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Maternal Smoking During Pregnancy and Risk of Appendicitis in the Offspring

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Background: Existing evidence on the link between smoking and appendicitis is scarce and ambiguous. We therefore conducted a population-based cohort study in Denmark to investigate whether smoking during pregnancy is associated with an increased risk of appendicitis in offspring.

Methods: We used the Danish Birth Registry to include all singletons born during 1991–2017 and to identify maternal smoking status during pregnancy. We followed the children from birth until date of appendicitis, emigration, death, or administrative end of study (31 December 2018), whichever came first. We calculated crude and adjusted hazard ratios (HRs) of appendicitis with 95% confidence intervals (CIs) comparing children of mothers who smoked during pregnancy to children of nonsmokers. Further, we conducted a bias analysis and sibling analysis.

Results: We included 1,659,526 singletons of whom 19% were born to mothers who smoked during pregnancy. After maximum 28 years of follow-up, hazard rates for children of smokers were slightly higher than for children of nonsmokers [adjusted HR: 1.07 (95% CI = 1.04, 1.10)]. Stratification by sex revealed no association for males [adjusted HR: 1.02 (95% CI = 0.99, 1.06)], but a higher HR for females [adjusted HR: 1.13 (95% CI = 1.09, 1.18)]. This association increased with increasing length of follow-up, indicating that the association may be mediated by later-life exposures. The bias analysis indicated that misclassification of maternal smoking could attenuate a true association, while the sibling analysis showed no association.

Conclusions: Maternal smoking during pregnancy and appendicitis in the offspring may be associated.

Keywords: Smoking; Pregnancy; Appendicitis; Epidemiology; Cohort studies

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Appendicitis is the most common cause of acute abdominal surgery with an estimated lifetime risk of 7%–9% in the Western population.[1] The disease can occur at all ages, but incidence peaks around age 10–30 years with a slight male preponderance.[2] Little is known about the etiology of the disease, albeit it is likely multifactorial involving both genetic and environmental factors.[1] Thus, the reason for declining incidence rates during the second half of the 20th century[2,3] remains unknown. During the same period, the prevalence of smoking has dropped markedly.[4,5]

In 1999, two studies linked smoking to risk of appendicitis.[6,7] Montgomery et al.[6] found a doubling in odds of appendectomy for everyday smokers compared with never-smokers. Butland et al.[7] found being exposed to passive smoking in childhood increased the odds of appendectomy with 60%. However, in the latter study, smokers themselves did not have an increased risk of appendectomy, nor did smoking during pregnancy affect the offspring. In 2008, the association between smoking and risk of appendectomy was supported by an Australian twin-study by Oldmeadow et al.[8] The study revealed a 65% increased risk of appendectomy among current smokers. All three studies were, however, relatively small, comprising approximately 5,000–7,600 participants each.

The existing evidence on smoking and appendicitis is thus scarce and ambiguous. In this study, we aimed to investigate if smoking during pregnancy is associated with increased risk of appendicitis in the offspring.

METHODS

Setting

The Danish health care system covers the entire Danish population, providing access to tax-funded health services at both primary and secondary care free of charge.[9] At immigration or birth, all Danish residents are assigned a unique

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personal registration number: the civil personal registration number, hereafter referred to as the registration number.[10] The registration number is recorded along with residents' contacts with the health care system in national health registries. Data from health and administrative registries can be linked using the registration number.

Study population and design

We used Danish national health and administrative registries to conduct this population-based cohort study. We used the Danish Medical Birth Registry (MBR)[11] to identify and include all singletons born alive during the period of January 1, 1991, to December 31, 2017, in our study cohort (see flow chart, eAppendix 1, http://links.lww.com/EDE/B981, which illustrates assembly of the study population). This registry was established in 1973 and contains information on all live and stillbirths in Denmark. The registration number of the child, mother, and father along with information on the pregnancy and labor are registered by the midwives and hospital physicians involved in the pregnancy. Since 1991, the registry has recorded information on maternal smoking status during pregnancy.[12,13] During the antenatal care offered to all pregnant women, hospital staff registers whether the pregnant woman ever smoked during the current pregnancy. A record will be made for each pregnancy. During 1991–1996, the answer was recorded in the MBR as a yes/no variable. From 1997 onwards, smoking intensity has also been recorded.[14] We used this information to ascertain the status of exposure: whether or not the mother of the child smoked during the pregnancy.

Appendicitis

Since 1977, all hospital admissions have been recorded in the Danish National Patient Registry (DNPR).[15] In 1995, registration of outpatient clinic contacts began. At each contact, one primary and possible secondary diagnoses are registered along with the patient's registration number, dates of admission and discharge, and procedure codes. Until 1994, the diagnoses were recorded using the International Classification of Diseases 8th Revision (ICD-8). From 1994 and forth, the diagnoses have been registered using the International Classification of Diseases 10th Revision (ICD-10). Surgical procedures have been coded using a Danish version of the Nordic Medico-Statistical Committee (NOMESCO) Classification of Surgical Procedures since 1996. Previous to this, surgeries were coded according to the Danish Classification of Surgical Procedures and Therapies.

We used the DNPR to identify appendicitis (ICD-8 code 540 and ICD-10 code K35). Appendicitis requires inpatient care and thus, to increase specificity, we only used inpatient, primary diagnoses. The date of admission was defined as date of diagnosis.

Covariates

We used the MBR to collect the following variables concerning the mother: age at birth (≤24, 25–29, 30–34, 35–39, or ≥40 years), parity (first child or second or later child), body mass index (BMI), and marital status. Recording of BMI began in 2004 and was thus unavailable before this year. BMI was categorized as: not available because of birth before 2004, missing, or <15, 15–18.4, 18.5–24.9, 25–29.9, or 30 or more. Marital status was classified into three categories: (1) married or in a civil partnership, (2) single, widow, divorced, or not registered/annulled civil partnership, or (3) unknown. Further, year of birth was obtained from the MBR and categorized into five groups (1991–1995, 1996–2000, 2001–2005, 2006–2011, and 2012–2017).

The occupation, income, employment status, and level of education of all Danish citizens can be found in the Danish Integrated Database for Labor Market Research.[16] From this registry, we attained the following information on the mother: employment status, highest level of education, and level of income as a proxy for socioeconomic status. Employment status was categorized as employed, unemployed, early retirement, state pension, enrolled in education, or missing. Education was characterized as low (primary or lower secondary education), medium (upper secondary education or academic profession degree), high (university education at bachelor degree or higher), or unknown. Level of income was categorized according to percentiles as either low (0–<25th percentile), intermediate (≥25th–<50th percentile), high (≥50th–<75th percentile), very high (≥75th–100th percentile), or missing. For both employment status and level of income, we used data obtained from the year preceding the birth.

Descriptive birth outcomes

Variables collected from the MBR on the child or the birth included: sex of the child, birth weight in grams (300–2,499, 2,500–3,999, 4,000–7,000, or, <300, >7,000, or missing), gestational age (<28 weeks, 28–31 weeks, 32–37 weeks, ≥38 weeks, or missing), Apgar score at five minutes (>7, ≤7, or missing), and whether or not the child was small for gestational age (birth weight below the 10th percentile for children born at the same gestational age) or was delivered by cesarean section.

Statistical analyses

We followed individuals from date of birth until a diagnosis of appendicitis, death, emigration, or administrative end of follow-up (December 31, 2018), whichever came first. Treating death as a competing risk, we computed the cumulative incidence of appendicitis for the children of maternal smokers and nonsmokers. We calculated the crude and adjusted hazard ratios (HRs) with 95% confidence intervals (95% CIs) comparing children of mothers who smoked during pregnancy with children of mothers who did not. We made adjustments for year of birth as a categorical variable, maternal age, parity, maternal employment, and level of income. Because of the number of missing values for maternal education, we chose not to adjust for this variable. Further, adjusting for three correlated indicators of socioeconomic position

could have caused unnecessary adjustment affecting precision.[17] We computed HRs for follow-up of 0–15 years, 0–20 years, and 0–28 years. We excluded children with missing information on maternal smoking status or covariates from the analyses (n = 83,749). We used robust variance estimation to account for the presence of siblings in the data. We also accounted for siblings using generalized estimating equations (GEE) to address potential informative cluster size, that is, if family size was related to risk of appendicitis. We evaluated the proportional hazards assumption by visual inspection of log-log plots and found a small variation over time. However, the hazard functions did not cross. Thus, while the computed HRs may have ignored some time-varying effect, the ratios will estimate the average impact of the exposure.[18]

Sensitivity analyses

We conducted six sensitivity analyses.

We computed cumulative incidences and HRs with appendectomy and with appendectomy and a diagnosis of appendicitis as outcome (using surgical codes 43001 and 43000 before 1996 and hereafter NOMESCO codes KJEA00, KJEA01, and KJEA10 for appendectomy). Appendectomy is the standard treatment for acute appendicitis and is performed merely on the suspicion of appendicitis. We therefore assumed that sensitivity and specificity would differ when using different definitions of the outcome. In particular, specificity of the outcome would likely be higher in the analysis where a diagnosis code as well as a procedure code defined the outcome. From 1997 and forth, smoking status in the MBR has been further classified into the following groups: never smoked during pregnancy, smoked during pregnancy but stopped, and ≤5, 6–10, 11–20 or ≥21 cigarettes a day. For a subcohort restricted to the period 1997–2017, we computed the HRs for each smoking group with "never smokers" as reference group, to assess a potential dose response relationship. To assess potential impact of restricting the time period, we repeated the main analysis restricted to the 1997–2017 period.

From 2004 and forth, BMI has been recorded in the MBR. For a subcohort restricted to the 2004–2017 period, we computed HRs adjusted for BMI in addition to year of birth, maternal age, parity, maternal employment, and level of income. We adjusted for BMI for as a continuous variable modeled as a cubic spline. For comparison, we repeated the main analysis restricted to 2004–2017. As BMI and smoking status may be associated, we conducted this analysis to investigate whether BMI was confounding the main analysis.

To assess potential impact of children with missing information on maternal smoking status, we computed crude and adjusted HRs of appendicitis comparing these children first to the children of women who smoked during pregnancy and second to children of women who did not smoke during pregnancy.

Further, we conducted a sibling design analysis to control for bias owing to family-related factors such as genetics or other shared risk factors. We computed the HRs comparing children of maternal smokers with children of maternal nonsmokers of the same sex using a stratified Cox model with one stratum per family. In this type of analysis, there is one baseline rate function per family that represents the common genetic and environmental factors of the family. From this setup, it follows that only siblings discordant for maternal smoking status during pregnancy and for the outcome, appendicitis, contribute to the effect estimates. We compared same-sex siblings because of effect modification by sex in the main analyses. We adjusted for year of birth, maternal age, parity, maternal employment, and level of income. We expected women to be more likely to smoke during their first than their later pregnancies. Thus, length of follow-up could depend on exposure. However, we accounted for this by adjusting for parity.

The recorded smoking status in the MBR is what the pregnant woman reports herself. Harmful effects of smoking are well known, and it is therefore likely that women who smoke will report themselves as nonsmokers. This misclassification is unlikely to depend on whether the offspring is diagnosed with appendicitis later and would therefore be nondifferential. We conducted a bias analysis to assess the potential effect of nondifferential misclassification of maternal smoking during pregnancy. The risk ratio and odds ratio were computed using the observed data. Further, we computed 14 different scenarios of the "true" association assuming the observed data had a sensitivity of 95%, 90%, 85%, or 80% within levels of specificity of 100%, 95%, and 90%.

We performed all statistical analyses using version 9.4 of the SAS statistical software package (SAS Institute, Cary, NC, USA). We reported the study to the Danish Data Protection Agency (record number 2016-051-000001, serial number 605) and no further approval was required according to Danish law.

RESULTS

Descriptive data

We identified 1,659,526 singletons born alive during the period of January 1, 1991, to December 31, 2017. Of these, 19% were born to mothers who smoked during their pregnancy. In total, 77% were born to mothers reported as nonsmokers and, thus, smoking status was missing for 4%. Median follow-up time was approximately 18 years for children born to mothers who smoked. Children of nonsmokers had a shorter median follow-up time of approximately 13 years. Women smoking during their pregnancy were younger, more likely to be unmarried and have a low or intermediate income, and less likely to be employed or have completed a higher level of education (Table 1). BMI status was missing for a larger proportion of the smoking women owing to them giving birth before 2004 (where BMI registration began). Parity was similar between smokers and nonsmokers.

TABLE 1. Baseline Maternal Characteristics According to Maternal Smoking Status During Pregnancy

	Nonsmoker n (%)	Smoker n (%)
All births	1,281,428 (100)	310,051 (100)
Age at birth (years of age)		
≤24	155,767 (12)	77,234 (25)
25–29	453,207 (35)	108,091 (35)
30–34	452,114 (35)	84,055 (27)
35–39	186,556 (15)	34,696 (11)
≥40	33,171 (3)	5,915 (2)
Missing	613 (0)	60 (0)
Parity		
First child	564,119 (44)	136,487 (44)
Second or later child	717,309 (56)	173,564 (56)
Marital status		
Married, civil partnership	772,227 (60)	126,929 (41)
Single, widow, divorced, or not registered/annulled civil partnership	508,711 (40)	183,037 (59)
Unknown	490 (0)	85 (0)
Maternal BMI (kg/m^2)		
Missing or <15	28,291 (2)	5,359 (2)
15–18.4	26,433 (2)	6,754 (2)
18.5–24.9	423,437 (33)	55,361 (18)
25–29.9	139,582 (11)	22,537 (7)
≥30	79,282 (6)	15,870 (5)
No BMI (before 2004)	584,403 (46)	204,170 (66)
Maternal employment status		
Employed	991,942 (77)	203,078 (66)
Unemployed	136,249 (11)	55,997 (18)
Early retirement	82,075 (6)	36,746 (12)
State pension	847 (0)	870 (0)
Enrolled in education	56,634 (4)	11,770 (4)
Missing	13,681 (1)	1,590 (1)
Maternal highest level of education[a]		
Low	201,845 (16)	137,804 (44)
Medium	583,358 (46)	129,813 (42)
High	417,344 (33)	31,825 (10)
Unknown	78,881 (6)	10,609 (3)
Maternal income level[b]		
Low	292,781 (23)	99,073 (32)
Intermediate	286,929 (22)	105,450 (34)
High	324,622 (25)	72,703 (23)
Very high	368,385 (29)	31,994 (10)
Missing	8,711 (1)	831 (0)

[a]Low: primary or lower secondary, medium: upper secondary or academic profession degree, high: university education at bachelor degree or higher.
[b]Low: 0–<25th percentile, intermediate: ≥25th–<50th percentile, high: ≥50th–<75th percentile, very high: ≥75th–100th percentile.

Birth outcomes

Sex distribution of the children was similar across smoking status, with a slight overrepresentation of males (Table 2). Children born to mothers who were smoking were more likely to be born during the earliest categories of year of birth. The birth weight appeared lower for children of smokers, and likewise these children were more likely to be small for gestational age. A higher percentage of the children of smokers were born before week 38. The percentage of children delivered by cesarean section and of children having an Apgar score above 7 at 5 minutes after birth did not differ between children of nonsmokers and smokers. (For descriptive tables of those with missing maternal smoking status see Tables A,B, eAppendix 2, http://links.lww.com/EDE/B981).

Risk of appendicitis

We observed 28,577 cases of appendicitis during follow-up. In total, 7,365 were children of smokers, 19,675 were children of nonsmokers, and 1,537 were children where maternal smoking status was missing. Figure 1 shows the cumulative incidence of appendicitis for children of

TABLE 2. Baseline Characteristics of Singleton Births According to Maternal Smoking Status During Pregnancy

	Nonsmoker n (%)	Smoker n (%)
All births	1,281,428 (100)	310,051 (100)
Sex of the child		
Male	657,358 (51)	159,434 (51)
Female	624,070 (49)	150,617 (49)
Year of birth		
1991–1995	210,511 (16)	94,299 (30)
1996–2000	229,601 (18)	73,711 (24)
2001–2005	243,566 (19)	57,073 (18)
2006–2011	304,881 (24)	49,294 (16)
2012–2017	292,869 (23)	35,674 (12)
Birth weight in grams		
300–2,499	35,064 (3)	17,966 (6)
2,500–3,999	979,320 (76)	255,579 (82)
4,000–7,000	261,375 (20)	35,257 (11)
Missing, <300 or >7,000	5,669 (0)	1,249 (0)
Gestational age (weeks)		
<28	1,388 (0)	490 (0)
28–31	5,027 (0)	1,908 (1)
32–37	102,803 (8)	32,116 (10)
≥38	1,169,345 (91)	274,269 (89)
Missing	2,865 (0)	1,268 (0)
Small for gestational age[a]		
No	1,177,273 (92)	258,283 (83)
Yes	100,185 (8)	50,869 (16)
Missing	3,970 (0)	899 (0)
Cesarean section		
No	1,063,481 (83)	261,200 (84)
Yes	217,947 (17)	48,851 (16)
Apgar score at 5 minutes		
>7	1,256,509 (98)	303,404 (98)
≤7	16,519 (1)	4,620 (2)
Missing	8,400 (1)	2,027 (1)
Follow-up time, years		
Median (IQR)	13.4 (7.1–20.2)	18.5 (11.6–23.6)

[a]Birth weight below the 10th percentile for children born at the same gestational age.

smokers in comparison with children of nonsmokers. After 15 years of follow-up, cumulative incidence was 1.6% (95% CI = 1.6, 1.7) for children of mothers who did not smoke during pregnancy and 1.8% (95% CI = 1.7, 1.8) for children of mothers who smoked during pregnancy. After 28 years of follow-up, cumulative incidence was 3.9% (95% CI = 3.8, 4.0) for children of mothers who did not smoke during pregnancy and 4.2% (95% CI = 4.1, 4.4) for children of mothers who smoked during pregnancy (Table 3). Figures 2 and 3 represent the risks for male and female offspring, respectively.

The crude HR comparing children of maternal smokers with children of maternal nonsmokers for the total follow-up period of 28 years was 1.12 (95% CI = 1.09, 1.15) (Table 3). Adjusting for year of birth, maternal age, parity, maternal employment, and level of income reduced the HR to 1.07 (95% CI = 1.04, 1.10). There appeared to be no difference in the hazard rates of appendicitis based on maternal smoking status among the male offspring [28-year follow-up adjusted HR: 1.02 (95% CI = 0.99, 1.06)]. Female offspring of maternal smokers had a higher hazard rate than the offspring of nonsmokers [28-year follow-up adjusted HR: 1.13 (95% CI = 1.09, 1.18)]. The HRs for the female offspring increased with increasing length of follow-up period: 15-year follow-up adjusted HR: 1.07 (95% CI = 1.01, 1.12), 20-year follow-up adjusted HR: 1.11 (95% CI = 1.06, 1.16), 28-year follow-up adjusted HR: 1.13 (95% CI = 1.09, 1.18). The same tendency did not appear among the male offspring: 15-year follow-up adjusted HR: 1.04 (95% CI = 0.99, 1.09), 20-year follow-up adjusted HR: 1.03 (95% CI = 0.98, 1.07), and 28-year follow-up adjusted HR: 1.02 (95% CI = 0.99, 1.06).

Sensitivity analyses

Using appendectomy or the combination of appendectomy and diagnosis of appendicitis as outcome had little impact on the estimated HRs [adjusted HRs after 28 years: 1.10 (95% CI = 1.07, 1.13) and 1.06 (95% CI = 1.03, 1.09), respectively] (see Tables A,B, eAppendix 3, http://links.lww.com/EDE/B981).

Classifying exposure into cigarettes per day did not reveal a pattern consistent with a dose–response relationship (see Tables A–C, eAppendix 4, http://links.lww.com/EDE/B981). We found the highest HRs for children of mothers who smoked 1-5 cigarettes a day [adjusted HR: 1.16 (95% CI = 1.08, 1.25)] followed by 11-20 cigarettes a day [adjusted HR: 1.08 (95% CI = 1.00, 1.17)]. Estimated HRs of smoking for 6-10 cigarettes a day were 1.04 (95% CI = 0.98, 1.12) and, for >20 cigarettes a day, 1.05 (95% CI = 0.85, 1.28). Children of mothers who smoked but stopped during the pregnancy had a hazard similar to that of those born to mothers who never smoked [adjusted HR: 1.00 (95% CI = 0.88, 1.13)]. Repeating the main analysis to the time period where smoking intensity was available did not appear to affect the estimates [adjusted HR comparing children of smokers with children of nonsmokers, 1997–2018: 1.08 (95% CI = 1.04, 1.13)] (see Table D, eAppendix 4, http://links.lww.com/EDE/B981).

Adding the adjustment for BMI had no effect on the HR as the adjusted HR both with and without adjustment for BMI

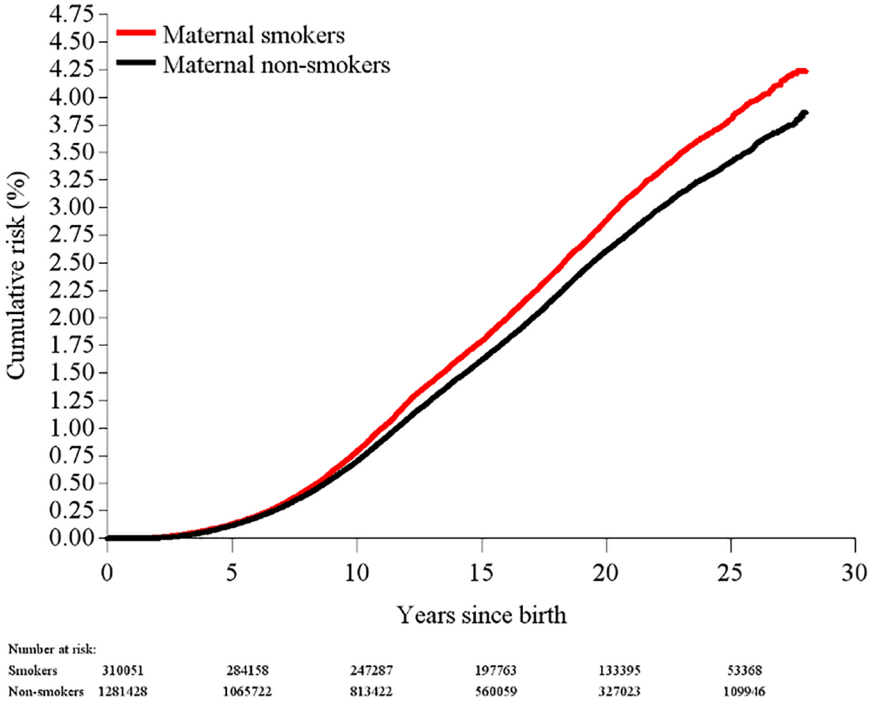

FIGURE 1. Cumulative incidence of appendicitis according to maternal smoking status during pregnancy—all.

TABLE 3. Cumulative Incidence, Crude, and Adjusted HRs of Appendicitis With 95% CIs Comparing Children of Women Who Smoked During Pregnancy to Children of Women Who Did Not Smoke During Pregnancy

Category	Follow-up (years)	Cumulative Incidence % (95% CI)		Crude HR (95% CI)	Adjusted HR[a] (95% CI)
		Nonsmokers	Smokers		
All	0–15	1.6 (1.6, 1.7)	1.8 (1.7, 1.8)	1.11 (1.07, 1.15)	1.05 (1.01, 1.09)
	0–20	2.6 (2.6, 2.7)	2.9 (2.8, 3.0)	1.11 (1.08, 1.14)	1.06 (1.03, 1.10)
	0–28	3.9 (3.8, 4.0)	4.2 (4.1, 4.4)	1.12 (1.09, 1.15)	1.07 (1.04, 1.10)
Males	0–15	1.8 (1.7, 1.8)	1.9 (1.9, 2.0)	1.09 (1.04, 1.14)	1.04 (0.99, 1.09)
	0–20	2.8 (2.8, 2.9)	3.0 (2.9, 3.1)	1.07 (1.03, 1.11)	1.03 (0.98, 1.07)
	0–28	4.1 (3.9, 4.3)	4.1 (4.0, 4.3)	1.06 (1.02, 1.10)	1.02 (0.99, 1.06)
Females	0–15	1.5 (1.4, 1.5)	1.6 (1.6, 1.7)	1.13 (1.08, 1.19)	1.07 (1.01, 1.12)
	0–20	2.4 (2.4, 2.5)	2.8 (2.7, 2.9)	1.17 (1.12, 1.22)	1.11 (1.06, 1.16)
	0–28	3.6 (3.5, 3.8)	4.4 (4.2, 4.6)	1.18 (1.14, 1.23)	1.13 (1.09, 1.18)

[a]Adjusted for year of birth, maternal age, parity, maternal employment, and level of income.

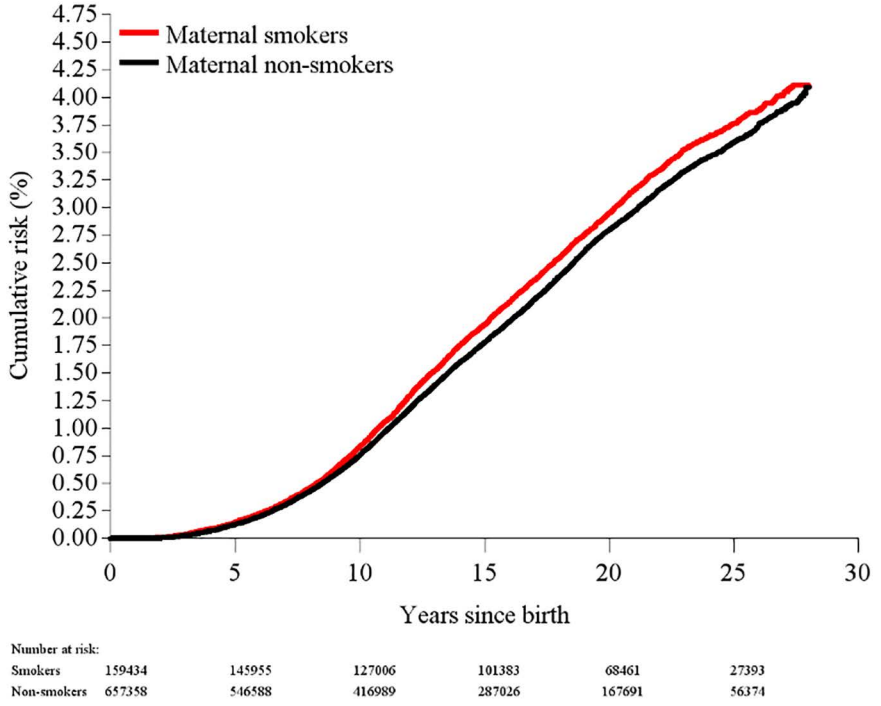

FIGURE 2. Cumulative incidence of appendicitis according to maternal smoking status during pregnancy—male offspring.

was 1.09 (95% CI = 0.99, 1.19) (see eAppendix 5, http://links.lww.com/EDE/B981).

Comparing children with missing maternal smoking status to children of maternal nonsmokers and to children of maternal smokers yielded slightly higher HRs when comparing to children of nonsmokers than to children of smokers. However, HRs from both comparisons across all lengths of follow-up and for both sexes were one or close to one (see Tables A,B, eAppendix 6, http://links.lww.com/EDE/B981).

In the sibling analysis, we included 35,629 sibling pairs, of which 18,610 were male and 17,019 were female sibling pairs (see eAppendix 7, http://links.lww.com/EDE/B981 for flow chart of inclusion of siblings). The comparison of siblings of the same sex but discordant on exposure reduced the adjusted HR to 0.92 (95% CI = 0.81, 1.04) after 28 years of follow-up (see eAppendix 7, http://links.lww.com/EDE/B981). However, a low number of children in this design made CIs rather wide.

Using the observed data, the relative risk of appendicitis comparing children of smokers to children of nonsmokers was 1.54 (1.50–1.59) and the odds ratio was 1.56 (1.52–1.60) (see Tables A–P, eAppendix 8, http://links.lww.com/EDE/B981). If a specificity of 100% and a sensitivity of 80% were assumed, the true relative risk would have been 1.60 and the odds ratio 1.62 (1.57–1.66) (Table E, eAppendix 8, http://links.lww.com/EDE/B981). The computed "worst case scenario," where

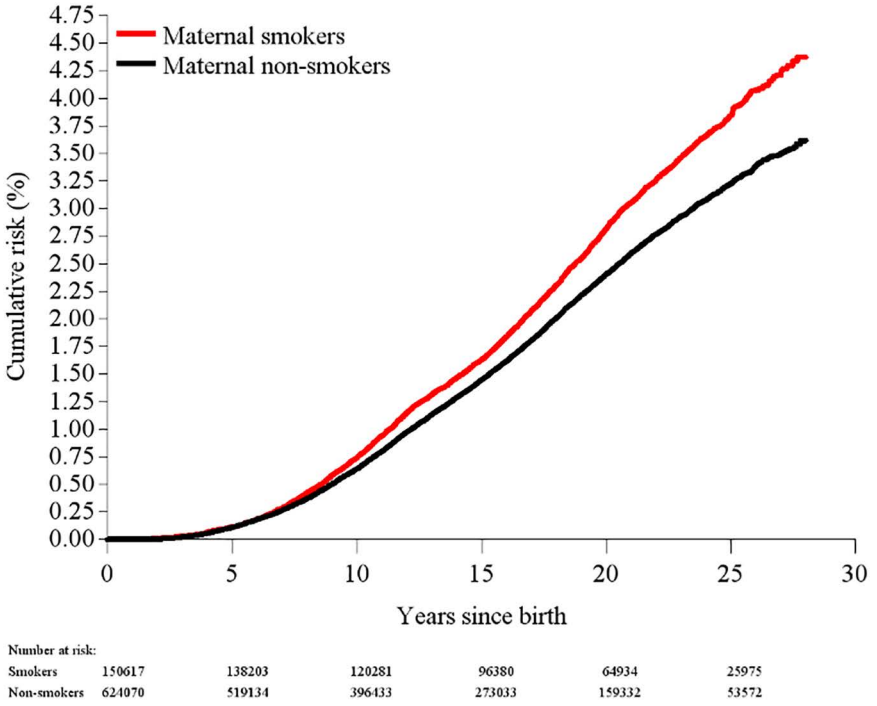

FIGURE 3. Cumulative incidence of appendicitis according to maternal smoking status during pregnancy—female offspring.

a specificity of 90% and a sensitivity of 80% were assumed, yielded a true relative risk of 2.08 and an odds ratio of 2.11 (2.02-2.20) (see Table P, eAppendix 8, http://links.lww.com/EDE/B981).

DISCUSSION

In this population-based cohort study, children of mothers who smoked during pregnancy had a 12% higher hazard of being diagnosed with appendicitis after 28 years of follow-up compared with children of mothers who did not smoke during pregnancy. Adjusting for year of birth, maternal age, parity, maternal employment, and level of income reduced this to 7%. The higher hazards among children of mothers who smoked during pregnancy seemed to be ascribable to the female offspring. With increasing length of follow-up, HR for the females increased. Conducting a sibling design to account for family-related factors such as genetics or shared environmental risk factors during childhood attenuated the association. Conversely, the bias analysis that revealed a true association could have been attenuated if maternal smoking status was misclassified.

Previous studies have reported an association between maternal smoking during pregnancy and risk of childhood asthma and of adult-onset asthma in the offspring.[19–21] These associations may partly be owing to tobacco smoke affecting the development of the immune system.[22] Possibly, such an impact on the immune system could be of importance for other inflammatory or infectious diseases such as appendicitis. However, to our knowledge, only one study has investigated whether smoking during pregnancy is associated to the risk of appendicitis in the offspring. This case-control study by Butland et al.[7] resulted in an adjusted odds ratio of appendectomy of 0.93 (95% CI = 0.63, 1.37) comparing children of mothers smoking 10 or more cigarettes a day during pregnancy with offspring of nonsmokers. Thus, the scarce existing evidence does not support an association between smoking during pregnancy and appendicitis in the offspring. Furthermore, our data on the female offspring showed an increasing HR with increasing length of follow-up, suggesting that if smoking during pregnancy was linked to appendicitis in the offspring, it may have been mediated by later-life exposures. One such potential mediator could be personal smoking habits. Two previous studies have reported an increased risk of appendicitis among smokers. Comparing smokers to never-smokers resulted in a doubling in odds of appendectomy [odds ratio 2.13 (95% CI = 1.63, 2.78)] in a study by Montgomery et al.,[6] and a relative risk of appendectomy of 1.65 (95% CI = 1.41, 1.93) in a cohort study by Oldmeadow et al.[8] Contrary to this, Butland et al. did not find an association between personal smoking habits and appendectomy. However, in their study, exposure to passive smoking in childhood was associated with appendectomy.[7] This could suggest passive smoking as another potential mediator. To our knowledge only the study by Oldmeadow et al. has reported estimates for men and women separately. In their analysis comparing ever-smokers to never-smokers, an association with smoking appeared among women but not among men. However, these subgroups were relatively small yielding large confidence intervals for

both sexes. Nonetheless, it could be that a potential impact of smoking differs according to sex. Sex differences in immune response are well known. Women tend to have a stronger immune response and thereby likely higher risks of autoimmune diseases while men appear to be more susceptible to certain infections. Further, impact on the immune system of other environmental factors, such as nutrition, has also been shown to differ according to sex.[23] Thus, a smoking-induced attenuation of the otherwise strong female immune response could mediate the effect we found.

Strengths of this study include the large cohort size with long and almost complete follow-up. Because of the use of registry data attained from the Danish universal health care system, risk of selection bias was negligible. Smoking during pregnancy could lead to pregnancy loss. Thus, this can create some form of selection of who enters the study. This type of selection is unavoidable and remains a condition for all birth registry studies.[24] Further, the study was limited by potential misclassification bias. The physician or midwife registers the smoking status that the pregnant woman herself reports. Since the harmful effects of smoking are well known, it is likely that some women who smoke will report being nonsmokers. This misclassification is unlikely to depend on whether or not the child is diagnosed with appendicitis later in life and would therefore be nondifferential and bias the results towards the null. Thus, as illustrated by the bias analysis, a possible true association could have been masked by such a misclassification. The extent of the misclassification may, however, not be as severe as illustrated in the example with an assumed sensitivity of 80% and specificity of 90%. Specificity might be closer to 100%, as it seems less likely that women would report being smokers when they were in fact nonsmokers. Further, according to the Danish Health Authority, 18% of the Danish population smoked daily or occasionally in 2020.[25] In our study, the proportion of children born by a mother who smoked during the pregnancy in the birth year category of 2012–2017 was, as expected, smaller (11%). This difference could be because of misclassification but it also likely that women will cease smoking when planning to become pregnant. Naturally, the outcome could also be affected by misclassification. However, appendicitis requires acute medical care and therefore, all patients will be attended to in the public health care system in Denmark. They should, thus, be registered in the DNPR. Recently, comparison of the diagnosis code from the DNPR with pathology specimens has revealed a sensitivity of 92.8%, specificity of 99.5%, positive predictive value of 76.9%, and negative predictive value of 99.9%.[26] Finally, as mentioned, confounding could be a problem. The sibling analysis illustrated that there may indeed be some genetic or other environmental factors confounding the main analyses. However, while this type of study design will remove confounding by factors which are shared by siblings, it can actually introduce bias. In this type of design, a true association could have been attenuated if random measurement error of the maternal smoking status occurred. Furthermore, bias could be introduced by confounders that were not shared by the siblings.[27,28] Potential confounders and mediators are, however, difficult to identify as very little is known about the etiology of appendicitis.[1] Further research is needed if risk factors of the disease are to be identified.

CONCLUSIONS

Overall, we found a weak association between smoking during pregnancy and appendicitis among the female offspring. Although the bias analysis showed a true association may have been attenuated if nondifferential misclassification of the maternal smoking occurred, the sibling analysis showed no association.

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Because of Danish legislation, the data cannot be shared with third parties. Analytic code is presented in eAppendix 9.