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Prevalence of medication and psychoactive substances in first-trimester blood samples from Danish pregnant women

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Abstract

Objective: The aim of this study was to investigate the prevalence of medicine and psychoactive substances such as caffeine, nicotine and illicit drugs in blood samples of first-trimester Danish pregnant women unaware of the screening.

Design: A cross-sectional study, including 436 unselected anonymized residual blood samples obtained during 2014 as part of the nationwide prenatal first-trimester screening program. The samples were analyzed by ultra-performance liquid chromatography with high-resolution time-of-flight mass spectrometry (UPLC-HR-TOFMS).

Setting: The antenatal clinic in a Danish city with 62,000 inhabitants where more than 95% of the pregnant women joined the screening program.

Primary and secondary outcome measures: The prevalence and patterns of caffeine, nicotine, medication and illicit drug intake during the first trimester of pregnancy.

Results: The prevalence of prescription and over-the-counter drugs was 17.9%, including acetaminophen (8.9%) and antidepressants (3.0%) of which citalopram (0.9%) was the most frequent. The prevalence of illegal drugs was 0.9%, indicators of smoking (nicotine/cotinine) 9.9%, and caffeine 76.4%. Only 17.4% had no substance identified in their sample.

Conclusions: The study emphasizes the need for further translational studies investigating lifestyle habits during pregnancy and the underlining molecular mechanisms through which xenobiotic substances may affect placental function and fetal developement.

Keywords: pregnancy, medicine, drug abuse, acetaminophen, caffeine, nicotine

STRENGTH AND LIMITATIONS OF THE STUDY

- The pregnant women were unaware of the screening, eliminating information and re-call bias as the blood samples analyzed were anonymized sur-plus blood samples from the prenatal screening

- The UPLC-HR-TOFMS is a broad targeting method which is validated for the 225 most toxicologically relevant drugs and metabolites and is also supplying the pattern of co-exposure as all substances are detected at once.
- As the study was based on anonymous blood samples we have no opportunity to follow the children prospectively.
- We only have a single blood sample from the first trimester and none from the late pregnancy which would have given further information by confirming exposure patterns and/or identify changes in exposure during pregnancy

INTRODUCTION

Since the thalidomide catastrophe in the 1960's [1], the focus on avoiding teratogenic exposures has increased, but with the Developmental Origins of Health and Disease Hypothesis (DOHaD) [2, 3] the need for a deeper understanding of less evident treats to fetal health has become more apparent. According to DOHaD, early pregnancy constitutes a highly sensitive period, during which environmental factors can affect fetal development. The intrauterine environment is thus believed to "program" the fetus and placenta through subtle molecular changes i.e. chances of the epigenome that alter gene regulation and affect disease risk in later life [2-4]. Such epigenetic alterations have already been linked to the development of many chronic diseases including type 2-diabetes [5] and cardio-vascular illnesses [6]. To unravel potential links between disease development in later life and epigenetic changes a better understanding of the exposures affecting pregnant women of today is needed.

The use of prescription medication in the first trimester pregnancy has increased more than 60% in the past three decades [7]. Previous international studies have found that 79-94% of all pregnant women reported having taken some form of medication at least once during their pregnancy [7-10]; most frequently (66.9 %) over-the-counter drugs, but as many as 17% received treatment for a long-term or chronic disorder [8]. Cultural differences in the use of medicine and other potential harmful exposers is evident. In the USA and the Western Europe, it is estimated that 15-25% of young women in the fertile age are habitual smokers [11-15], although recent data from the Danish Health Protection Agency found that only 12% of pregnant women smoke daily [16]. Many adverse effects to smoking are known [17-23]; however, the patterns of co-exposure between smoking and other xenobiotics not well-described hereby making it difficult to track harmful combinations and decribe the mechanisms involved. Also, the prevalence of harmful exposures like illicit drug use during pregnancy is relatively unknown in a Danish setting, despite the knowledge that numerous adverse pregnancy outcomes are related to drug abuse [11, 14]. The latest Danish population survey from 2013 found that 46% of young Danes under the age of 35 years had experimented with cannabis and approximately 9% of them had at least once tried other illegal drugs beside cannabis, with cocaine and amphetamine as the most commonly used drugs [24]. An increasing use of certain forms of psycho-stimulants like methylphenidate (e.g. Ritalin®) has also been reported among Danish pregnant women [25]. Epidemiological studies link intrauterine exposure to ADHD medications (methylphenidate or atomoxetin) to lower Apgar score at birth and an increased risk of miscarriage [26]. This underlines the need for more knowledge of illicit drug use during pregnancy.

Most previous studies investigating the use of medication during pregnancy are based upon retrospective self-report or search in national prescription registries and may thus be affected by recall and selection bias. Hence, the aim of this study was to characterize the use of prescription type medicine, over-the-counter medicine and psychoactive substances in the blood of first-trimester-pregnant Danish women unaware of the screening. We aimed to investigate both the prevalence and pattern of substance use in pregnant women including the use of over-the-counter medications, which has not been otherwise described when using the national prescription registries.

MATERIALS AND METHODS

Design and study group

The study was designed as a cross-sectional study, in which the study population consisted of the pregnant women from the municipality Randers, Denmark, who had participated in the national prenatal screening program in gestational week 8-13. The remains of the blood samples (for the double test) collected as part of this screening program are always stored in a biobank at minus 80°C [27] for quality control purposes. Blood samples analyzed the first 8 days of each month in 2014 were included in this study.

All blood samples were drawn either by family general practitioners (GPs) or in outpatient settings connected to Randers Regional Hospital. Serum was isolated and samples shipped to the Department of Clinical Biochemistry, Aarhus University Hospital, within four hours, re-centrifuged and stored at 4 degrees until the double test analysis were performed 12-36 hours after collection. The remains of the samples were stored in the biobank at -80°C [27] until our analysis was performed in the spring of 2016.

Pregnant women who had participated in the prenatal screening program and attended the nuchal translucent scan with risk assessment in 2014 were identified using a treatment code. Their blood samples were subsequently identified in the biobank through barcode numbers, anonymized and delivered for analysis. A total of 436 samples fitting the inclusion criteria were analyzed in this study. This corresponds with 23.5% of the participants in first trimester nuchal translucent scans at Randers Regional Hospital in 2014.

Ethical aspects

The study was approved by the Central Denmark Region Committee on Biomedical Research Ethics (1-10-72-22-16) and the Danish Data Protection Agency (1-16-02-255-16). All data were handled and stored de-identified.

Sample and data analysis

Serum samples were thawed, and 300 µl of each serum sample were transferred to an Eppendorf plate and analyzed for the presence of substances as described in the paper by Telving et al. [28]. Approximately 500 different substances comprising toxic compounds, illegal and legal drugs and some of their metabolites can be identified using protein precipitation followed by UPLC-HR-TOFMS analysis [28]. The method is validated for the 225 most toxicologically relevant drugs and

metabolites. The presence of a substance and all related metabolites from each blood sample were counted as one exposure in a given sample e.g. nicotine and/or cotinine as indicators of smoking. In some cases, substances were identified solely based on the presence of their metabolites (e.g. benzoylecgonin, metabolite of cocaine).

The laboratory is accredited by an external independent organization, DANAK (Danish Accreditation), and participates in various screening and quantification proficiency tests. All results from the UPLC-HR-TOFMS analysis were evaluated by two experienced persons at the Section for Forensic Chemistry as described by Telving et al. [28].

RESULTS

In 360 (82.6%) of the 436 serum samples from unselected first trimester pregnant women, we identified either caffeine, medication, indicators of smoking, illicit drugs, or a combination of these. Thus, only in 17.4% of the samples no trace of the exogenous substances was detected. However, in 62% of samples only one substance was indentified whereas 16.2% contained two substances, and 3.6% of the samples contained evidence of three substances or more. Table 1 shows all the indentified substances.

TABEL 1: Substances identified

Pharmacological groups	Number of women	Percentage of women	Recommendations during pregnancy from promedicine.dk (Danish website with information for doctors)
Analgesics	**51**	**11.7%**	
- Acetaminophen (paracetamol)	41	9.4	Can be used if necessary
- Codeine	5	1.1%	Should not be used
- Ibuprofen	2	0.5%	Only under certain conditions
- Lidocaine + prilocaine (Emla®[a])	1	0.2%	Can be used if necessary
- Morphine (from codeine[b])	1	0.2%	Should not be used
- Salicylic acid (from acetylsalicylic acid)	2	0.5%	Only under certain conditions
- Tramadol	1	0.2%	Only under certain conditions
Antidepressants	**13**	**3.0%**	
- Amitriptyline	2	0.5%	Can be used if necessary
- Citalopram	4	0.9%	Can be used if necessary
- Fluoxetine	2	0.5%	Only under certain conditions
- Sertraline	3	0.7%	Can be used if necessary
- Venlafaxine	2	0.5%	Can be used if necessary
Antipsychotics	**3**	**0.7%**	
- Chlorprothixene (first generation, HD)	1	0.2%	Should not be used
- Quetiapine (second generation)	1	0.2%	Only under certain conditions
- Risperidone (second generation)	1	0.2%	Only under certain conditions
Anxiolytics	**1**	**0.2%**	
- Diazepam	1	0.2%	Only under certain conditions
Antiepileptics	**2**	**0.5%**	
- Gabapentin	1	0.2%	Should not be used
- Lamotrigine	1	0.2%	Only under certain conditions
- Levetiracetam	1	0.2%	Only under certain conditions
- Oxcarbazepine	1	0.2%	Should not be used

Central stimulators	**1**	**0.2%**	
- Methylphenidate (ritalinic acid)[c]	1	0.2%	**Should not be used**
Antihistamines	**8**	**1.8%**	
- Cetirizine	6	1.4%	**Can be used if necessary**
- Cyclizine	1	0.2%	**Only under certain conditions**
- Meclozine	1	0.2%	**Only under certain conditions**
Antidiabetics	**8**	**1.8%**	
- Metformin	8	1.8%	**Only under certain conditions**
Sympathomimetic	**1**	**0,2%**	
- Salbutamol	1	0.2%	**Can be used if necessary**
Quinin (possibly from tonic water)	**2**	**0.5%**	**Only under certain conditions**
Illegal substances	**4**	**0.9%**	
- Cannabis[d] (THC-COOH and THC-COOH-glucuronid)	4	0.9%	
- Amphetamine	1	0.2%	
- Cocaine[e] (benzoylecgonine, levamisole and phenacetin)	1	0.2%	

[a]Emla® is a local anesthetic containing lidocaine and prilocaine. [b]Morphine was found in a serum sample that also contained codeine indicating that the morphine presumably is a metabolite from codeine. [c]Ritalinic acid is a metabolite of methylphenidate indicating use of methylpheniate. [d]The metabolites THC-COOH and THC-COOH glucuronide is detected indicating cannabis use. [e]The metabolite benzoylecgonine and the "cocaine cutting agents" levemisole and phenacetin are detected, indicate cocaine use.

Over-the-counter and prescription medicine

In 17.9% (n=78) of the samples, we identified traces of medicine finding traces of a total of 27 different forms of medications (for details see table 1). The frequency of over-the-counter medications (acetaminophen, ibuprofen, aspirin, analgesic bandages or antihistamines) was 11.9% (n=52), and medication only available with a prescription was found in 7.1% (n = 34) of the samples samples with two or more drugs in 2.1% (n=9) of all samples. A combination of over-the-counter and prescription medicine was found in 1.1% (n=5) of our samples, and the combination of medication and caffeine was present in 13.5% (n=59) of all samples. The frequencies of the different medications are given in table 1 with analgesics (11.7%) including acetaminophen (9.4%) and codeine (1.1%) being the most frequently used drug category.

Psychoactive medications (defined as antidepressants, antipsychotics, anxiolytics, methylphenidate) were identified in 3.7% with antidepressants (3.0%) as the most frequent type and citalopram (0.9%) as the drug most frequently used. Antipsychotic and anxiolytic drugs were identified in 0.7% and 0.2% of the samples respectively. In addition, 0.5% of the samples contained antiepileptic drugs.

Antihistamines (1.8%); antidiabetic drugs (1.8%); and the astma mediciation salbutamol were also identified in (1,8%), (1,8%) and (0.2%), of all samples respectively.

Illicit drugs

We identified Illicit drugs and/or their metabolites in 0.9% (n=4) of the serum samples (table 1). Cannabis was identified in four samples (0.9%). Moreover, amphetamine and benzoylecgonine (a cocaine metabolite) were also present in one of these samples.

Caffeine and nicotine

Caffeine was the most frequent substance used and identified in a total of 76.4% of the samples. The indicators of smoking (nicotine and cotinine, a metabolite of nicotine) were found in 9.9%. Both caffeine, nicotine and cotinine have psychoactive properties and if considered as psychoactive substances alongside psychoactive medications and illicit drugs, as many as 79.4% of the pregnant women had used some form of psychoactive substance.

DISCUSSION

In this study, we found a high prevalence (82.6%) of medicine and psychoactive substances such as caffeine, nicotine and illicit drugs in blood samples from the first trimester Danish pregnant women. To the best of our knowledge, this is the first study investigating prevalence and pattern of xenobiotic substance use in early pregnancy of women unaware of the screening. It also adds to the strength of the study that we investigate samples from Danish pregnant women who have a very high attentdancy (95%) in the prental screening programs [29]. Furthermore, this is the first study using this type of broad targeted substance screening on serum samples using the UPLC-HR-TOFMS analysis which can identify most relevant psychoactive medications, over-the-counter medications and illicit drugs found in the Danish population, albeit, not the use of alcohol [28].

Importantly, the cross-sectional design reflects only the use within a short period of time, and it should be remembered that habits may change during the pregnancy. Moreover, differences in pharmacokinetics of various xenobiotics could also affect the likelyhood of identifying a given substance. However, knowing that the first trimester is the most sensitive and critical period in fetal development, it still calls for reflection that only 17.4% of the pregnant women had no evidence of xenobiotic substances in their blood, indicating that early intervention is needed to prevent or regulate the use of medication during pregnancy. Due to ethical considerations, this analysis of samples from women unaware of the screening required anonymization of the samples hence limiting the present study by preventing the inclusion of perinatal outcomes in the analysis, which would of course have been highly relevant in this context.

Pharmacological compounds available as over-the-counter drugs were the most frequent finding in this study, and we detected acetaminophen in 9.4% of the samples. This raises concern as the use of acetaminophen during pregnancy might be associated with an increased risk of asthma [30], cryptorchidism [31, 32], autism, hyperkinetic disorders and ADHD-like behavior in children [33, 34] all though not associated with major birth defects [35]. Thus, the high prevalence of acetaminophen use in this and other studies [32-35] raises concern emphasizing the need for further research into the more subtle effects of acetaminophen on human placenta and early development.

The frequency of iprobufen and other over-the counter drugs of the NSAID family associated with adverse outcomes such as increased risk of miscarriage [36-38] and congenital cardiac defects [39, 40] was however low (0.5%). Also, the frequency of antihistamines was only 1.8% which we find surprisingly low as it is recommended for common conditions like hyperemesis and allergies and reported widely use in previous studies [41].

In the present study, prescription drugs were found in 7.1 % of the samples, whereas other European studies have found a 79% prevalence of prescription drug use during pregnancy with up to 48% of all pregnant women receiving a prescription in first trimester [7, 9, 10, 42]. Likely, this partly reflects the design of this study showing only a snapshot of the use of medicine among Danish pregnant women however the fact that certain drug types such as antibiotics and antiemetic drugs are not detected in with our analysis most likely also contributes to the difference.

The most frequent group of prescription drugs in our study was antidepressants with a frequency of 3%, however, this is considerably lower than that in a US study were 6.6% of the pregnant women used antidepressants [43]. It remains uncertain whether prenatal exposure to antidepressents and other psychoactive substances can impair brain development, cause postnatal neurobehavioral differences [26, 44] or increase the risk of miscarriage [25, 26, 45-48] and major malformation [49-55], stillbirth and preterm birth [56]. The continued use among the pregnant population thus calls for continued optimization of obstetric guidelines in the years to come. Reflecting the use of such guidelines, metformin was only identified in 1.8% of our samples, which is in accordiance with the Danish guidelines advocating discontinuation during pregnancy when used for diabetes or PCOS.

The presence of illicit drugs (0.9%) in our population appears relatively low compared to international studies finding frequencies of up to 4.4% [11, 14]. A Danish study from 1998 found that 2.1% of Danish pregnant women had used cannabis and that 0.2% had used other illicit drugs within the last three months before recognition of pregnancy [57]. Naturally, some form of “healthy worker selection” or change of abuse pattern prior to the doctor’s visit cannot be excluded.

Our findings show a high frequency of caffeine (76.4%) even though only 40 % of Danish pregnant women reported drinking coffee in previous studies [58, 59]. This descrepancy may be explained by the intake of caffeine-containing beverages and energy drinks. Regardless the source, caffeine may affect the fetus as it crosses the placenta [60] and can cause miscarriage [61, 62], fetal growth restriction [62-67], and childhood leukemia [68]. Furthermore, animal studies indicate that caffeine exposure might potentiate the adverse effects of some medications [69, 70].

We found evidence of (habitual) cigarette smoking in almost 10% of the samples despite the fact that smoking in pregnancy is a well-known risk factor [71] associated with an increased risk of placenta dysfunction [18] and adverse birth outcomes such as low birth weight and preterm birth [17, 19]. As a previous report stated that 8% of pregnant women continue to smoke throughout pregnancy [72], this finding might reflect a continuous increase in smoking cessation during pregnancy. In recent years, prenatal exposure to cigarette smoke has also been associated with development of diseases in later life such as asthma and allergy [73], metabolic diseases [74] and certain cancer forms [17]. Moreover, prenatal exposure to cigarette smoke has been associated with behavioral deficiencies and neuropsychiatric disorders [20-23]. Many smokers reduce or quit smoking during pregnancy [72], however, even short term exposure during the first trimester might still result in adverse effects on the fetus. In this respect, first trimester exposure to maternal cigarette smoke has been shown to reduce the number of gonadal cells in both male and female human fetuses [75]. Thus, our findings underline the importance of maintaining focus on early smoking cessation during pregnancy.

CONCLUSION

With the limitations of being a one-time-only analysis, this study brings unbiased knowledge about medication and substance abuse among Danish pregnant women seeking prenatal diagnostic advise at the end of the first trimester. The quite high use of medications (17.9%) in first trimester found in the present study indicates that early intervention is needed to prevent or regulate the use of medication during pregnancy as well as address the continued exposure to caffeine and smoking in early pregnancy. The study emphasizes the need for further studies on the mechanistic effects of xenobiotic exposures during pregnancy as both legal and illegal drugs may be teratogenic or have adverse effects on placental function and finally subtle effects as epigenetic alterations.

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Foot notes

Contributors: Project design: NU, PB, IL, AL, MFA. Management of bloodsample and data selection: NT. Blood analyzing: SA, MFA, RT. Data analyzing: SA, AV under supervision of PB, IL, AL, MFA, NU. Drafting of the manuscript: SA, AV. Critical revision of the manuscript: PB, IL, AL, NU, MFA. All authors have commented on the manuscript.

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Competing interests The authors declare that no conflicts of interests exists regardning the context of this manuscript.

Ethics approval The study was approved by the Central Denmark Region Committee on Biomedical Research Ethics (1-10-72-22-16) and the Danish Data Protection Agency (1-16-02-255-16).

Data sharing statement We do not have any unpublished data, except from what is in this article.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2-3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	3-4
Bias	9	Describe any efforts to address potential sources of bias	*3-4*
Study size	10	Explain how the study size was arrived at	3-4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	3-4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	3-4
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	3
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	3-5
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	5-7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-7
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	5-8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-9
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Prevalence of xenobiotic substances in first-trimester blood samples from Danish pregnant women: a cross-sectional study

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Title: Prevalence of xenobiotic substances in first-trimester blood samples from Danish pregnant women: a cross-sectional study

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Prevalence of xenobiotic substances in first-trimester blood samples from Danish pregnant women: a cross-sectional study

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Abstract

Objective: The aim of this study was to investigate the prevalence of xenobiotic substances, such as caffeine, nicotine and illicit drugs (e.g., cannabis and cocaine), in blood samples from first-trimester Danish pregnant women unaware of the screening.

Design: A cross-sectional study examined 436 anonymized residual blood samples obtained during 2014 as part of the nationwide prenatal first-trimester screening program. The samples were analyzed by ultra-performance liquid chromatography with high-resolution time-of-flight mass spectrometry (UPLC-HR-TOFMS).

Setting: An antenatal clinic in a Danish city with 62,000 inhabitants, where more than 95% of pregnant women joined the screening program.

Primary and secondary outcome measures: The prevalence and patterns of caffeine, nicotine, medication and illicit drug intake during the first trimester of pregnancy.

Results: The prevalence of prescription and over-the-counter drug detection was 17.9%, including acetaminophen (8.9%) and antidepressants (3.0%), of which citalopram (0.9%) was the most frequent. The prevalence of illegal drugs, indicators of smoking (nicotine/cotinine), and caffeine was 0.9%, 9.9%, and 76.4%, respectively. Only 17.4% of women had no substance identified in their sample.

Conclusions: This study emphasizes the need for further translational studies investigating lifestyle habits during pregnancy, as well as the underlying molecular mechanisms through which xenobiotic substances may affect placental function and fetal development.

Keywords: pregnancy, medicine, drug abuse, acetaminophen, caffeine, nicotine

STRENGTHS AND LIMITATIONS OF THE STUDY

- The pregnant women were unaware of the screening, eliminating information and recall bias as potential sources of error, as the blood samples analyzed were anonymized surplus blood samples from the prenatal screening program.

- UPLC-HR-TOFMS is a broad targeting method that has been validated for the 225 most toxicologically relevant drugs and metabolites that also supplies patterns of co-exposure, as all substances are detected at once.
- As the study was based on anonymous blood samples, we had no opportunity to follow the children prospectively.
- We only had a single blood sample from the first trimester and no samples from late pregnancy, which would have provided further information by confirming exposure patterns and/or identifying changes in exposure during pregnancy.

INTRODUCTION

Since the thalidomide catastrophe of the 1960s [1], in which a seemingly safe antiemetic drug caused major congenital malformations in children worldwide, focus on avoiding teratogenic exposures during pregnancy has increased. However, with the Developmental Origins of Health and Disease Hypothesis (DOHaD) [2, 3], the need for a deeper understanding of less-recognized threats to fetal health has become more apparent. According to the DOHaD, early pregnancy constitutes a highly sensitive period during which environmental factors can affect fetal development. The intrauterine environment is thus believed to "program" the fetus and placenta through subtle molecular changes, i.e., changes of the epigenome that alter gene regulation and affect disease risk later in life [2-4]. Such epigenetic alterations have already been linked to the development of many chronic diseases, including type 2-diabetes [5] and cardiovascular illnesses [6]. To unravel potential links between disease development later in life and epigenetic changes, a better understanding of the exposures currently affecting pregnant women is needed. In this respect, attention should be focused on all xenobiotic substances (substances not normally found in the human body, ranging from medications to nicotine or caffeine), which might constitute a potential health risk during pregnancy or later in the life of the child.

The use of prescription medications in the first trimester of pregnancy has increased more than 60% over the past three decades [7]. Previous international studies have found that up to 94% of all pregnant women take some form of medication at least once during their pregnancy [7-9]; most frequently over-the-counter drugs (66.9%), but also treatments for long-term or chronic disorders (as much as 17% of women) [8]. Cultural differences in the use of medicines and other potential harmful exposures are evident. In the USA, it is estimated that 17-25% of young women of fertile age are habitual smokers [10-12]. However, recent data from the Danish Health Protection Agency found that only 12% of Danish pregnant women smoke daily [13]. Many adverse effects of smoking are well-known [14-17], but the patterns of co-exposure between smoking and other xenobiotics are not well-described, making it difficult to track harmful combinations and describe the mechanisms involved. Additionally, the prevalence of harmful exposures such as illicit drug use during pregnancy is relatively unknown in the Danish setting, despite the fact that numerous adverse pregnancy outcomes are related to drug abuse [12, 18]. The latest Danish population survey from 2013 found that 46% of young Danes under the age of 35 had experimented with cannabis and approximately 9% of them had at least once tried other illegal drugs in addition to cannabis, with cocaine and amphetamines being the most commonly reported [19]. An increasing use of certain forms of psycho-stimulants such as methylphenidate

(e.g., Ritalin®) has also been reported among Danish pregnant women [20]. Epidemiological studies have linked intrauterine exposure to ADHD medications (methylphenidate or atomoxetine) with lower Apgar scores at birth and an increased risk of miscarriage [21]. This underscores the need for more knowledge of illicit drug use during pregnancy.

The majority of previous studies investigating the use of medication during pregnancy have been based upon retrospective self-reporting or searching in national prescription registries, and may thus be affected by recall and selection bias. Hence, the aim of this study was to characterize the use of prescription-type medicine, over-the-counter medicine and psychoactive substances in the blood of first-trimester-pregnant Danish women who were unaware of the screening. We aimed to investigate both the prevalence and pattern of xenobiotic substance use, such as medicines, caffeine, nicotine and illicit drugs (e.g., cannabis and cocaine), in pregnant women. We also aimed to include the use of over-the-counter medications, which has not been otherwise described when using the national prescription registries.

MATERIALS AND METHODS

Design and study group

The study was designed as a cross-sectional study, in which the study population consisted of the pregnant women from the municipality of Randers, Denmark who had participated in the national prenatal screening program between gestational week 8-13. As part of the screening program, blood samples (for the double test) are always drawn and the unused portion of the samples is stored in a biobank at minus 80°C [22] for quality control purposes. All women with residual blood in the biobank were theoretically eligible to participate in the study. However, our inclusion criteria were that the original double test analysis must have occurred during the first 8 days of each month in 2014. Each woman contributed one sample. There were no specific exclusion criteria.

All blood samples were drawn either by family general practitioners (GPs) or in outpatient settings connected to Randers Regional Hospital. Serum was isolated and samples were shipped to the Department of Clinical Biochemistry, Aarhus University Hospital, within four hours, re-centrifuged and stored at 4°C until the double test analysis could be performed, 12-36 hours after sample collection. The unused portions of the samples were stored in the biobank at -80°C [22] until our analysis was performed in the spring of 2016.

Pregnant women who had participated in the prenatal screening program and attended the first trimester scan with risk assessment in 2014 were identified using a treatment code. Their blood samples were subsequently identified in the biobank through barcode numbers, anonymized and delivered for analysis. A total of 436 samples fitting the inclusion criteria were analyzed in the present study. This corresponds to 23.5% of the pregnant women who underwent first trimester nuchal translucent scans at Randers Regional Hospital in 2014.

Ethical aspects

The study was approved by the Central Denmark Region Committee on Biomedical Research Ethics (1-10-72-22-16) and the Danish Data Protection Agency (1-16-02-255-16). All data were handled and stored in a de-identified manner.

Sample and data analysis

Serum samples were thawed, and 300 µl of each serum sample were transferred to an Eppendorf plate and analyzed for the presence of substances using a forensic analytical method as described by Telving et al. [23]. Approximately 500 different substances comprising toxic compounds, illegal and legal drugs and several of their metabolites could be identified using protein precipitation followed by UPLC-HR-TOFMS analysis [23]. A list of the substances included in this method can be found in the supplementary section of the methods in the article by Telving et al. [23]. This method has been validated for the 225 most toxicologically relevant drugs and metabolites. The supplementary section [23] also summarizes the cut-off values for these substances in antemortem whole blood. The method cannot detect alcohol or all types of medications used by pregnant women, i.e., only certain antiemetic drugs (e.g., metoclopramide and ondansetron) and the most frequently used antihypertensive drugs (e.g., amlodipine, furosemide, diltiazem and metoprolol). Furthermore, antibiotics and thyroid medications, such as levothyroxine, could not be assessed by the UPLC-HR-TOFMS analysis.

The presence of a substance and all related metabolites from each blood sample were counted as one exposure in a given sample, e.g., nicotine and/or cotinine as indicators of smoking. In several cases, substances were identified solely based on the presence of their metabolites (e.g., benzoylecgonine, a metabolite of cocaine).

The analyzing laboratory is accredited by an external independent organization, DANAK (Danish Accreditation), and participates in various screening and quantification proficiency tests. All results from the UPLC-HR-TOFMS analysis were evaluated by two experienced persons at the Section for Forensic Chemistry, as described by Telving et al. [23].

RESULTS

We found the prevalence of xenobiotic substances (medications, caffeine, nicotine and illicit drugs) in first trimester Danish pregnant women to be 82.6%. Thus, only in 17.4% of the samples was no trace of any exogenous substance detected. However, in 62% of samples, only one substance was identified, whereas 16.2% contained two substances, and 3.6% of the samples contained evidence of three or more substances. Table 1 shows all the identified substances. As several of the women had traces of more than one substance, these women are counted more than once in Table 1.

TABLE 1: Substances identified

Pharmacological groups	Number of women	Percentage of women	Recommendations during pregnancy from promedicin.dk[1]

Analgesics	**51**	**11.7%**	
- Acetaminophen (paracetamol)	41	9.4	**Can be used if necessary**
- Codeine			
- Ibuprofen	5	1.1%	**Should not be used**
- Lidocaine + prilocaine (Emla®[a])			
- Morphine (from codeine[b])	2	0.5%	**Only under certain conditions**
- Salicylic acid (from acetylsalicylic acid)			
- Tramadol	1	0.2%	**Can be used if necessary**
	1	0.2%	**Should not be used**
	2	0.5%	**Only under certain conditions**
	1	0.2%	**Only under certain conditions**
Antidepressants	**13**	**3.0%**	
- Amitriptyline	2	0.5%	**Can be used if necessary**
- Citalopram			
- Fluoxetine	4	0.9%	**Can be used if necessary**
- Sertraline			
- Venlafaxine	2	0.5%	**Only under certain conditions**
	3	0.7%	**Can be used if necessary**
	2	0.5%	**Can be used if necessary**
Antipsychotics	**3**	**0.7%**	
- Chlorprothixene (first generation, HD)	1	0.2%	**Should not be used**
- Quetiapine (second generation)			
- Risperidone (second generation)	1	0.2%	**Only under certain conditions**
	1	0.2%	**Only under certain conditions**
Anxiolytics	**1**	**0.2%**	
- Diazepam	1	0.2%	**Only under certain conditions**
Antiepileptics	**2**	**0.5%**	
- Gabapentin	1	0.2%	**Should not be used**
- Lamotrigine			
- Levetiracetam	1	0.2%	**Only under certain conditions**
- Oxcarbazepine			
	1	0.2%	**Only under certain conditions**
	1	0.2%	**Should not be used**
Central stimulators	**1**	**0.2%**	
- Methylphenidate (ritalinic acid)[c]	1	0.2%	**Should not be used**
Antihistamines	**8**	**1.8%**	

- Cetirizine	6	1.4%	**Can be used if necessary**
- Cyclizine			
- Meclozine	1	0.2%	**Only under certain conditions**
	1	0.2%	**Only under certain conditions**
Antidiabetics	**8**	**1.8%**	
- Metformin	8	1.8%	**Only under certain conditions**
Sympathomimetic	**1**	**0,2%**	
- Salbutamol	1	0.2%	**Can be used if necessary**
Quinin (possibly from tonic water)	**2**	**0.5%**	**Only under certain conditions**
Illegal substances	**4**	**0.9%**	
- Cannabis[d] (THC-COOH and THC-COOH-glucuronide)	4	0.9%	
- Amphetamine			
- Cocaine[e] (benzoylecgonine, levamisole and phenacetin)	1	0.2%	
	1	0.2%	

[1]Promedicin.dk is a Danish website developed by Danish Medicine Information for medical doctors and other healthcare personnel and contains detailed information on all marketed human medications. It is the most frequently used source of information on medication safety among Danish health professionals. [a]Emla® is a local anesthetic containing lidocaine and prilocaine. [b]Morphine was found in a serum sample that also contained codeine, suggesting that the morphine could be a metabolite from codeine. [c]Ritalinic acid is a metabolite of methylphenidate, indicating the use of methylphenidate. [d]The detected THC-COOH and THC-COOH glucuronide metabolites indicate the use of cannabis. [e]The detection of the metabolite benzoylecgonine and the "cocaine cutting agents" levamisole and phenacetin indicate cocaine use.

Over-the-counter and prescription medicine

In 17.9% (n=78) of the samples, we identified traces of medicine; a total of 27 different forms of medications were identified (see Table 1 for details). The prevalence of over-the-counter medications (acetaminophen, ibuprofen, aspirin, analgesic bandages or antihistamines) was 11.9% (n=52), and the prevalence of medication available only with a prescription in Denmark was 7.1% (n = 34). Samples with two or more drugs accounted for 2.1% (n=9) of all samples. A combination of over-the-counter and prescription medicine was found in 1.1% (n=5) of our samples, and the combination of medication and caffeine was present in 13.5% (n=59) of all samples. The frequencies of the different medications are given in Table 1, with analgesics (11.7%) including acetaminophen (9.4%) and codeine (1.1%) being the most frequently used drug category.

Psychoactive medications (defined as antidepressants, antipsychotics, anxiolytics, methylphenidate) were identified in 3.7% of samples, with antidepressants (3.0%) being the most frequent type and citalopram (0.9%) being the drug most frequently used. Antipsychotic and anxiolytic drugs were identified in 0.7% and 0.2% of the samples, respectively. In addition, 0.5% of the samples contained antiepileptic drugs.

Antihistamines, antidiabetic drugs, and the asthma medication salbutamol were also identified in 1.8%, 1.8%, and 0.2% of all samples, respectively.

Illicit drugs

We identified illicit drugs (including cannabis) and/or their metabolites in 0.9% (n=4) of the serum samples (Table 1). Cannabis was identified in all four illicit drug-positive samples (0.9%). Moreover, amphetamine and benzoylecgonine (a cocaine metabolite) were also present in one of these samples.

Caffeine and nicotine

Caffeine was the most frequent substance used, identified in a total of 76.4% of the samples. Indicators of smoking (nicotine and cotinine, a metabolite of nicotine) were found in 9.9% of samples. Caffeine, nicotine and cotinine have psychoactive properties and, if considered as psychoactive substances alongside psychoactive medications and illicit drugs, as many as 79.4% of the pregnant women had used some form of psychoactive substance.

DISCUSSION

In this study, we found a high prevalence (82.6%) of xenobiotics (medicine, caffeine, nicotine and illicit drugs) in blood samples from first trimester Danish pregnant women. To the best of our knowledge, this is the first study investigating the prevalence and pattern of xenobiotic substance use in early pregnancy in women unaware of the screening. This study was strengthened by the inclusion of samples from Danish pregnant women who generally had a very high attendance rate (95%) in prenatal screening programs [24]. Furthermore, this is the first study using this type of broad targeted substance screening on serum samples using UPLC-HR-TOFMS analysis, which can identify most relevant psychoactive medications, over-the-counter medications and illicit drugs found in the Danish population, although not the use of alcohol [23].

Importantly, the cross-sectional study design only reflects the substance use within a short period of time, and it should be considered that habits may change during the pregnancy. Moreover, differences in the pharmacokinetics of various xenobiotics could also affect the likelihood of identifying a given substance. However, as the first trimester is the most sensitive and critical period in fetal development, it is critical to note that only 17.4% of pregnant women analyzed in this study had no evidence of xenobiotic substances in their blood, indicating that early intervention is needed to prevent or regulate the use of medications during pregnancy. Due to ethical considerations, this analysis of samples from women unaware of the screening required anonymization of the samples; hence, the present study is limited by the prevention of inclusion of perinatal outcomes in the analysis, which would very likely have been highly relevant in this context.

Pharmacological compounds available as over-the-counter drugs were the most frequent finding in this study. Acetaminophen was identified in a total of 9.4% of the samples. This finding raises concerns, as the use of acetaminophen during pregnancy might be associated with an increased risk of asthma [25], cryptorchidism [26, 27], autism, hyperkinetic disorders and ADHD-like

behavior in children [28, 29], despite not being associated with major birth defects [30]. Thus, the markedly high prevalence of acetaminophen use in this and previous studies [27-30] emphasizes the need for further research concerning the more subtle effects of acetaminophen in the human placenta and early development.

The prevalence of ibuprofen and other over-the counter drugs of the NSAID family associated with adverse outcomes, such as increased risk of miscarriage [31, 32] and congenital cardiac defects [33], was low (0.5%). Similarly, the frequency of antihistamine use was only 1.8%, which we consider surprisingly low as they are recommended for common conditions such as hyperemesis and allergies and have previously been reported to be widely used [34].

In the present study, prescription drugs were found in 7.1% of the samples; other European studies have found a 79% prevalence of prescription drug use during pregnancy, with up to 48% of all pregnant women receiving a prescription in first trimester [7, 35]. This value likely reflects the design of this study, showing only a snapshot of the use of medicine among Danish pregnant women. The fact that certain types of drugs, such as antibiotics and antiemetic drugs, are not detected in the analytical method used [28] also contributes to the differences between these and previously reported results. The analytical method used includes substances that are relevant in forensic cases.

The most frequent group of prescription drugs identified in our study was antidepressants, with a frequency of 3%. However, this was considerably lower than in a US study, which found that 6.6% of pregnant women used antidepressants [36]. It remains unclear whether prenatal exposure to antidepressants and other psychoactive substances can impair brain development, cause postnatal neurobehavioral differences [21, 37] or increase the risk of miscarriage [38-40], major malformation [41-43], stillbirth and preterm birth [44].

Among the prescription drugs identified, several are suspected of having adverse effects on fetal development and are thus not recommended for pregnant women (Table 1). However, many of these drugs are also vital in the treatment of chronic diseases, highlighting the complexity of the safe use of medications in early pregnancy. Studies have shown that insufficient treatment of conditions such as depression [39], psychosis [45], epilepsy [46] or inflammatory bowel [47] disease during pregnancy can have negative effects on both the mother and child. Thus, when treating psychiatric patients or patients with other chronic diseases who are pregnant, it is important to consider the potential teratogenicity with the risk associated with the untreated diseases [39, 45, 47]. However, a deeper understanding of lifestyle patterns and the intake of non-prescription medications is needed to limit the risks to necessary medical treatments, just as emphasis should be placed on avoiding unnecessary multi-drug treatments. The use of medications among the pregnant population calls for continued optimization of obstetric guidelines. Reflecting the use of such guidelines, metformin was only identified in 1.8% of our samples, which is in accordance with the Danish guidelines advocating discontinuation during pregnancy when used for treatment of diabetes or PCOS.

The presence of illicit drugs (0.9%) in our population appears relatively low compared to previous international studies, which have reported frequencies of up to 4.4% [12, 18]. A Danish study from 1998 found that 2.1% of Danish pregnant women had used cannabis, and 0.2% had used other

illicit drugs within the last three months before recognition of pregnancy [48]. Naturally, some form of "healthy worker selection" or change of abuse pattern prior to the doctor's visit cannot be excluded, as cannabis is currently only approved for a severely limited number of conditions in Denmark and is presumably not used in the treatment of pregnant women. Thus, in other countries in which medical cannabis is legal, the prevalence rate among pregnant women may likely be higher.

Our findings show a high frequency of caffeine (76.4%), even though only 40% of Danish pregnant women reported drinking coffee in previous studies [49, 50]. This discrepancy may be explained by the intake of caffeine-containing beverages and energy drinks. Regardless of the source, caffeine may affect the fetus as it crosses the placenta [51], and can cause miscarriage [52, 53], fetal growth restriction [54, 55], and childhood leukemia [56]. Furthermore, animal studies indicate that caffeine exposure might potentiate the adverse effects of certain medications [57, 58].

We found evidence of (habitual) cigarette smoking in nearly 10% of the samples, despite the fact that smoking in pregnancy is a well-known risk factor [59] associated with an increased risk of placenta dysfunction [15] and adverse birth outcomes, such as low birth weight and preterm birth [14, 60]. As a previous report stated that 8% of pregnant women continue to smoke throughout pregnancy [61], this finding might reflect a continuous increase in smoking cessation during pregnancy. In recent years, prenatal exposure to cigarette smoke has also been associated with the development of diseases later in life, such as asthma and allergies [62], metabolic diseases [63] and certain forms of cancer [14]. Moreover, prenatal exposure to cigarette smoke has been associated with behavioral deficiencies and neuropsychiatric disorders [16, 17]. Many smokers reduce or quit smoking during pregnancy [61]; however, even short-term exposure during the first trimester may still result in adverse effects on the fetus. In this respect, first trimester exposure to maternal cigarette smoke has been shown to reduce the number of gonadal cells in both male and female human fetuses [64]. Thus, our findings underline the importance of maintaining focus on early smoking cessation during pregnancy.

CONCLUSION

With the recognized limitation of being a one-time-only analysis, this study provides unbiased knowledge regarding medication and substance abuse among Danish pregnant women seeking prenatal diagnostic advise at the end of the first trimester. The fact that almost one in five randomly selected Danish pregnant women displayed traces of medication in a single first trimester blood sample in the present study underscores the critical need for early intervention to prevent or regulate the use of medication during pregnancy, as well as to address continuous exposure to caffeine and smoking in early pregnancy. The study emphasizes the need for further studies on the mechanistic effects of xenobiotic exposures during pregnancy, as both legal and illegal drugs may be teratogenic or have adverse effects on placental function and ultimately have subtle effects on epigenetic alterations.

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Endnotes

Contributors: Project design: NU, PB, IL, AL, MFA. Management of blood samples and data selection: NT. Blood analysis: SKA, MFA, RT. Data analysis: SKA under supervision of PB, IL, AL, MFA, NU. Drafting of the manuscript: SKA, AV. Critical revision of the manuscript: PB, IL, AL, NU, MFA. All authors have commented on the manuscript.

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Competing interests: The authors declare that no conflicts of interests exist regarding the context of this manuscript.

Ethical approval: The study was approved by the Central Denmark Region Committee on Biomedical Research Ethics (1-10-72-22-16) and the Danish Data Protection Agency (1-16-02-255-16).

Data sharing statement: We have no unpublished data. All data are presented in the article.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2-3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	3-4
Bias	9	Describe any efforts to address potential sources of bias	*3-4*
Study size	10	Explain how the study size was arrived at	3-4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	3-4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	3-4
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	3
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	3-5
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	5-7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-7
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	5-8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-9
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.