Original Article

Phenolphthalein treatment in pregnant women and congenital abnormalities in their offspring: A population-based case-control study

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ABSTRACT: Phenolphthalein is frequently used laxative drug since 1930s, but the possible teratogenic effect of phenolphthalein was not checked in case-control eptedmiological study. In addition US Food and Drug Administration (FDA) declared the mutagenic and carcinogenic effect of phenolphthalein in 1999, thus we decided to evaluate the birth outcomes particularly congenital abnormalities (CAs) of newborn infants born to women treated with phenolphthalein during pregnancy. Cases with CA and their matched controls without CA born to mothers with phenolphthalein use during pregnancy were compared in the population-based large data set of the Hungarian Case-Control Surveillance System of Congenital Abnormalities. Of 22,843 cases with CA, 191 (0.83%) while of 38,151 controls, 247 (0.64%) were born to mothers with phenolphthalein treatment (adjusted OR with 95% CI: 1.3, 1.0-1.5). The mean gestational week at delivery was somewhat longer in both the case (0.3 week) and control (0.2 week) groups while the mean birth weight was somewhat larger in cases (46 g) and controls (12 g) born to mothers with phenolphthalein treatment during the study pregnancy compared with mothers without phenolphthalein treatment. These differences were in agreement with the lower rate of preterm births and low birth weight in controls born to mothers with phenolphthalein treatment during pregnancy. The detailed analysis of different CA groups showed an association between maternal phenolphthalein treatment during pregnancy and a higher risk for Hirschsprung's disease (p = 0.01) based on 4 cases in the so-called other isolated CA-group. In conclusion phenolphthalein treatment in pregnant women associates with a higher risk for Hirschsprung's disease in their children, but this finding is only a signal which needs confirmation or rejection in other studies.

Keywords: Phenolphthalein, Congenital abnormalities, Hirschsprung's disease, Birth outcomes, Population-based case-control study

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1. Introduction

Among maternal diseases during pregnancy, constipation is one of the most frequent pathological conditions which affects 11-38% of pregnant women (*1,2*). However, some clinical reports mentioned the complaints of constipation in over half of pregnant women (*3*). The recommended first line therapy of constipation includes diet with increased intake of bran and wheat fibre, in addition of fluid intakes, regular defecation and increased exercise. The second line of therapy comprises of osmotic laxatives such as magnesium hydrochloride and lactulose. The third line of therapy is based on stimulant medications, mainly senna (*4-6*), however, phenolphthalein was also used for the treatment of constipation in Hungary during the study period frequently by pregnant women as well.

The phenolphthalein is diphenylmethane ($C_{20}H_{14}O_4$) derivative laxative that act as a relatively nontoxic stimulant on the colon and take at least 6 hours to produce a fecal evacuation (*7*). Phenolphthalein was discovered as a laxative in 1902 by Zoltan Vámossy (1868-1953) in Hungary (*8,9*) and marketed in 1937 as laxative tablet without prescription. However, FDA declared the mutagenic and carcinogenic effect of phenolphthalein in 1999, and though EMEA did not accept this statement, the use of phenolphthalein was recommended only after prescription (*10*).

We found only one study regarding the human teratogenic effect of phenolphthalein that did not indicate

any increase in the rate of structural birth defects, *i.e*. congenital abnormalities (CAs) (*11*). The objective of our study was to compare the occurrence of pregnant women by phenolphthalein treatment during pregnancy who had later informative offspring ("cases") with different CAs and their matched control newborns without CA in the population-based large data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA) (*12*).

2. Materials and Methods

2.1. *Protocols*

The protocol of the HCCSCA included five steps.

The first step was the selection of cases with CA from the data set of the Hungarian Congenital Abnormality Registry (HCAR), 1980-1996 (*13*) for the HCCSCA. Notification of CAs is mandatory for physicians from the birth until the end of first postnatal year to the HCAR in Hungary. Most cases with CA are reported by obstetricians and pediatricians. In Hungary practically all deliveries take place in inpatient obstetric clinics and the birth attendants are obstetricians. In addition all infants affected with CA are treated in the neonatal units of inpatient obstetric clinics, or in various general and special (surgical, cardiologic, orthopedic, *etc*.) inpatient and outpatient pediatric clinics. Autopsy was mandatory for all infant deaths and common in stillborn fetuses during the study period. Pathologists sent a copy of the autopsy report to the HCAR if defects were identified in stillbirths and infant deaths. Since 1984 fetal defects diagnosed in prenatal diagnostic centres with or without elective termination of pregnancy have also been included into the HCAR. Isolated minor anomalies (*e.g.*, umbilical hernia, small hemangioma, hydrocele) were recorded in the HCAR but not evaluated at the calculation of different CA rates. 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 1,000 *informative offspring* (live-born infants, stillborn fetuses and electively terminated malformed fetuses) in the HCAR, 1980-1996, and about 90% of major CAs were recorded in the HCAR during the 17 years of the study period (*14*).

The major objective of the HCCSCA is a postmarketing surveillance of drug teratogenicity (*12*). Thus there were three exclusion criteria of cases with CAs from the HCAR for the data set of the HCCSCA. (i) Cases reported after three months of birth or pregnancy termination were excluded. The longer time between birth or pregnancy termination and data collection decreases the accuracy of information about pregnancy history. However, 77% of cases were reported during the first three-month time window, and the rest of most cases were affected with mild CA. (ii) Three mild CAs (such as congenital dysplasia of hip, congenital inguinal hernia, and large hemangioma), and (iii) CA-syndromes caused by major gene mutations or chromosomal aberrations with preconceptional origin were also excluded.

The second step was to ascertain appropriate *controls* 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 in the year when the case was born, and district of parents' residence.

The third step was to obtain the necessary *maternal and exposure* data from three sources:

(1) *Prospective medically recorded data*: an explanatory letter was mailed to mothers immediately after the selection of cases and controls to inform them on the purpose of the HCCSCA, the benefit of this public health activity for them and in general for the prevention of CAs. Mothers were asked to send us the *prenatal care logbook* and other *medical records* particularly discharge summaries concerning their diseases during the study pregnancy and their child's CA for three weeks. Prenatal care was mandatory for pregnant women in Hungary (if somebody 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, an average 7 times in their pregnancies. The first visit was between the 6th and 12th gestational week. The role of licensed obstetricians is to record all pregnancy complications, maternal diseases and related drug prescriptions in the prenatal care logbook.

(2) *Retrospective self-reported maternal information*: a structured *questionnaire* with a list of medicinal products (drugs and pregnancy supplements) and diseases, plus a printed informed consent form were also mailed to the mothers. The questionnaire requested information on pregnancy complications and maternal diseases, on medicinal products taken during pregnancy according to gestational months, and on family history of CAs. To standardize the answers, mothers were asked to read the enclosed lists of medicinal products and diseases as a memory aid before they filled in the questionnaire. We also asked mothers to give a signature for informed consent form which permitted us to record their name and address in the HCCSCA.

The mean ± S.D. time elapsed between the birth or pregnancy termination and the return of the "information package" (questionnaire, logbook, discharge summary, and informed consent form) in our prepaid envelope was 3.5 ± 1.2 and 5.2 ± 2.9 months in the case and control groups, respectively.

(3) *Supplementary data collection*: regional nurses were asked to visit all non-respondent case mothers, in addition 200 non-respondent control mothers. Regional nurses helped mothers to fill in the same questionnaire used in the HCCSCA; obtained data regarding smoking and drinking habit through cross interview of mothers and their close relatives; they evaluated the available

medical records and asked mothers to sign informed consent form. Regional nurses did not visit all non-respondent control mothers because the committee on ethics considered this follow-up to be disturbing to the parents of all healthy children (*15*).

The flow of cases from the HCAR and controls from the Central Statistical Office to the HCCSCA and the achievement of final data set were published previously (*16*). Overall, the necessary information was available on 96.3% of cases (84.4% from reply to the mailing, 11.9% from the nurse visit) and 83.0% of the controls (82.6% from reply, 0.4% from visit). Prenatal care logbooks were available in 88.4% of cases and in 93.8% of controls who were evaluated. Informed consent form was signed by 98% of mothers, names and addresses were deleted in the rest of subjects.

The fourth step was the *evaluation of phenolphthalein treatment* according to 12 different aspects.

1) The source of information. Three groups of phenolphthalein treatments were differentiated: (a) data only from the prenatal care logbooks and/or other medical record; (b) data from the questionnaire, and (c) concordant data from both medical records and the questionnaire.

2) The type of treatment. Two groups were differentiated: (a) phenolphthalein alone and (b) phenolphthalein plus other drugs.

3) The route of administration. In Hungary phenolphthalein was used in three medicinal products: (i) Phenolphthaleinum® (Alkaloida) tablets contain 500 mg, (ii) Bilagit® (Chinoin) tablets contain phenolphthalein 20 mg, methylhomatropine 1 mg, papaverine 20 mg, methenamine 80 mg, sodium choleinicum 60 mg and menthol 7.5 mg and (iii) Artin® (Biogal) tablets contain phenolphthalein 32 mg, aloin 16 mg, ipecacuanhae radix 4 mg, strychnin siccum extr. 4 mg, and belladonae siccum extr. 2.4 mg for oral treatment. However, Artin® was used only by 3 control and 2 case mothers, thus these pregnant women were excluded from the study due to the small numbers of subjects, in addition we wanted to evaluate a homogeneous sample as much as possible.

4) The dose of phenolphthalein treatment. The recommended oral treatment is ½-1 tablet of phenolphthalein in the evening, *i.e*. 250-500 mg per day or 2-3 times 1-2 tablets of Bilagit® tablets per day.

5) The duration of treatment.

6) Maternal diseases as underlying medical conditions particularly constipation as confounders.

7) Pregnancy complications.

8) Other drug uses as confounders.

9) Pregnancy supplements. The use of pregnancy supplements may indicate the level of pregnancy care, and indirectly may show the socio-economic status and the motivation of mothers to prepare and/or to achieve a healthy baby. In addition it is necessary to consider folic acid and folic acid-containing multivitamins in the evaluation of preventable CAs (*17-19*).

10) 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. This is the 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.

11) Medically recorded birth weight and gestational age in the discharge summary of mothers after delivery. In addition the rate of low birth weight (less than 2,500 gram) and preterm birth (less than 37th gestational week) was also calculated and evaluated.

12) Other confounding factors, such as maternal age, birth order, marital and employment status. Employment status of mothers showed a strong correlation with their education and a moderate correlation with their income (*20*), thus this variable was considered as indicator of socioeconomic status.

2.2. *Statistical analyses*

Statistical analyses were performed using the software package SAS version 8.02 (SAS Institute Inc., Cary, NC, USA). First, the occurrence of phenolphthalein treatment during the study pregnancy was compared between the study groups and crude odds ratios (OR) with 95% confidence interval (CI) was calculated. Second, frequency tables were made for the main maternal variables in order to describe the study groups of mothers with phenolphthalein treatment and of mothers without phenolphthalein treatment as reference. Third, the prevalence of pregnancy complications, acute and chronic maternal diseases, other drug treatments and pregnancy supplements used during the study pregnancy were compared between case and control mothers with phenolphthalein treatment, and crude OR with 95% CI were calculated. Fourth, the prevalence of phenolphthalein treatment was evaluated according to gestational period in 16 different CA groups (including at least 2 cases born to mothers with phenolphthalein treatment during pregnancy) in the second and/or third gestational months and this prevalence was compared with the frequency of phenolphthalein treatment in their all matched controls, and adjusted OR with 95% CI were evaluated in a conditional logistic regression model. The latter OR were adjusted for maternal age (< 20 yr *vs*. 20-29 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) use of folic acid and fever related acute maternal diseases (as a dichotomous variable).

3. Results

The case group consisted of 22,843 malformed newborns or fetuses ("informative offspring") with CA, of whom 191 (0.83%) had mothers with oral phenolphthalein treatment (43 pregnant women were treated by Phenolphthalein® and 148 pregnant women by Bilagit® tablets). The total number of births in Hungary was 2,146,574 during the study period between 1980 and 1996. Thus the 38,151 controls without CA represented 1.8% of all Hungarian births, and among those controls, 247 (0.64%) were born to mothers treated orally with phenolphthalein tablets (crude OR with 95% CI: 1.3, 0.7-1.6). Of these 247 pregnant women, 49 were treated by Phenolphthalein® and 198 by Bilagit® tablets.

Of 191 case and 247 control mothers, 7 (3.7%) and 38 (15.4%) had medically recorded oral phenolphthalein treatments in the prenatal logbooks and/or discharge summaries ($\chi^2_1 = 16.0$; $p < 0.0001$). Most pregnant women took one Phenolphthalein® tablet (*i.e.* 500 mg) or 3 times 1-2 Bilagit® tablets (60-120 mg) per day. Of 191 case and 247 controls mothers, only 2 and 1 used only Phenolphthalein® or Bilagit® tablets during the study pregnancy, respectively, thus pregnant women with phenolphthalein plus other drug treatments were evaluated together.

The onset and duration of phenolphthalein treatments in case and control mothers are shown in Table 1. About one-third of pregnant women used phenolphthalein in the first gestational month, however, of these 63 cases and 87 control mothers, only 22 and 35 continued this treatment in the second gestational month, respectively. The mean duration of phenolphthalein treatment was 2.2 and 2.5 months in the case and control mothers ($t = 1.2$; $p = 0.23$), respectively, but it depended on the onset of treatment. The earlier onset associated with the longer duration of treatment. The distribution of gestational months according to the onset of phenolphthalein treatment did not show significant difference between case and control mothers ($\chi^2_8 = 4.1$; $p = 0.85$).

Table 2 summarises the birth data of cases and controls born to mothers with oral phenolphthalein treatment during the study pregnancy. There was no difference in the sex ratio between treated and untreated case and control subgroups. The obvious general male excess is explained by the higher rate of CAs in male genital organs such as hypospadias and undescended testis and controls were matched to the sex of cases.

Here mainly the birth outcomes of controls are commented because CAs may have a more drastic effect for birth outcomes than phenolphthalein itself. The mean gestational week at delivery was somewhat longer in both the case (0.3 week) and control (0.2 week) groups

Table 1. Onset and duration of phenolphthalein treatment according to gestational month and mean duration of treatment in case and control mothers

Gestational month	Case mothers				Control mothers			
	No.	%	Mean	S.D.	No.	%	Mean	S.D.
I.	63	33.0	3.3	3.4	87	35.2	3.8	3.7
II.	17	8.9	1.8	1.1	21	8.5	2.4	2.4
III.	19	9.9	2.2	1.8	17	6.9	2.4	2.3
IV.	11	5.8	3.3	2.3	16	6.5	2.1	1.9
V.	23	12.0	1.4	0.9	32	13.0	1.9	1.6
VI.	16	8.4	1.4	0.7	29	11.7	1.7	1.3
VII.	22	11.5	1.3	0.6	26	10.5	1.5	0.8
VIII.	12	6.3	1.3	0.5	13	5.3	1.4	0.5
IX.	8	4.2	0.0	0.0	6	2.4	0.0	0.0
Total	191	100.0	2.2	2.4	247	100.0	2.5	2.7

Table 2. Birth outcomes of cases and controls born to mothers with or without phenolphthalein treatment (PT) during pregnancy

Variables	Cases				Controls				Comparison of cases and controls born to mothers with PT
	with PT ($N = 191$)		without PT ($N = 22,652$)		with P T ($N = 247$)		without PT ($N = 37,904$)		
Categorical	No.	%	No.	%	No.	%	No.	%	OR (95% CI)
Sex ratio (boy)	124	64.9	14,773	65.2	174	70.4	24,625	65.0	0.7 (0.5 - 1.2)
Stillbirths	5	2.6	392	1.7	0	0.0	0	0.0	-
Elective terminations	2	1.1	102	0.5	0	0.0	0	0.0	-
Livebirths	184	96.3	22,158	97.8	247	100.0	37,904	100.0	-
Twins	5	2.7	416	1.9	2	0.8	408	1.1	3.3 (0.6 - 17.2)
Preterm births	33	17.9	3,732	16.8	21	8.5	3,475	9.2	2.2 (1.3 - 4.0)
Low birthweight newborns	38	20.7	4,591	20.7	11	4.5	2,156	5.7	5.3 (2.6 - 10.7)
Quantitative	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Student *t* test
Gestational age at delivery (wk)*	38.9	3.1	38.6	3.2	39.6	2.1	39.4	2.1	$t = 2.8$, $p = 0.006$
Birth weight (g)*	3,023	766	2,977	704	3,288	508	3,276	511	$t = 4.3$, $p < 0.0001$

* calculated for livebirths

while the mean birth weight was somewhat larger in cases (46 g) and controls (12 g) born to mothers with phenolphthalein treatment during the study pregnancy compared with mothers without phenolphthalein treatment. These differences were in agreement with the lower rate of preterm births and low birth weight in controls born to mothers with phenolphthalein treatment during pregnancy.

Table 3 shows the basic characteristics of mothers with phenolphthalein treatment and without phenolphthalein treatment as reference. The mean maternal age was somewhat higher in pregnant women with phenolphthalein treatment due to the larger proportion of women over 30 years of age. However, the mean birth order was somewhat lower in case mother with phenolphthalein treatment due to the much lower proportion of primiparae. On the other hand the mean birth order was higher in treated control than untreated control mothers. There was no significant difference in the proportion of marital status of mothers among the study groups. Maternal employment status as an indicator of socioeconomic status showed also some differences because treated mothers were more frequent among professional and managerial than among untreated mothers. However, there was no significant difference in the distribution of employment status between case and control mothers with phenolphthalein treatment.

Among pregnancy supplements, the use of folic acid and multivitamins was higher in control mothers with phenolphthalein treatment than in the untreated reference group while their use was less frequent in treated case mothers. Thus, there was a significant difference in the occurrence of folic acid supplementation between treated case and control mothers.

Non-respondent 2,822 mothers who delivered malformed babies were visited at home and the proportion of phenolphthalein treatment occurred in 26 (0.9%) pregnant women. Of these 26 pregnant women, 5 (19.2%) smoked, while of 2,796 pregnant women without phenolphthalein treatment, 551 (19.7%) were smoker. In the control groups, only 200 non-respondent pregnant women were visited at home. The rate of smokers was 19% while pregnant women with phenolphthalein treatment did not occur among them. The proportion of regular/hard drinkers during the study pregnancy was 1.2% and 1.0% in the non-respondent case and control mothers.

We evaluated those pregnancy complications which were recorded in the prenatal care logbook; the exception was nausea and vomiting in pregnancy because this variable was analyzed on the basis of maternal information as well. Nearly all pregnancy complications showed a higher incidence in both case and control pregnant women with phenolphthalein treatment than in case and control mothers without the use of phenolphthalein (Table 4). The exception was pre-eclampsia that showed a lower occurrence in both case and control pregnant women with phenolphthalein treatment. The higher occurrence of anaemia was connected with the frequent haemorrhoids of treated pregnant women due to their chronic constipation. However, there was no significant difference in the incidence of pregnancy complications between case and control mother with phenolphthalein treatment.

The prevalence of all acute specified maternal disease groups was higher in pregnant women with

Table 3. Main variables of case and control pregnant women with phenolphthalein treatment (PT) and without phenolphthalein treatment as reference

Variables	Case mothers				Control mothers				Comparison of cases and controls born to mothers with PT
	without PT (N = 22,652)		with PT (N = 191)		without PT (N = 37,904)		with PT (N = 247)		
Quantitative	No.	%	No.	%	No.	%	No.	%	
Maternal age (yr)									
- 19	11,326	50.0	84	44.0	18,611	49.1	96	38.9	
20 - 29	7,407	32.7	58	30.2	13,304	35.1	97	39.3	$\chi^2_2 = 3.8$, $p = 0.15$
30 -	3,919	17.3	49	25.8	5,989	15.8	54	21.8	
Mean, S.D.	25.5 ± 5.3		26.6 ± 5.2		25.5 ± 4.9		26.5 ± 4.8		$t = 0.2$, $p = 0.84$
Birth order									
1	10,624	46.9	79	41.4	18,111	47.8	98	39.7	$\chi^2_1 = 0.1$, $p = 0.72$
2 or more	12,028	53.1	112	58.6	19,793	52.2	149	60.3	
Mean, S.D.	1.9 ± 1.1		1.8 ± 1.0		1.7 ± 0.9		1.9 ± 0.9		$t = 1.1$, $p = 0.27$
Categorical	No.	%	No.	%	No.	%	No.	%	
Unmarried	1,259	5.6	10	5.2	1,464	3.9	7	2.8	
Employment status									
Professional	1,883	8.3	18	9.4	4,317	11.4	36	14.6	
Managerial	4,905	21.7	63	33.0	10,038	26.5	96	38.9	
Skilled worker	6,270	27.7	59	30.9	11,631	30.7	59	23.9	
Semiskilled worker	3,844	17.0	25	13.1	5,751	15.2	32	13.0	$\chi^2_6 = 7.0$, $p = 0.32$
Unskilled worker	1,495	6.6	8	4.2	1,850	4.9	9	3.6	
Housewife	2,121	9.4	7	3.7	2,032	5.4	6	2.4	
Others	2,134	9.4	11	5.8	2,285	6.0	9	3.6	
Pregnancy supplements									OR (95% CI)
Folic acid	11,188	49.4	91	47.6	20,632	54.4	143	57.9	0.7 (0.5 - 0.9)
Multivitamins	1,321	5.8	9	4.7	2,490	6.6	19	7.7	0.6 (0.3 - 1.3)

phenolphthalein treatment compared with pregnant women without phenolphthalein treatment both in the case and in the control group (Table 5). However, only the rate of influenza-common cold was significantly higher in treated mothers particularly among case mothers.

Among chronic maternal disorders (Table 5), constipation was reported by nearly all pregnant women with phenolphthalein treatment, therefore these data are not shown in Table 5. There was a higher prevalence of haemorrhoids in treated case and control mothers than in untreated mothers.

Table 6 summarizes the frequently used other drugs (at least 4 pregnant women either in case or control mothers with phenolphthalein treatment). There was a much higher frequency of drugs used for the treatment of pregnancy complications, *i.e*. threatened abortion (promethazine) and preterm delivery (pholedrin), in addition nausea and vomiting in pregnancy (vitamin B6). Some others drugs such as acetylsalicylic acid, clotrimazole, dipyrone, penamecillin were used for the treatment of acute maternal diseases. Only the higher use of Reparon® and Demalgon® suppositories was used for the treatment of haemorrhoids, but the treatment of spasmodic drotaverine, analgesic Quarelin® and digesting Dipankrin® tablet might also be associated with complications of constipation. Three drugs (acetylsalicylic acid, Demalgon® and dipyrone) were used somewhat more frequently by case mothers than by control mothers with phenolphthalein treatment.

The main objective of the study was to evaluate cases with different CA groups and their *all matched controls* (Table 7). Our study protocol includes 25 CA-groups, but only 16 had at least 2 cases born to mother with phenolphthalein treatment. There was a higher rate of phenolphthalein treatment during the entire pregnancy in the mothers of cases with total CAs (OR with 95% CI: 1.3, 1.0-1.5) but among different CA-groups only cases with neural-tube defects were born to mother with significantly higher rate of phenolphthalein treatment. However, we focused our analysis into the second and/or third gestational months because most major CAs have the critical period in this time window. (Pregnant women who used phenolphthalein in the first gestational month and continued in the second gestational month were included.) There was no CA-group with higher

Table 4. Occurrence of pregnancy complications in case and control mothers with phenolphthalein treatment (PT) and without PT as reference

Pregnancy complications	Cases mothers				Controls mothers				Comparison of cases and controls born to mothers with PT
	without PT (*N* = 22,692)		with PT (*N* = 191)		without PT (*N* = 37,904)		with PT (*N* = 247)		
	No.	%	No.	%	No.	%	No.	%	OR (95% CI)
Nausea and vomiting									
All	10,772	47.6	98	51.3	19,826	52.3	142	57.5	0.8 (0.5 - 1.1)
Medically recorded (severe)	1,729	7.6	17	8.9	3,831	10.1	38	15.4	0.5 (0.3 - 0.9)
Threatened abortion	3,463	15.3	38	19.9	6,459	17.0	53	21.5	0.9 (0.6 - 1.4)
Pre-eclampsia*	1,928	8.4	11	5.7	3,486	9.1	22	8.8	0.6 (0.3 - 1.3)
Placental disorders**	290	1.3	4	2.1	587	1.5	5	2.0	1.0 (0.3 - 3.9)
Polyhydramnios	206	0.9	6	3.1	188	0.5	3	1.2	2.6 (0.7 - 10.7)
Threatened preterm delivery	2,820	12.5	20	15.7	5,945	15.7	41	16.6	0.6 (0.3 - 1.0)
Gestational diabetes	139	0.6	2	1.0	269	0.7	1	0.4	2.6 (0.2 - 28.9)
Anaemia	3,198	14.1	42	22.0	6,302	16.6	54	21.9	1.0 (0.6 - 1.6)

* hypertonia, edema, albuminuria; ** placenta previa, premature separation of placenta, antepartum hemorrhage.

Table 5. Prevalence of acute and chronic maternal diseases in case and control mothers with phenolphthalein treatment (PT) and without PT as reference

Maternal diseases	Cases mothers				Controls mothers				Comparison of cases and controls born to mothers with PT
	without PT (*N* = 22,652)		with PT (*N* = 191)		without PT (*N* = 37,904)		with PT (*N* = 247)		
	No.	%	No.	%	No.	%	No.	%	OR (95% CI)
Acute									
Influenza - common cold	4,893	21.6	74	38.7	7,001	18.5	60	24.3	2.0 (1.3 - 3.0)
Respiratory system	2,095	9.2	23	12.0	3,418	9.0	37	15.0	0.8 (0.4 - 1.3)
Digestive system	712	3.1	30	15.7	903	2.4	30	12.1	1.3 (0.8 - 2.3)
Urinary tract	1,574	6.9	15	7.9	2,292	6.0	16	6.5	1.2 (0.6 - 2.5)
Genital organs	1,665	7.4	15	7.9	2,878	7.6	20	8.1	1.0 (0.5 - 1.9)
Others	384	1.7	5	2.6	507	1.3	8	3.2	0.8 (0.3 - 2.5)
Chronic									
Diabetes mellitus	55	0.2	1	0.5	51	0.1	1	0.4	1.3 (0.1 - 20.8)
Epilepsy	76	0.3	0	0.0	76	0.2	1	0.4	-
Headache	551	2.4	14	7.3	701	1.8	12	4.9	1.5 (0.7 - 3.4)
Varicose veins in lower extremities	305	1.3	7	3.7	910	2.4	11	4.5	0.8 (0.3 - 2.1)
Thrombophlebitis	327	1.4	5	2.6	565	1.5	1	0.4	6.6 (0.8 - 57.1)
Haemorrhoids	548	2.4	21	11.0	1,244	3.3	24	9.7	1.1 (0.6 - 2.1)

rate of phenolphthalein treatment in the second and/or third gestational months according to adjusted OR. Cases with CA of eyes had different buphthalmos and congenital cataract. However, it is necessary to mention that the critical period of some CAs such as hypospadias, undescended testis, clubfoot is after the third gestational month. Our further analysis calculated with their specific critical periods without any positive associations.

Finally we evaluated 19 cases with other isolated CAs in detail (Table 8). Of these 19 cases, 6 belonged to one of CA groups in the protocol of the HCCSCA but had only one case therefore they were omitted from Table 7. Among further 13 cases, 4 were affected with Hirschsprung's disease (Table 9) and 3 with torticollis.

Table 6. Occurrence of other frequent drug treatments in case and control mothers with phenolphthalein treatment (PT) and without PT as reference

Drugs	Cases mothers				Controls mothers				Comparison between case and control mothers with PT
	without PT (*N* = 22,652)		with PT (*N* = 191)		without PT (*N* = 37,904)		with PT (*N* = 247)		
	No.	%	No.	%	No.	%	No.	%	OR (95% CI)
Acetylsalicylic acid	979	4.3	22	11.5	1,380	3.6	15	6.1	2.0 (1.0 - 4.0)
Allylestrenol	3,449	15.2	32	16,8	5,320	14.0	37	15.0	1.1 (0.7 - 1.9)
Aminophenazone+carbromal (Demalgon®)	371	1.6	12	6.3	336	0.9	5	2.0	3.2 (1.1 - 9.4)
Aminophylline	1,362	6.0	12	6.3	2,267	6.0	17	6.9	0.9 (0.4 - 1.9)
Ampicillin	1,607	7.1	17	8.9	2,573	6.8	19	7.7	1.2 (0.6 - 2.3)
Bacterium coli + phenol (Reparon®)	169	0.7	9	4.7	400	1.1	16	6.5	0.7 (0.3 - 1.6)
Chlordiazepoxide	190	0.8	11	5.8	261	0.7	6	2.4	2.5 (0.9 - 6.8)
Clotrimazole	1,619	7.1	22	11.5	3,051	8.0	26	10.5	1.1 (0.6 - 2.0)
Diazepam	2,723	12.0	23	12.0	4,098	10.8	32	13.0	0.9 (0.5 - 1.6)
Dimenhydrinate	898	4.0	16	8.4	1,711	4.5	15	6.1	1.4 (0.7 - 2.9)
Dipyrone	1,336	5.9	46	24.1	1,872	4.9	39	15.8	1.7 (1.1 - 2.7)
Drotaverine	2,005	8.9	48	25.1	3,428	9.0	53	21.5	1.2 (0.8 - 1.9)
Hydroxyethylrutoside	563	2.5	4	2.1	1,129	3.0	14	5.7	0.4 (0.1 - 1.1)
Irons	14,624	64.6	120	62.8	26,589	70.1	185	74.9	0.6 (0.4 - 0.8)
Noraminophenazone + caffeine + droteverine (Quarelin®)	195	0.9	10	5.2	270	0.7	15	6.1	0.9 (0.4 - 1.9)
Pancreatin+duodenum siccum (Dipankrin®)	44	0.2	9	4.7	88	0.2	15	6.1	0.8 (0.3 - 1.8)
Penamecillin	1,570	6.9	26	13.6	2,223	5.9	23	9.3	1.5 (0.8 - 2.8)
Pholedrin	758	3.3	10	5.2	1,490	3.9	19	7.7	0.7 (0.3 - 1.5)
Potassium + magnesium (Panangin®)	765	3.4	7	3.7	1,392	3.7	13	5.3	0.7 (0.3 - 1.7)
Promethazine	3,608	15.9	40	20.9	5,974	5.8	51	20.6	1.0 (0.6 - 1.6)
Senna	443	2.0	27	14.1	818	2.2	37	15.0	0.9 (0.5 - 1.6)
Terbutalin	2,325	10.3	25	13.1	3,966	10.5	28	11.3	1.2 (0.7 - 2.1)
Vitamin B6	1,988	8.8	25	13.1	4,045	10.7	41	16.6	0.8 (0.4 - 1.3)

Table 7. Results of conditional logistic regression analysis of cases and their all matched controls without CA born to mothers with phenolphthalein treatment during the entire pregnancy and in the second and/or third gestational month

Study groups	Grand total	Entire pregnancy				II-III months			
	No.	No.	%	OR*	95% CI	No.	%	OR*	95% CI
Controls	38,151	247	0.6	Referent		73	0.2	Referent	
Isolated CAs									
Neural-tube defects	1,202	15	1.3	2.7	1.2 - 6.4	4	0.3	1.3	0.3 - 5.2
Hydrocephaly, congenital	314	3	1.0	2.3	0.4 - 11.7	2	0.6	3.5	0.3 - 39.1
Eye CAs	99	2	2.0	-	-	2	2.0	-	-
Cleft lip ± palate	1,374	15	1.1	1.3	0.6 - 2.8	5	0.4	1.3	0.3 - 4.7
Cleft palate only	582	2	0.3	0.8	0.1 - 5.1	0	0.0	-	-
Cardiovascular CAs	4,479	33	0.7	1.1	0.7 - 1.7	13	0.3	1.8	0.8 - 4.0
Obstructive CAs of urinary tract	271	2	0.7	0.7	0.1 - 3.9	0	0.0	-	-
Hypospadias	3,038	22	0.7	1.3	0.7 - 2.2	8	0.3	1.3	0.5 - 3.2
Undescended testis	2,051	10	0.5	0.9	0.4 - 1.9	3	0.2	3.8	0.4 - 38.4
Poly/syndactyly	1,744	20	1.1	1.8	0.9 - 3.4	4	0.2	1.0	0.3 - 3.7
Limb deficiencies	548	7	1.3	2.0	0.6 - 6.0	4	0.7	3.6	0.6 - 19.9
Clubfoot	2,424	25	1.0	1.3	0.8 - 2.2	2	0.1	0.3	0.1 - 1.2
Diaphragmatic CAs	243	2	0.8	0.6	0.1 - 3.1	1	0.4	0.6	0.0 - 6.6
Exomphalos/gastroschisis	238	3	1.3	1.1	0.2 - 5.2	1	0.4	0.7	0.1 - 8.7
Other isolated CAs	2,887	19	0.7	1.0	0.5 - 1.8	6	0.2	0.9	0.3 - 2.7
Multiple CAs	1,349	11	0.8	1.3	0.5 - 3.0	3	0.2	1.3	0.2 - 6.7
Total CAs	22,843	191	0.8	1.3	1.0 - 1.5	58	0.3	1.2	0.9 - 1.8

* adjusted for maternal age, birth order, maternal employment status, use of folic acid and influenza - common cold during pregnancy.

Table 8. Distribution of cases with "other isolated CA"

CA groups/cases	Grand total	Entire pregnancy		Comments
		No.	%	
CA-groups evaluated				
CAs of ear: microtia	354	1	0.3	Bilagit (VIII-IX)
Esophageal atresia/stenosis	217	1	0.5	Bilagit (V)
Pyloric stenosis, congenital	241	1	0.4	Phenolphthalein (IV-IX)
Anal atresia	220	1	0.5	Bilagit (I-II)
CAs of genital organs: intermediate sex	211	1	0.5	Bilagit (III-VIII)
CAs of skeletal system: pectus excavatum	155	1	0.7	Bilagit (I-II)
Subtotal	1,398	6	0.4	
Cases with isolated CA				
Branchial cyst	21	1	4.8	Bilagit (I-II)
Hirschsprung's disease	35	4	11.4	See Table 9
Other CAs of digestive system: Megaloduodenum with transposition of intestine	64	1	1.6	Bilagit (IX)
CA of gallbladder, bile ducts, liver: Congenital cystic liver, Atresia of bile duct	26	2	7.7	Bilagit (I-II), Bilagit (IX)
Torticollis	301	3	1.0	Bilagit (I-IX), Bilagit (VII-VIII), Bilagit (VI)
CA of urachus	6	1	16.7	Phenolphthalein (VIII)
Teratoma	58	1	1.7	Bilagit (I-III)
Others	978	0	0.0	
Subtotal	1,489	13	0.9	
Total	2,887	19	0.7	

The critical period of torticollis caused by the intrauterine deformation of sternocleidomastoid muscle is during the last months of pregnancy, and all cases had mothers with phenolphthalein treatment between the 6th and 9th months. All other CAs occurred only once, thus they are not analysed due to the old rule: one case – no case. However it is worth mentioning, that of these 19 cases, 10 are connected with the digestive system.

The data of four cases with Hirschsprung's disease with a male predominance are shown in detail in Table 9, and there is a 5th case with megaloduodenum (Table 8). The data set of the HCCSCA includes 35 cases with Hirschsprung's disease and 4 (11.4%) had mothers with phenolphthalein treatment while the mothers of 54 matched controls were not treated with phenolphthalein ($\chi^2_1 = 6.4$; $p = 0.01$).

We attempted to evaluate only medically recorded phenolphthalein treatments during the critical period of the previously discussed specified CAs, but the number of cases was too low for any estimation.

4. Discussion

The objective of our study was to evaluate the possible association between oral phenolphthalein treatment during the critical period of different CA groups and the risk for different CAs. Our data did not show an association of phenolphthalein treatment in second and/or third gestational month of pregnancy with any CA group. However, the detailed analysis of the group of the so-called other isolated CAs showed that 4 cases with Hirschsprung's disease and 3 cases with torticollis had mother with phenolphthalein treatment. In addition we analyzed birth outcomes of controls born to mothers with phenolphthalein treatment and we did not find any clinically important association.

At the evaluation of these findings we have to consider the indication of phenolphthalein and Bilagit® treatment, *i.e.* constipation. (However, Bilagit® is used for the treatment of gallbladder's diseases as well.) Severe constipation which needed drug treatment is more frequent in elder primiparae with a higher socioeconomic status and higher proportion of folic acid supplementation (at least in control mothers). However, these pregnant women had a controversial pattern. On the one hand nearly all pregnancy complications and maternal diseases occurred more frequently in pregnant women with phenolphthalein treatment. On the other hand the birth/pregnancy outcomes of these pregnant women did not show the adverse affect of the above risk factors. The explanation for this discrepancy may be the more health conscious lifestyle of pregnant women with severe constipation and their more attentive care from medical doctors. In addition it is worth mentioning that pre-eclampsia occurred less frequently in pregnant women with constipation and phenolphthalein treatment.

There were 3 cases with torticollis, but the prevalence at birth of this deformation type CA is 0.88 per l,000 in Hungary (*14,21*), thus this observed rate did not result in a significant deviation from the expected rate.

The expected incidence of cases with Hirschsprung's disease (HSCR) is between 1 in 8,000 (*22*) and 1 in 25,000 (*23*) births. The data set of the HCCSCA is not appropriate for the estimation of HSCR's incidence but it is worth noting that the rate of cases with HSCR born to mothers with phenolphthalein treatment was higher in our data set than the rate of congenital hydrocephaly (0.7/1,000), cleft palate (0.5/1,000), and diaphragmatic CAs (0.2/1,000) (*14*) with much higher prevalence at birth (their Hungarian rates are shown in brackets). In addition the comparison of cases with HSCR and their matched controls indicated a very significant association with maternal phenolphthalein (mainly Bilagit®) treatment. However, we have to consider that

Table 9. Data of cases with Hirschsprung's disease

No.	Sex[a]	Cases		Mother		Father		Sibs		Pregnancy cmplications	Maternal diseases	Drug treatments	Pregnancy supplements
		Gestational age (wk)	Birth weight (g)	Age (yr)	ES[b]	Age (yr)	ES[b]	No.	CA				
4407	M	37	3,000	26	SSW	28	M	1	None	TPD (V-VII)[c]	Tonsillitis (I) Cholelithiasis (I) Constipation (V) Common cold (V)	Acetylsalicylic acid (I) Bilagit® (I)[e] Senna (V) Allylestrenol (V-VII) Diazepam (V-VII) Terbutaline (V-VII)	Iron (V) Multivitamin (V)
2216	M	37	3,750	23	M	23	P	0	–	–	Common cold (I) Constipation - haemorrhoid (I) Cholelithiasis (VIII)	Almagel® = aluminium hydroxide + magnesium hydroxide (VIII) Reparon®(ung) = Bacterium coli + phenol Bilagit® (VIII)[e]	Folic acid (VIII) Iron (VIII) Multivitamin (II)
1415	M	36	1,900	23	M	29	P	1	None	Mild NVP (I)[d] anaemia (V)	Migraine (III) Cholecystitis (III)	Kefalgin® = ergotamine + aminophenazone + caffeine + belladonna extr. (III) Bilagit® (III)[e]	Iron (V) Vitamin D (V-VIII)
1455	F	36	1,900	39	SW	31	SW	0	–	Mild NVP (II-III)[d]	Constipation (I-IX)	Ampicillin (III) Penamecillin (III) Dipyrone (III) Drotaverine (III) Acetylsalicylic acid (III) Phenolphthalein (I-IX) Senna (I-IX)	Vitamin B6 (II-III) Caldea® = retinol + ergocalciferol + calcium hydrogenphosphate + calcium lactate (IV)

[a] M = male, F = female; [b] ES = Employment status; P = professional; M = managerial; SW = skilled worker; SSW = semiskilled worker; [c] TPD = threatened preterm delivery; [d] NVP = nausea and vomiting in pregnancy; [e] Bilagit® = papaverine + methylhomatropine + phenolphthalein + sodium choleinicum + methenamine + menthol.

multiple comparisons result in a statistically significant association in every 20th estimation because of chance.

HSCR or congenital aganglionic megacolon or colon aganglionosis was described by Harald Hirschsprung in 1888 (*24*) and is caused by the congenital absence of the intramural myenteric parasympathetic nerve ganglia and sympathetic nerve plexus in a segment of colon that extends proximally from the anus for a varying distance. Aganglionosis is limited to the recto-sigmoid colon in 70% of cases (short-segment HSCR), but total colonic aganglionosis and small intestinal aganglionosis were found in 1-10% of cases (long-segment HSCR) in different studies. The aganglionic colon is unable to transmit the coordinated peristaltic waves from the proximal colon producing variable degrees of intestinal obstruction. Hyperperistaltic activity results in increasing hypertrophy and dilatation of the normal colon.

HSCR is common in males (3-5:1). Familial occurrence is obvious because the recurrence risk for sibs is 4% though the occurrence of HSCR cases is 0.02% in the population (*25*). The etiology of this CA can be explained by gene-environmental interactions. The polygenic background of HSCR is supported by the observation that (i) the recurrence risk increases with the number of affected first degree relatives, and (ii) greater when involvement (long-segment) is more severe, in addition (iii) the familial risk is higher in the relatives of females (7.2% *vs*. 2.6% in males) (*26*). The locus of major genes of HSCR was localized in chromosome 10q11.2 (*27*). Recently the mutations of genes operating either alone or in combination in the origin of HSCR have been revealed. These mutations include dominant mutations in the RET gene (*28*) and a recessive mutation in the endothelium receptor type B gene (EDNR-B) (*29*). However, the triggering environmental factors are less known, previously hyperthermia in early gestation was described as a triggering factor in the origin of HSCR (*30*) but later this finding was not confirmed (*31*). Our study showed a higher risk for HSCR thus further studies will be needed to differentiate the possible teratogenic/ triggering or mutagenic effect of phenolphthalein in the origin of this disease.

The strengths of HCCSCA can be explained by the population-based large data set including 438 pregnant women with phenolphthalein treatment in the ethnically homogeneous Hungarian (Caucasian) people. Additional strengths include the matching of cases to controls without CA, available data for potential confounders, and finally that the diagnosis of medically reported CAs was checked in the HCAR (*13*) and later modified, if necessary, on the basis of recent medical examination within the HCCSCA (*12*). Our study design regarding birth outcomes were based on medically recorded gestational age at delivery and birth weight.

However, this data set also has limitations. (i) Most pregnant women were treated by Bilagit® containing methylhomatropine, papaverine, methenamine, sodium choleinicum, and menthol beyond phenolphthalein and of 4 cases with HSCR, 3 had mothers with Bilagit® treatment. The other components of Bilagit® had no teratogenic effect (*32*), nevertheless a drug interaction cannot be excluded. (ii) The response rate was 83% in controls and 84% in cases, but there was an active follow up for all non-respondent case mothers, but for only 200 non-respondent control mothers. However, it is worth noting that there was no significant difference in the prevalence of other frequent maternal diseases and drug treatments between the subgroups of respondents and non-respondents (*15*), thus, the effect of selection bias seems to be limited in the study. (iii) The mean time between the birth/pregnancy termination and the return of the information package was 1.7 months longer in the group of control mothers ($t = 4.4$; $p < 0.001$). However, this degree of time difference does not cause recall bias in long term treatment such as phenolphthalein (*15*). (iv) Most women with phenolphthalein treatment were treated with other drugs as well, but in general their proportion was similar in case and control mothers. (v) Only a very small proportion of case and control mothers had prospectively and medically recorded phenolphthalein treatment during the study pregnancy because these drugs were not prescribed in the prenatal care clinic. Thus, we have to consider recall bias, because the birth of an infant with CA is a serious traumatic event for most mothers who therefore try to find a causal explanation such as diseases or drug uses during pregnancy for CA of their babies. This does not occur after the birth of a healthy newborn infant. Thus recall bias might inflate an increased risk for CAs. Our previous analysis showed that a case-control surveillance of this type may cause spurious association between drugs and CAs with biased OR up to a factor of 1.9 (*33*). However, at the planning of our study design we wanted to limit recall bias. Thus we evaluated different CAs separately because if we find a significant association of phenolphthalein treatment with only one or a few CA, it is an argument against the recall bias because it is general for all CAs. In addition we focused our analysis for the critical period of CAs because we expect an underreporting of phenolphthalein treatment in both the critical and non-critical periods of CAs in the control group. Unfortunately of our 834 malformed controls (Down syndrome), only 11 had mothers with phenolphthalein treatment, thus we were not able for the comparative analysis of cases and malformed controls.

The mechanism of action of the laxative phenolphthalein is similar to that of the anthraquinone purgatives such as senna. Small amounts of the laxatives are absorbed into the systematic circulation. As far as we know results of investigation regarding the cross of phenolphthalein through the placenta have not been published, however, its molecular weight is low enough (approximately 318) for placental transfer (*34*). No investigations reporting the use of phenolphthalein in

experimental animals have been located (*32*). Previously only Heinonen *et al.* (*11*) studied the teratogenic effect of phenolphthalein, and they did not find an increase in the expected rate of CAs among offspring of 236 women who took this laxative during the first four lunar months compared to the expected rate. They reported similar findings in 806 women who took phenolphthalein anytime during pregnancy.

Phenolphthalein is an old-fashioned drug but its mutagenic/carcinogenic effect is debated, therefore the data of our study may contribute the final conclusion.

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