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Tobacco smoking during pregnancy and risk of adverse behaviour in offspring: A follow-up study

Dorte Wiwe Dürr[a], Birgit Bjerre Høyer[a], Line Høgenhof Christensen[a], Henning Sloth Pedersen[b], Andrii Zinchuk[c], Bo AG Jönsson[d], Christian H Lindh[d], Jens Peter Bonde[e], Gunnar Toft[a,f,*]

[a] Danish Ramazzini Center, Department of Occupational Medicine, Aarhus University Hospital, Nørrebrogade 44, build.2c, 8000 Aarhus C, Denmark
[b] Primary Health Care Clinic, Postbox 570, DK-3900 Nuuk, Greenland
[c] Departement of Social Medicine and Organization of Public Health, Kharkiv National Medical University, 61022 Kharkiv, Ukraine
[d] Division of Occupational and Environmental Medicine, Lund University, S-221 85 Lund, Sweden
[e] Department of Occupational and Environmental Medicine, Copenhagen University Hospital, Bispebjerg Bakke 23, 2400 Copenhagen NV, Denmark
[f] Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Alle 43-45, 8200 Aarhus N, Denmark

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ABSTRACT

Objectives: This study examines associations between prenatal exposure to tobacco smoking and adverse behaviour in the offspring.
Methods: We included 1016 pregnant women from Greenland and Ukraine (526 from Greenland and 490 from Ukraine). Serum cotinine measurements were used to identify smoking pregnant women. When the children were from five to nine years of age, the parents assessed the child's behaviour using the Strength and Difficulties Questionnaire (SDQ).
Results: Overall, smoking in pregnancy was not associated with a higher probability of adverse behaviour assessed by the total SDQ score. However, in the crude analysis smoking was associated with a higher mean difference of SDQ-total score. In Greenland the SDQ-total mean difference (MD) was (MD (95% CI) = 1.31 points (0.42; 2.19)) and in Ukraine (MD (95% CI) = 0.18 points (−1.2; 0.91)), whereas the adjusted mean differences were statistically non-significant.
Conclusions: In utero exposure to tobacco smoking was not associated with a significant higher risk of adverse behaviour in the offspring, but elevated risk of adverse behaviour among children prenatally exposed to smoking cannot be excluded.

1. Introduction

Smoking during pregnancy is considered to be a preventable cause of foetal death and is well known to be associated with reduced birth weight, preterm birth and other obstetric difficulties [1–5]. Thousands of chemical compounds are found in cigarette smoke and nicotine is known to be a psychoactive component [6]. In recent years, smoking during pregnancy has been suspected of causing behavioural disorders in children [7]. Animal studies, as well as epidemiological studies have found that nicotine, and its metabolite cotinine is a potent neuroteratologic substance which could potentially damage the normal regulation of the neurotransmitter system [6,8–10]. Nicotine has properties that enable the emulation of the neurotransmitter acetylcholine. The nicotinic acetylcholine receptor (nAChR) triggers developmental events, generally attributed by acetylcholine and creates a deregulation of the transmitter system [3,7,9]. One study which examines the effect on prenatal nicotine exposure on rodents and primates support a teratological effect of nicotine on the developing brain, especially in the areas of the prefrontal cortex [9].

More recently, epidemiological studies have assessed the neurobehavioral changes in brain structure and function in children exposed to prenatal smoking, using ultrasound measurements for the foetal period and magnetic resonance imaging (MRI) scans of children's brains during development [6]. In some studies, prenatal smoking exposure was associated with reduced volume in the cerebellum, the corpus callosum and reduction in parts of the

Abbreviations: SDQ, strength and difficulties questionnaire; ADHD, attention deficit hyperactivity disorder; LOD, lower limits of detection.

* Corresponding author at: Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Alle 43-45, 8200 Aarhus N, Denmark. Fax: +45 87167215.
E-mail address: gunnar.toft@clin.au.dk (G. Toft).

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frontal and temporal lobe [11,12]. Prefrontal abnormalities, as well as irregular and decreased dopamine activity related to the deficit in cognitive and social functions are associated with Attention Deficit Hyperactivity Disorder (ADHD) symptoms [11,13–15].

Previous epidemiological studies have examined the relationship between smoking and adverse behaviour in children [13,16–19]. The majority of the studies are based on case–control studies, cohort studies, and other comparative designs, such as siblings' studies with different smoking exposure and in vitro fertilization [13,16,17,20]. Overall, the previously represented studies show divergent results. Case–control studies mostly provide support for the hypothesis that prenatal exposure to smoking might result in adverse behaviour [21,22]. Studies found from no association, to twofold or fourfold higher risk of attention deficit hyperactivity disorder (ADHD) in offspring exposed to prenatal tobacco smoking [21–23]. The studies had different numbers of cases, exposure information was self-reported and collected retrospectively and the method of case definition was mostly based on the criteria according to Diagnostic and Statistical Manual of Mental Disorders, third edition (DSM-III) [21–23]. Cohort studies also provide support for the hypothesis, but they do not find the same high risk in the offspring, as found in case–control studies [13,16,18,19,24,25]. Several studies found a dose- depended association between exposure to smoking and inattentive and hyperactive behaviour in the offspring [16,18]. Other studies found the same association, but only in boys [26]. To date literature is inconclusive on whether the association between prenatal smoking exposure and behavioural disorders is caused by the teratological effect of nicotine during pregnancy or other aspects. The majority of studies which examines the topic have used different types of smoking assessments, some collected retrospectively other prospectively [13,16,18,24]. Smoking status was also measured differently; some on an ordinal scale, dichotomized and in most cases smoking status was based on self-report [13,16,18,24]. In the literature, self-reported smoking behaviour is described as problematic among pregnant women and previous results may be due to misclassification [7,16,27–30]. It is well known that pregnant women might underreport smoking prenatally because of the social stigma against smoking during pregnancy. Previous research has also found that self-reported smoking data, could be underestimated compared to levels of serum cotinine [19,29–32]. Several studies have concluded that the cotinine value was higher than the self-reported smoking prevalence [29,32]. This suggesting that, using serum cotinine to examine the association between prenatal smoking exposure and adverse behaviour might give a more precise result. At present, only one study has examined the effect of prenatal smoking on behavioural disorders by using serum cotinine to verify maternal smoking. That study did not find a higher risk of adverse behaviour in off-spring. However, the assessment was based on the mothers report using only three items to assess the children's activity level at 5 years of age [19].

The present study is based on assessments of children's behaviour, as observed by the parents. The purpose of this follow-up study was to investigate the associations between prenatal exposure to tobacco smoking measured by serum cotinine levels and subsequent adverse behaviour, including hyperactivity, impulsivity, behavioural and emotional problems in children, aged from five to nine years.

2. Methods

2.1. Study cohort

The current study is based on the INUENDO cohort, a European and Arctic cohort established to study human fertility in relation to biopersistent organochlorines in the environment [33]. The study included women and their offspring from two regions of Europe and Greenland/ the Arctic, in order to evaluate coherence between exposure- outcome associations across European and Greenlandic/Arctic populations [33]. The present study includes data from this cohort collected from pregnant women and their offspring living in Greenland and Ukraine. During the period from May 2002 to February 2004 the pregnant women enrolled in the INUENDO cohort were approached through their obstetric care provider or antenatal health care clinics [33]. The participating women had to be at least eighteen years of age and born in the country of study, to be eligible for the study. At baseline, 665 women were eligible in Greenland of whom 599 (90%) participated. In Ukraine, 2478 women were eligible of whom 651 (26%) participated [33].

At baseline, the 1250 pregnant women (599 from Greenland and 651 from Ukraine) were interviewed regarding socio-demographic, obstetric, psychosocial conditions and lifestyle [33]. To participate in the present study, the women should have contributed with baseline and follow-up information, including a serum sample analysed for cotinine. In total, 1177 pregnant women (569 from Greenland and 608 from Ukraine) agreed to provide a blood sample. A follow-up was conducted from January 2010 to May 2012, when the children were from five to nine years old. The parents' responded questions in face- to- face interviews concerning behaviour and other characteristics. A total of 1016 mothers (526 from Greenland and 490 from Ukraine) gave permission for follow-up and only single born children were included in the follow-up. In all, 1016 participated with serum cotinine and information concerning parentally assessed child behaviour (Fig. 1).

The participation in the project was voluntary and participants did not receive any compensation for participating. The participating mothers received written and oral information on the purpose of the study, and they gave a written informed consent. Approval of the study was obtained from Ethical Committee for Human Research in Greenland (approval no. 2010-13) and the commission on Ethics and Bioethics Kharkiv National Medical University in Ukraine (protocol number 7, October 2009).

2.2. Maternal serum cotinine measurement

We used maternal serum cotinine as a proxy for foetal exposure to tobacco smoke compounds. Cotinine is the primary metabolite of nicotine, therefore cotinine levels also can be elevated in users of nicotine- containing products such as nicotine gum [32]. It is widely applied as a marker of tobacco use because it has a longer half-life (18–20 h) than nicotine (2–3 h) [7,31,35]. Thus, it becomes possible to estimate whether the women had been smoking within the last two or three days [7,35]. Serum cotinine was dichotomized into $\leq$10 ng/ml defined as non-smokers and >10 ng/ml defined as smokers [36]. Information on women's smoking during pregnancy was obtained at baseline by measuring a biochemical serum cotinine level, when the women were in 2nd or 3rd trimester. In Greenland the samples were collected at median 24.7 weeks of pregnancy, 25th–75th percentile (18.0–32.5) and in Ukraine at median 22.8 weeks of pregnancy, 25th–75th percentile (12.7–33.3).

The collected blood samples were centrifuged and serum was immediately frozen to −20 °C. The samples were shipped on dry ice to the University Hospital in Lund, Sweden for further analyses [36]. The serum samples were analysed using a liquid chromatography connected to a hybrid triple quadruple linear ion trap tandem mass spectrometer (LC/MS/MS) (QTRAP 5500 AB Sciex, Foster City, CA, USA) [37]. Deuterium labelled internal standard were added to the serum samples and were treated with glucuronidase thereafter the proteins were precipitated using acetonitrile [37]. The supernatant was analysed by LC/MS/MS [37]. The limit of detection (LOD)