child's father among mothers of cases compared with controls.

Vascular disruption defects are a broad category of malformations of heterogeneous etiology arising from disturbances in the normal development of embryofetal vasculature and often resulting from environmental insults [van Allen, 1981]. We believe that the wide range of anomalies we observed associated to misoprostol indicates that the drug may act either directly on vasculature or indirectly through the increase in uterine contractility. Situations of increased uterine contractility and failed abortion can predispose to events such as vascular disruptions or some degree of rupture of the amniotic membranes [Holmes, 1995; Fawcett et al., 1998]. The type of limb defect observed among the cases exposed to misoprostol (terminal transverse) is indistinguishable from those seen as a result of premature rupture of membranes. Indeed, Genest et al. [1999] observed pathological features of early amnion rupture in a 17-week-old fetus following first trimester misoprostol exposure.

In our sample the predominant diagnostic category in the vascular disruption group was Moebius (29/93) followed by terminal transverse limb defects (27/93). These were also the categories with higher odds ratio (OR = 49.0 and 24.0, respectively) for maternal exposure to misoprostol compared with a lower OR of 7.5 for the rest of the vascular disruption anomalies as a group. This fact can indicate a possible differential susceptibility for the genesis of these anomalies or, alternatively, a specific higher sensitive timing around the 4th through the 6th week of pregnancy when a significant number of exposures in our series occurred (14/

TABLE V. Odds Ratio for Maternal Exposure to Misoprostol According to Cases Anomaly Group

Cases: anomaly group	Number of discordant matched sets: case exposed/ controls not exposed	Number of discordant matched sets: case not exposed/ one control exposed[a]	Mantel-Haentzel matched odds ratio	95% Confidence interval
All vascular disruptions	26	4	22.0	7.3 < OR < 81.3
Moebius	14	1	49.0	7.07 < OR < 1,907
Transverse terminal limb reduction	7	1	24.0	3.00 < OR < 99.1
Vascular disruptions excluding Moebius and limb reductions	5	2	7.5	1.23 < OR < 78.7

[a]We did not find any discordant set with more than one control exposed to a case nonexposed.

30). The exposure to misoprostol in our control group (4.3%) is quite similar to that reported by the study of one group of normal babies born in Brazil (6.0%) [Costa and Vessey, 1993], confirming the notion that abortion attempt with misoprostol is not a rare event in Brazil.

In conclusion, our study adds epidemiological basis to the growing body of evidence that prenatal exposure to misoprostol is related to the occurrence of vascular disruption defects in some exposed fetuses. The precise spectrum of malformations involved in this phenotype, as well as an estimate of the potential teratogenicity of misoprostol is still to be determined. Such an issue becomes even more important when we are faced with the fact that approximately 5% of Brazilian women currently have to resort to the illegal use of misoprostol to deal with unwanted pregnancies.

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