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Trimethoprim use in early pregnancy and the risk of miscarriage: a register-based nationwide cohort study

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SUMMARY

The antibiotic trimethoprim acts as a folate antagonist. Since trophoblasts are very sensitive to drugs that interfere with the folic acid cycle and thereby inhibit DNA synthesis, use of trimethoprim during the first trimester could be associated with miscarriage. A nationwide cohort study including all women in Denmark with a registered pregnancy between 1997 and 2005 was conducted. We used nationwide registers to identify all women giving birth, having a record of miscarriage or induced abortion. Data on exposure to trimethoprim were obtained from the National Prescription Register. Cox proportional hazard regression analysis with exposure to trimethoprim as a time-dependent variable was used to estimate the risk of miscarriage. The adjusted hazard ratio of having a miscarriage after exposure to trimethoprim in the first trimester compared to non-exposure was 2·04 (95% confidence interval 1·43–2·91). Our results indicate that trimethoprim exposure in the first trimester is associated with a doubling of the hazard of miscarriage.

Key words: Antibiotics, birth defects, epidemiology, pregnancy.

INTRODUCTION

Trimethoprim is an antibiotic that acts as a folate antagonist [1]. It inhibits dihydrofolate reductase (DHFR) and thereby the synthesis of DNA [1]. The trophoblasts of the fetus are very sensitive to drugs that interfere with the folate cycle and inhibit DNA synthesis [2]. Therefore, if trimethoprim is taken early in pregnancy it could in theory interfere with fetal development. Moreover, studies have shown trimethoprim to be teratogenic when used in the first trimester of pregnancy [3, 4] and use of folic acid supplementation before and during pregnancy has been associated with a reduced risk of having offspring with neural tube [5–7], oral cleft [5, 8], limb [8, 9], urinary tract [8, 10], and cardiovascular [5, 10, 11] defects. Trimethoprim can decrease folate levels for up to 50 days after exposure [12]. Due to this

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folate lowering effect and due to its mode of action trimethoprim could, in theory, induce miscarriage when used in early pregnancy and in the pre-conceptional period. This issue has not previously been addressed although another DHFR inhibitor, methotrexate, can be used as an abortifacient in the first trimester [13, 14].

Worldwide, trimethoprim is used in urinary tract, respiratory tract and gastrointestinal infections. It is used as daily prophylaxis in AIDS treatment and HIV infections in sub-Saharan Africa [15]. In Denmark, at least in younger women, trimethoprim is primarily used in urinary tract infections.

Trimethoprim is not recommended for use in pregnancy but due to its common use, large numbers of pregnant women could be exposed to the drug in the first trimester and in the pre-conceptional period. We therefore conducted a nationwide cohort study testing the hypothesis if use of trimethoprim in the first trimester or before pregnancy was associated with a higher frequency of miscarriage in pregnant women in Denmark.

MATERIALS AND METHODS

Study population

A cohort was constructed by identifying all pregnancies in Denmark using the National Hospital Register [16] and the Danish Fertility Database [17]. Pregnancies were defined as all incidences of miscarriage, induced abortion and birth, with time of conception in the study period from 1 January 1997 to 31 March 2007 ($n=934\,840$). We excluded 2976 pregnancies due to coding errors. Records of miscarriage were defined as women diagnosed with O02 or O03 codes between the fifth and 20th week of gestation. Induced abortion was defined as women diagnosed with O04, O05 or O06 codes. All diagnoses are classified according to the International Classification of Diseases – 10th Danish revision. Births were identified in the Danish Fertility Database.

The National Hospital Register contains information on all hospitalizations in the country, including admittance data and discharge diagnoses. It holds more than 99% of discharge records from all Danish hospitals [18]. Information on gestational age for induced abortion and miscarriage has been available since 1 January 1997.

The Danish Fertility Database consists of individual-level data on the mother and the father, including a unique identification number, age, previous births and abortions, as well as the child's birth weight, death and cause of death, sex and gestational age. The time of conception is based on ultrasound analyses or information on the last menstrual period. More than 99·5% of all births in Denmark since 1978 are registered in the Danish Fertility Database [17].

Information on drug use was obtained from the Register of Medicinal Product Statistics (The National Prescription Register) [19]. Exposure was defined as dispensing of a prescription drug containing trimethoprim for systemic use [Anatomical Therapeutic Chemical Classification (ATC) J01EE01 and J01EA01 codes]. The register contains individual-level data on all prescribed drugs dispensed at all pharmacies in Denmark since 1995. The register contains no information on over-the-counter drugs or indication of treatment.

Urinary tract infections could in theory be abortogenic. Sulfamethizole is in Denmark primarily used in urinary tract infections. Using exposure to sulfamethizole as a proxy for urinary tract infection we investigated whether there was an association between redeeming a prescription of sulfamethizole (ATC J01EB02) and miscarriage.

Statistics

The hazard ratio of miscarriage was estimated using four time-dependent Cox proportional hazard regression models with first-trimester exposure to trimethoprim as a time-dependent variable and time from conception to miscarriage as outcome. Model 1 was unadjusted, model two was adjusted for age (five groups, <20, 20–24, 25–29, 30–34, >35 years), number of previous miscarriages (four groups, 0, 1, 2, $\geqslant 3$), income (four groups as quartiles) and education (four groups).

In the third (unadjusted) and fourth (adjusted) models we defined case time for trimethoprim exposure to 50 days, thereby defining cases as women having a miscarriage within 50 days after start of treatment. The relationship between exposure before conception and miscarriage was analysed using an unadjusted and an adjusted Cox proportional hazard regression model with time from conception to miscarriage as outcome. Adjustment variables were the same as the previous adjusted models.

The assumption of proportional hazards in both univariate and multivariate Cox models is met. Time of conception was defined as 14 days after the first day

Table 1. *Basic characteristics*

	Women redeeming a trimethoprim prescription in the first trimester ($n=265$)	Women not redeeming a trimethoprim prescription in the first trimester ($n=931\,239$)	P value
No. of miscarriages	31	77 522	<0·001
Age (years)			<0·001
<20	16 (6·0%)	30 810 (3·3%)	
20–24	53 (20·0%)	120 725 (13·0%)	
25–29	69 (26·0%)	296 397 (32·0%)	
30–34	70 (26·4%)	309 059 (33·2%)	
>35	57 (21·5%)	174 248 (18·7%)	
Educational level			<0·001
Low	133 (50·6%)	343 839 (37·8%)	
Medium	78 (29·7%)	270 109 (29·7%)	
High	45 (17·1%)	264 231 (29·1%)	
No information	7 (2·6%)	31 174 (3·4%)	
Income			0·007
Lowest quartile	74 (28·0%)	232 727 (25·0%)	
Low quartile	89 (33·6%)	232 666 (25·0%)	
Medium quartile	57 (21·5%)	232 802 (25·0%)	
High quartile	45 (17·0%)	233 034 (25·0%)	
No. of previous miscarriages			0·12
0	227 (85·7%)	770 759 (82·8%)	
1	26 (9·8%)	129 395 (13·9%)	
2	8 (3·0%)	24 320 (2·6%)	
⩾3	4 (1·5%)	6765 (0·7%)	

of the last menstrual period based on either ultrasound or menstrual information. Information on age, income and number of previous miscarriages had <1% missing values. If data on highest achieved educational level on the birth year was missing, data from the following year was included; 2·3% of records had educational information missing [0·8% in the trimethoprim-exposed group and 2·4% in the unexposed group ($P=0·09$)].

For all analyses, a two-sided P value of $P<0·05$ was considered statistically significant and hazard ratios (HRs) are presented with 95% confidence intervals (CIs). All data management and analyses were performed using SAS software, version 9.2 (SAS Institute Inc., USA).

Ethics

All data were linked in computers held by Statistics Denmark and were made available with encrypted personal information. This ensured that no individuals could be identified. Ethical permission is not required in Denmark for retrospective register studies. The Danish Data Protection Agency approved the study (No. 2008-41-2517).

We report our findings according to strengthening the reporting of observational studies in epidemiology (STROBE) [20].

RESULTS

We identified 931 504 pregnancies during the study period; 705 837 (75·8%) live births, 148 114 (15·9%) induced abortions and 77 553 (8·3%) miscarriages. Women exposed to trimethoprim during the first trimester were more likely to have a lower educational level ($P<0·0001$), lower income ($P=0·007$) and differ in age compared to women not exposed to trimethoprim (Table 1). From conception to the end of the first trimester, 265 women were exposed to trimethoprim, of whom 31 (11·7%) experienced a miscarriage. In the same period, 77 522 (8·3%) unexposed women experienced a miscarriage (Table 2).

We found an association between being exposed to trimethoprim in the period from conception to the end of the first trimester and having a miscarriage, with an unadjusted HR of 2·09 (95% CI 1·46–2·99). When adjusting for education, income, prior abortions and age we found a HR of 2·04 (95% CI 1·43–2·91) (Table 2).

Table 2. *Results of exposure to trimethoprim*

	No. of exposed	No. of miscarriages	HR (95% CI)	HR (adjusted 95%CI)
Exposure in the trimester before conception				
Trimethoprim and combinations (J01EA01 and J01EE01)	812	73	1·15 (0·91–1·44)	0·88 (0·70–1·11)
Sulfamethizole and trimethoprim (J01EE01)	108	10	1·19 (0·64–2·20)	0·83 (0·45–1·55)
Trimethoprim (J01EA01)	704	63	1·14 (0·89–1·46)	0·89 (0·69–1·13)
Exposure in the trimester after conception				
Trimethoprim and combinations (J01EA01 and J01EE01)	265	31	2·09 (1·46–2·99)	2·04 (1·43–2·91)
Sulfamethizole and trimethoprim (J01EE01)	34	3	1·43 (0·46–4·43)	1·28 (0·41–3·97)
Trimethoprim (J01EA01)	233	28	2·12 (1·50–3·19)	2·27 (1·56–3·31)
Sulfamethizole (J01EB02)	23 286	1587	0·95 (0·90–1·00)	0·97 (0·92–1·02)

HR, Hazard ratio; CI, confidence interval.

Other analyses

When only including women having a miscarriage within 50 days after start of exposure the HR was 5·69 (95% CI 3·79–8·53). Adjusting for education, income, previous miscarriage, and age, HR was 5·62 (95% CI 3·74–8·46).

Post-hoc we divided the first trimester in three equal parts and analysed the hazard of having a miscarriage when exposed to trimethoprim in each part (first part: HR 2·31, 95% CI 1·56–3·42; second part: HR 1·85, 95% CI 0·70–4·88; third part: HR 1·42, 95% CI 0·36–5·68) (Fig. 1). Two women were exposed in both the first and second parts of the first trimester and were excluded from the analysis.

To investigate whether the results could be due to confounding by indication we analysed the hazard of having a miscarriage when exposed to sulfamethizole from the time of conception to the end of the first trimester. We found no association between sulfamethizole and miscarriage (HR 0·97, 95% CI 0·92–1·02) (Table 2). We did not find a significantly increased hazard of having a miscarriage when exposed to trimethoprim in the 12 weeks before conception (HR 1·15, 95% CI 0·91–1·44) with no significant change in the HR when adjusting (HR 0·88, 95% CI 0·70–1·11).

DISCUSSION

This is the first study to examine the abortogenic effects of trimethoprim use in the first trimester of pregnancy. We found an increased hazard of having a miscarriage when redeeming a prescription of trimethoprim between conception and the end of the first trimester.

We find these results biologically plausible since trimethoprim is a folate antagonist and since the trophoblasts are very sensitive to changes in the folate cycle [2]. Such changes could lead to miscarriage and congenital malformations due to the inhibition of DNA synthesis. Furthermore, it is known that trimethoprim can depress folate plasma levels for up to 50 days [12]. When limiting the case time of having a miscarriage after exposure to trimethoprim to 50 days we found a fivefold increase in the HR compared to women with no exposure to trimethoprim.

Another DHFR inhibitor, methotrexate, is a known and potent abortogenic agent and can be used for medically induced abortion [13, 14]. Although trimethoprim is a less potent inhibitor of DHFR, we find it likely that the known interference with the folate cycle, as for methotrexate, can induce miscarriage. Furthermore, it is has previously been shown that women exposed to trimethoprim during the first trimester have a higher risk of having children with congenital malformations and thereby presumably a higher risk of miscarriage [3. 4].

We analysed the hazard of trimethoprim exposure in three different parts of the first trimester and found an increased hazard in all parts of the first trimester but only statistically significant in the first part. Trophoblasts could be more vulnerable to changes in DNA synthesis in the early stages of development, which could explain our findings.

The observed association could be due to confounding by indication, e.g. if urinary tract infections induced miscarriage. We found no association

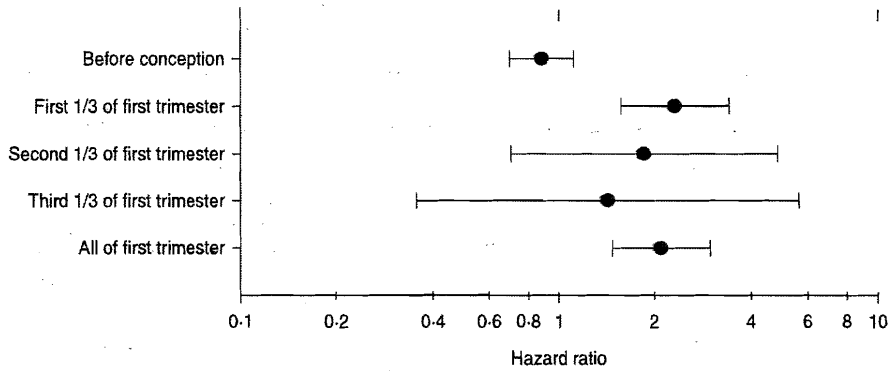

Fig. 1. Hazard ratio for miscarriage following trimethoprim exposure in three different parts of the first trimester.

between exposure to a commonly used antibiotic for urinary tract infections (sulfamethoxazole) and miscarriage. Therefore we find it unlikely that the observed association is due to confounding by indication.

Unexpectedly, we did not find a significantly higher hazard miscarriage when exposed to trimethoprim in the 12 weeks before conception. It might be that trimethoprim does not induce miscarriage when used before pregnancy, but due to the nature of the drug we find this unlikely. A plausible explanation for the lack of a significantly increased hazard of miscarriage before pregnancy might be that miscarriage occurs too early in pregnancy to be recognized or registered in the National Hospital Register or that the fertility of the exposed women was altered.

The registers used in this study have previously been validated and found to be accurately recorded. Pharmacies are, due to reimbursement of medical expenses from the state, required by law, to register all prescriptions redeemed. This ensures highly accurate data and eliminates recall bias. Completeness has previously been estimated as 97·5% [21]. More than 99·5% of all births since 1978 are registered in the Danish Fertility Database [17] and more than 99% of discharge diagnoses are registered in the National Hospital Register [18].

The study covers nationwide data including all registered miscarriages and induced abortions and all women giving birth. This minimizes selection bias and ensures high completeness of data independent of race, age and economic and educational status. Furthermore, only drugs redeemed and paid for at the pharmacy were included in the study.

The main limitation of the present study is the risk of unaddressed confounders. Women exposed to trimethoprim might differ from unexposed women in aspects causally related to the risk of miscarriage, for instance obesity, alcohol consumption, smoking and antiphospholipid syndrome [22, 23]. However, women exposed to trimethoprim before pregnancy did not have a significantly higher HR of having a miscarriage.

A further limitation includes the underreporting of miscarriage in the National Hospital Register. This underreporting consists of women having a miscarriage without hospitalization and has been estimated to be about 30% [24]. These cases are probably accounted for by miscarriage early in pregnancy.

Women exposed to trimethoprim or with urinary tract infections could be more likely to be hospitalized due to a miscarriage compared to women with no exposure or urinary infection. This possible skewness could lead to selection bias. Our data show that women exposed to sulfamethizole, as a proxy for urinary tract infection, during the first trimester and women exposed to trimethoprim before pregnancy did not have an increased HR of miscarriage. Therefore, we do not believe our results are due to selection bias.

The Prescription Register lacks information of treatment indication, for which reason we cannot completely rule out that the results are confounded by indication. Moreover, we have no information concerning compliance and the dosage prescribed, although we have knowledge of tablet strength and tablet size prescribed. For this reason we have restricted the analysis to exposed or unexposed. To address these limitations we have adjusted the analyses for risk factors available to us and used two reference groups: unexposed women and sulfamethizole-exposed women.

In addition, information on use of over-the-counter drugs is not available. If depletion of plasma folate induced by trimethoprim is a risk factor for

miscarriage, it would be plausible that supplementation with multivitamins containing folic acid could reduce this risk. Due to the nature of the registers we do not have this information.

Worldwide trimethoprim is used by millions of women. Most women use trimethoprim to treat urinary tract infections. Furthermore, it is recommended that adults living with AIDS and HIV should use trimethoprim as a daily prophylaxis against opportunistic infections. In sub-Saharan Africa this recommendation affects more than 12 million fertile women [25, 26]. Our findings could therefore have consequences for millions of pregnant women exposed to trimethoprim worldwide.

Although we find the results of the present study biologically plausible, it is the first time this hypothesis has been tested, and therefore it needs to be confirmed in other studies. In addition it is important to bear in mind that prophylaxis with trimethoprim-sulfamethoxazole in people living with HIV or AIDS has been shown to reduce mortality and morbidity [27–29].

In conclusion, we have found an association of redeeming a prescription for trimethoprim between conception to the end of the first trimester and a doubling of the hazard for miscarriage. There were no association found between trimethoprim before pregnancy and miscarriage. This calls for further investigation, both epidemiological and *in vivo* animal studies.

DECLARATION OF INTEREST

None.

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