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A PROSPECTIVE STUDY OF RESPIRATORY VIRAL INFECTION IN PREGNANT WOMEN WITH AND WITHOUT ASTHMA

(Short running head: Respiratory viral infection in pregnancy)

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Abbreviations list:

ACQ: Asthma control questionnaire

BMI: Body mass index

CCQ: Common cold questionnaire

CI: Confidence interval

FENO: Fractional exhaled nitric oxide

IQR: Interquartile range

MAP: Managing asthma in pregnancy

PCR: Polymerase chain reaction

RR: Relative risk

Abstract

Background: Respiratory viral infections are common in pregnancy, but their health impact, especially in asthma is unknown. The objective of the study was to assess the frequency, severity and consequences of respiratory viral infection in pregnancy in women with and without asthma.

Methods: In this prospective cohort study, common cold symptoms were assessed during pregnancy in 168 women with asthma, and 117 women without asthma, using the common cold questionnaire and by self-report. Nasal and throat swabs were collected for suspected infections and tested by polymerase chain reaction for respiratory viruses. Pregnancy and asthma outcomes were recorded.

Results: Pregnant women with asthma had more prospective self-reported and questionnaire detected common colds than pregnant women without asthma (incidence rate ratio 1.77, 95% confidence interval [1.30, 2.42], P<0.0001). Retrospectively reported common colds in early pregnancy and postpartum were increased in asthma compared to women without asthma. The severity of cold symptoms was also increased in asthma (total cold score median 8 interquartile range [5, 10] in asthma, vs 6 [5, 8] in controls, P=0.031). Among women with asthma, having a laboratory confirmed viral infection was associated with poorer maternal health, with 60% of infections associated with uncontrolled asthma and a higher likelihood of pre-eclampsia.

Conclusions: Pregnant women with asthma have more common colds during pregnancy than pregnant women without asthma. Colds during pregnancy were associated with adverse maternal and pregnancy outcomes. Prevention of viral infection in pregnancy may improve the health of mothers with asthma.

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Introduction

Pregnant women, especially those with asthma, experience significant problems associated with respiratory viral infections [1]. The outcomes from pandemic H1N1 were more severe in pregnant women and people with asthma [2] and retrospective studies report more infections in pregnant women with asthma than those without asthma [3,4]. The effects of viral infection may be more severe among pregnant women with asthma, with a 10-fold increased risk of respiratory related hospitalisation during the influenza season described for pregnant women with asthma compared to women without asthma [1].Respiratory viral infections are reported to be a significant cause of asthma exacerbations during pregnancy [5], and may be associated with adverse outcomes such as low birth weight [6].

The characteristics and mechanisms of these effects are not well understood. Among non-pregnant asthmatics, susceptibility to respiratory viral infection is not increased, but colds are more severe, with more lower respiratory tract symptoms which are longer lasting [7]. However, pregnant women may be more susceptible to viral infection due to a pregnancy related impairment in anti-viral interferon responses [8,9], or deficiencies in epithelial cell function, over production of mucous or alveolar macrophage dysfunction [10].

We hypothesised that during pregnancy, women with asthma experience more frequent and more severe respiratory viral infections than pregnant women without asthma. We assessed these effects prospectively during pregnancy, by assessing common colds by self-report and using the common cold questionnaire (CCQ) and polymerase chain reaction (PCR) testing, and retrospectively in early pregnancy and postpartum.

Materials and Methods

Study design

Pregnant women with and without asthma were recruited from April 2007 to November 2009 (Figure 1), at the antenatal clinic of John Hunter Hospital, Newcastle, Australia. Written, informed consent was obtained and ethics approval granted by the University of Newcastle and Hunter New England Area Health Service Research Ethics Committees (approval number 07/02/21/3.06). Women between 12 and 20 weeks gestation who were over 18 years of age were included. Exclusion criteria were the presence of a chronic medical disease (other than asthma), drug or alcohol dependence and an inability to attend study visits or perform spirometry. Control subjects had never received a diagnosis of asthma, while women with asthma had a doctor's diagnosis of asthma, and asthma symptoms or therapy in the prior 3 months.

Women completed monthly clinical visits and were telephoned fortnightly (e-Figure 1). The majority (157/168, 93%) of the asthmatic women also commenced participation in the Managing Asthma in Pregnancy (MAP) study [11,12]. Regardless of co-participation in MAP, all women, with and without asthma, had the same schedule of study visits and telephone contacts, and were eligible for additional visits based on the same criteria (current common cold). Some women consented to donate blood for in vitro studies of responses to viral infection [8,9].

Clinical measures

At each visit and telephone contact, asthma symptoms over the past 7 days were collected by self-report, and using the Asthma Control Questionnaire (ACQ7) [13] and exacerbations were assessed by direct questioning and defined as those requiring medical intervention (hospital

admission, emergency department presentation, unscheduled doctor visit or the use of oral corticosteroids).

Common colds were assessed by direct questioning (self-report: "Do you currently have a cold?") and using the CCQ (e-Figure 2) [14] at each contact. The CCQ assessed 9 symptoms over 4 domains (general: fevers, chills, muscle pains, Nasal: watery eyes, runny nose, sneezing, Throat: sore throat, Chest: cough, chest pain) which were scored as none (0), mild (1), moderate (2), or severe (3) [14]. A cold was "probable" when symptoms were moderate in at least 2 domains, or mild in at least 3 domains. Unless otherwise indicated, a common cold was defined as instances where the CCQ indicated a "probable cold". Common cold severity was assessed by the total CCQ score (possible score 0-27) and by the proportion of colds with a score ≥10 [14]. Colds in early pregnancy and postpartum were retrospectively assessed by self-report at the first study visit and 6 months postpartum respectively. Subjects with a current cold (women with and without asthma), or current asthma exacerbation were offered additional visits either at home or hospital within 48 hours. If a new cold was reported 14 days after a previous report, it was considered a separate clinical event [7].

Virus PCR testing

Nasal and throat swabs were collected from women with common colds, and viruses identified using real-time quantitative PCR for rhinovirus, enterovirus, respiratory syncytial virus A and B, influenza A and B, coronavirus and human metapneumovirus [15].

Statistical Methods

Statistical analysis was performed using Stata 11 (StataCorp, College Station, TX). Results are presented as mean ± standard deviation (SD) or median (interquartile range, IQR) with

Student's ttest and Wilcoxon ranksum tests as appropriate and Wilcoxon signed rank test for paired data. The Chi square test was used to compare proportions. Two-sided tests with $P<0{\cdot}05$ were considered significant, with the exception of data on the frequency of common colds and PCR positive colds ($P<0.025$, because this outcome was assessed by two similar methods). The rate difference between the groups for colds was compared using a Poisson regression model, adjusted for body mass index (BMI), atopy and parity with a robust option when data were over dispersed. Secondary outcomes were cold severity (analysed as panel data using Stata's xtreg with random effects and adjusted for baseline CCQ score, BMI, atopy and parity), impact of colds on asthma, and impact of colds on pregnancy outcomes. We assessed the relationship between PCR positive colds in asthma and pre-eclampsia/pregnancy-induced hypertension with logistic regression, adjusting for smoking, parity, age, BMI and multiple pregnancy.

Results

Subject characteristics (Table 1, e-Table 2)

285 pregnant women were recruited (168 asthma, 117 control, Figure 1). Pregnant women with asthma had significantly higher BMI ($P<0.002$), significantly worse lung function ($P<0.05$), and were more likely to have atopy ($P<0.0001$) than control women.

Frequency of common colds during pregnancy

Pregnant women with asthma had more questionnaire detected common colds during pregnancy (71%) than pregnant women without asthma (46%, Table 2, $P<0.0001$, Relative Risk (RR) 1.83, 95% confidence interval (CI) [1.39, 2.41]). More women with asthma had

multiple common colds than women without asthma (33% vs 16%, P=0.0028, RR 1.25, 95% CI [1.09, 1.42]). There were 223 common cold events in the asthma group, and 83 in the control group (e-Table 3). The rate of common cold events adjusted for follow-up time, atopy, parity and maternal BMI was significantly higher in the asthma group compared to the control group (Figure 2, incidence rate ratio (IRR) 1.77, 95% CI [1.30, 2.42], P<0.0001). The control group had the same proportion of common colds detected in the second and third trimesters, while the asthma group had significantly more common colds detected in the second compared to the third trimester (Table 2, P<0.0001), despite longer follow-up times for the third trimester. In addition to questionnaire-detected colds, women with asthma also self-reported more colds prospectively during pregnancy and retrospectively in early pregnancy and postpartum (e-Table 5).

Nasal and/or throat swab samples were collected from 80% of common cold events (20% were not collected due to refusal by the participant, or lack of a clinical visit at the time of the event), within a median time from symptom onset of 3.5 days (IQR 3, 7 days) in the control group and 4 days (IQR 2, 7 days) in the asthma group (e-Table 4). 31% of women with asthma and 18.8% of women without asthma had one or more PCR positive colds during pregnancy (RR 1.18, 95% CI [1.03, 1.34] asthma vs control, Table 3), but this did not reach our significance level of P<0.025 (P=0.0305). There were 26 PCR positive cold events in the non-asthmatic control group, and 60 PCR positive cold events in the asthma group (e-Table 4). There was no significant difference in the rate of PCR positive colds between groups (adjusted for follow-up time, atopy, parity and maternal BMI, IRR 1.18, 95% CI [0.72, 1.94], P=0.505, Table 3). The number of second trimester PCR positive colds was higher than the number of third trimester colds in the asthma group (P=0.0442) but not in the control group (P=0.4524, e-Table 4).

Severity of common colds during pregnancy

The median total CCQ score was higher among common cold events in the asthma group (8 [5, 10]) compared to the control group (6 [5, 8]) and was statistically significant when baseline values were adjusted for (xtreg, coefficient 1.16, 95% CI [0.11, 2.21], P=0.031, e-Table 6). However, in PCR positive colds, the total CCQ score was not different between groups (e-Table 6, xtreg, coefficient 0.86, 95% CI [-1.13, 2.85], P=0.397).

Impact of colds on asthma

One third of the PCR positive viral infections were associated with exacerbation requiring medical intervention and a further third with loss of control. Total CCQ score significantly correlated with ACQ score (Spearman r = 0.3187, P=0.0131, Spearman rank correlation, e-Figure 5).

Among the sub-group of asthmatic women participating in the MAP study, those randomised to fractional exhaled nitric oxide (FENO)-based management (n=69) were significantly less likely to report common colds (63.8% vs 82.2%, RR 0.492, 95% CI [0.274, 0.881]) or PCR positive colds (23.2% vs 42.5%, RR 0.749, 95% CI [0.592, 0.948]) compared to those randomised to clinical guidelines based management (n=73).

Impact of colds on neonatal outcomes

In the control group, women with at least one PCR positive cold had babies of significantly lower birth weight (P=0.0274) and length (P=0.0236), compared to control women with PCR negative colds (Table 4). Women with asthma with PCR positive colds had a significantly increased odds of pre-eclampsia or pregnancy induced hypertension, when adjusted for

known pre-eclampsia risk factors (maternal smoking, age, BMI, parity, multiple pregnancy, odds ratio 8.48, 95% CI 1.41, 51.11, P<0.02, Table 4), compared to asthmatic women with PCR negative colds.

Discussion

Pregnant women with asthma have more common colds during pregnancy than pregnant women without asthma, both by self-report and questionnaire. The severity of symptoms was higher in asthmatics with common colds than controls, when adjusted for baseline differences. While PCR positive colds were of similar severity in the two groups, virus-confirmed colds in asthmatics frequently resulted in exacerbations, and were associated with perinatal effects.

Previous studies in non-pregnant adults with asthma have suggested that asthmatics are no more susceptible to respiratory tract infections than non-asthmatics [7]. The evidence in the present study suggests that in pregnancy, women with asthma may be more susceptible to common colds than women without asthma, but that cold symptoms associated with PCR positive colds are similar to those in women without asthma. In a large prospective study, 14.4% of women had a common cold during pregnancy, with half of these colds medically recorded [16]. The CCQ we used identified more common colds than self-report, and not all of these were virus positive by laboratory testing. The prospective nature of our study likely contributed to a high rate of reporting of common colds.

It is possible that confounding by symptoms of rhinitis may have contributed to a high proportion of women with questionnaire-detected colds.

Common colds were more likely to occur in the second trimester than the third trimester in the asthma group only. Banhidy et al. found that there was a lower prevalence of the common cold in the 8th and 9th months of pregnancy, compared to the first 7 months [16]; however, it was unclear if follow-up time, and early deliveries had been accounted for. Asthma exacerbations also peak in the late second trimester [5,17]. Further evidence is required to determine if this is due to a pregnancy-specific rise in susceptibility to infection.

One third of PCR positive colds were associated with exacerbations requiring medical intervention. In previous studies, 60% of cases with a positive virus identification were associated with asthma exacerbation [18], while cold severity was predictive of subsequent asthma worsening [19]. Viral infection is a significant asthma trigger, possibly due to the inflammatory pathways activated during infection. Understanding the relationship between viral infection and asthma [20] is important as preventing viral infection could also prevent exacerbations. We have evidence that improved asthma management through FENO monitoring is associated with a reduction not only in exacerbations [11] but in PCR positive viral infections.

Pregnant women with asthma who had PCR positive colds were more likely to have pre-eclampsia than women without PCR positive colds, consistent with studies suggesting an association between maternal infection (bacterial or viral) and the risk of pre-eclampsia, possibly due to changes in the maternal immune system [21]. Pregnant women with asthma are at increased risk of pre-eclampsia compared to pregnant women without asthma [22 23,24 25]. No previous reports have linked asthma, pre-eclampsia and viral infection during pregnancy. It is possible that inflammation associated with the response to viral infection and/or asthma exacerbation may contribute to the underlying endothelial dysfunction in pre-eclampsia.

There are limitations to our study. The CCQ is an unvalidated tool which limits the conclusions we can make using this instrument, particularly since the CCQ is not validated to distinguish between viral infections and rhinitis. There was the possibility of recall bias for colds assessed retrospectively, although for the majority of pregnancy we collected data prospectively. The pregnant women with asthma had higher parity than pregnant women without asthma, which might increase their exposure to virus infections from other children [26,27]. However, we adjusted for this, as well as other confounders such as atopy and BMI (a known risk factor for exacerbations in pregnancy [28]), when considering the rate of colds. Our sample collection time was not ideal, with swabs collected within a median of 3-4 days from symptom worsening. We contacted women fortnightly by phone, and sent mobile phone text reminders every other week to try and increase participation, and offered home visits during colds. The CCQ covers only 2 days of the past 14, and since we did not administer it daily, it is possible colds were missed. The number of PCR positive colds experienced by asthmatic women, when adjusted for follow-up time was not significantly different from the control group. This may be due to a lack of power, since only a proportion of common colds were tested in the laboratory, or rhinitis-like symptoms and cough may be amplified by asthma or pregnancy themselves, resulting in more colds being detected that were not true infections. While we found second trimester colds to be more frequent than third trimester colds, it is possible that rhinitis of pregnancy may have contributed to this finding.

Conclusions

Common colds were more frequently reported among pregnant women with asthma, compared to women without asthma. They occurred more often in the second trimester than the third, perhaps explaining the greater exacerbation risk at this time. There was an impact

on maternal health, with one third of infections associated with exacerbations requiring medical intervention. Prevention of respiratory viral infections may improve asthma outcomes during pregnancy.

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Author's contributions:

Vanessa Murphy was involved in study conception and design, analysis and interpretation of data and wrote the manuscript.

Heather Powell was involved in analysis and interpretation of data, and revision of the article for important intellectual content.

Peter Wark was involved in acquisition of data and revision of the article for important intellectual content.

Peter Gibson was involved in study conception and design, interpretation of data and revision of the article for important intellectual content.

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Tables:

Table 1: Subject Characteristics

	Control (n=117)	**Asthma (n=168)**	**P value**
Maternal age (years)*	29.6 (4.6)	28.5 (5.6)	0.086
	Range 18 – 38	Range 18-43	
Gestational age at	16.6 (2.3)	16.9 (2.4)	0.193
recruitment (weeks)*	Range 12.6 – 21.3	Range 11.7 – 21.9	
Gravidity†	2 (1, 2)	2 (1, 3)	**0.010**
Parity†	1 (0, 1)	1 (0, 2)	**0.016**
Para 0‡	49 (41.9%)	56 (33.3%)	0.178
Maternal Atopy‡	51 (45.9%)	115 (71.9%)	**<0.0001**
	n=111	*n=160*	
Maternal BMI†	25.2 (22.8, 28.7)	27.7 (24.4, 32.1)	**0.0005**
	n=116	*n=165*	
Smoking Status:			
Never	66 (56.4%)	80 (47.6%)	
Ex	32 (27.4%)	52 (31.0%)	
Current‡	18 (15.4%)	35 (20.8%)	0.245
Smoking pack years†	3.0 (0.9, 6.0)	4.0 (1.5, 7.0)	0.070
Pre-bronchodilator	*n=117*	*n=142*	
spirometry:			
FEV_1*	3.29 (0.42)	2.96 (0.52)	**<0.0001**
% predicted FEV_1*	102.9 (11.1)	93.8 (14.6)	**<0.0001**
FVC†	3.93 (3.52, 4.24)	3.72 (3.34, 4.18)	**0.030**
% predicted FVC†	107.6 (98.0, 116.2)	103.0 (74.5, 112.6)	**0.037**

	n=116		
No ICS Treatment		120 (71.4%)	
ICS treatment		15 (8.9%)	
ICS/LABA treatment		33 (19.6%)	
ICS dose (among ICS users), BDP equivalents µg/day		800 (650, 1000) *n=47*	
ACQ7		0.86 (0.29, 1.57)	
Influenza vaccine for current season‡	10 (8.5%)	16 (9.5%)	P=0.8407

Data was collected at the first study visit. † median (IQR), Wilcoxon ranksum test, *mean (SD), Student's ttest, ‡n(%), Chi^2 test.

BMI: body mass index, FEV_1: forced expiratory volume at one second, FVC: forced vital capacity, ICS: inhaled corticosteroid, LABA: long acting beta-agonist, BDP: beclomethasone dipropionate, ACQ7: Asthma control questionnaire (7 item).

Table 2: Frequency of common colds during pregnancy

	Control (n=117)	**Asthma (n=168)**	**Effect size**	**P value**
Subjects with 1 or more colds (probable by CCQ) during pregnancy *	54 (46%)	120 (71.4%)	**RR 1.83, 95% CI (1.39, 2.41)**	**P<0.0001**
Subjects with more than 1 common cold during pregnancy*	19 (16.2%)	55 (32.7%)	**RR 1.25, 95% CI (1.09, 1.42)**	**P=0.0028**
Number of colds per person†	0 (0, 1) Range 0-6	1 (0, 2) Range 0-8		**P<0.0001**
Number of common cold events/person weeks‡	0.035	0.067	**IRR = 1.77, 95% CI (1.30, 2.42)**	**P<0.0001**
Cold events by season:				
Summer	13 (15.7%)	33 (14.8%)		
Autumn	24 (28.9%)	53 (23.8%)		
Winter	26 (31.3%)	75 (33.6%)		
Spring	20 (24.1)%	62 (27.8%)		

* n(%) Chi^2 test, Relative Risk (RR), † median (IQR) Mann Whitney test, ‡Incidence rate ratio (IRR) Poisson regression, adjusted for atopy, parity and BMI.

CCQ: common cold questionnaire, RR: relative risk, CI: confidence interval, IRR: incidence rate ratio. Seasons were Australian summer (December – February), autumn (March - May), winter (June - August), spring (September – November).

Table 3: Frequency of PCR positive colds during pregnancy

	Control (n=117)	**Asthma (n=168)**	**Effect size**	**P value**
Subjects with 1 or more PCR positive colds *	22 (18.8%)	52 (31.0%)	RR 1.18, 95% CI (1.03, 1.34)	P=0.0305
Subjects with multiple PCR positive cold events *	3 (2.6%)	8 (4.8%)		P=0.5254
PCR colds per person weeks †	26/2397= 0.0108	60/3305= 0.0182	IRR 1.18, 95% CI (0.72, 1.94)	P=0.505
Influenza A‡	1 (3.4%)	5 (7.7%)		
Influenza B‡	2 (6.9%)	3 (4.6%)		
Human Rhinovirus (RV) ‡	13 (44.8%)	25 (38.5%)		
Human Enterovirus (EV) ‡	1 (3.4%)	6 (9.2%)		
Coronavirus (CoV) ‡	3 (10.3%)	9 (13.8%)		
Respiratory Syncytial Virus (RSV) A‡	0 (0%)	1 (1.5%)		
RSV B‡	4 (13.8%)	1 (1.5%)		
Human metapneumovirus (MPV) ‡	5 (17.2%)	15 (23.1%)		
Total viruses detected	29	65		
Multiple infection	*RV+MPV* *RSVB+MPV* *RSVB+MPV*	*RV+EV* *RV+CoV* *CoV+MPV* *RSVA+RV* *FluA+MPV*		

*n(%) Chi^2 test, Relative Risk (RR), † Incidence rate ratio (IRR), Poisson regression adjusted for atopy, parity and BMI, ‡ n (% of all viruses detected).

PCR: polymerase chain reaction, RR: relative risk, CI: confidence interval, IRR: incidence rate ratio.

Table 4: Impact of PCR positive colds on pregnancy outcomes in the control group and the asthma group

	Control Group			**Asthma Group**		
	PCR- colds (n=24 pregnancies and babies)	PCR+ colds (n =22 pregnancies and babies)	P value	PCR- colds (n=53 pregnancies, n=55 babies)	PCR+ colds (n = 52 pregnancies, n=52 babies)	P value
Gestational age (weeks)*	40.8 (39.7, 41.3) *n=22*	39.9 (38.7, 41.0) *n=22*	0.1555	39.9 (38.6, 40.5) *n=54*	39.6 (38.4, 40.3) *n=52*	0.6265
Preterm delivery of infant (<37 completed weeks)†	0/22	3/22 (13.6%)	0.2316	9/54 (16.7%)	4/52 (7.7%)	0.2661
Birth weight (g)*	3600 (3384, 3885) *n=22*	3130 (2790, 3673) *n=22*	**0.0274**	3520 (3180, 3875) *n=51*	3280 (3010, 3520) *n=51*	0.0605
Low birth weight	0/22	4/22 (18.2%)	0.1157	5/51 (9.8%)	4/51 (7.8%)	0.727

(<2500 g) †						
Birth length (cm)*	52 (51.5, 53.5)	51 (49, 51.9)	**0.0236**	51 (49, 53)	50.5 (49, 52)	0.2622
	n=15	*n=18*		*n=45*	*n=44*	
Birth head	34.5 (33.5, 35.5)	34 (33, 35)	0.0974	34.5 (33.5, 35.4)	34 (33, 35)	0.3348
circumference (cm)*	*n=21*	*n=20*		*n=51*	*n=51*	
Apgar at 1 minute*	9 (8, 9)	8 (8, 9)	0.5328	9 (7, 9)	9 (6, 9)	0.8501
	n=21	*n=21*		*n=50*	*n=51*	
Apgar at 5 minutes*	9 (9, 9)	9 (9, 9)	0.5561	9 (9, 9)	9 (9, 9)	0.4819
	n=22	*n=22*		*n=50*	*n=51*	
Maternal pre-eclampsia†	0	1 (4.5%)	0.3177	0	6 (11.5%)	**0.0355**
Maternal pregnancy	0	1 (4.5%)	0.3177	2 (3.8%)	5 (9.6%)	0.4338
induced hypertension†						
Maternal gestational	0	0		2 (3.8%)	4 (7.7%)	0.6741
diabetes						
Still birth	0	0		1 (1.9%)	0	0.3241
Neonatal intensive care	3 (13.6%)	2 (9%)	0.6348	8 (4.8%)	7 (13.5%)	0.8416

admission†					
Congenital anomaly†	0	1 (4.5%)	0.3177	0	0

*median (IQR) Wilcoxon ranksum test or † n(%) Chi ^{2} test

Note: Data not available on all infants due to delivery at other hospitals.

Figure Legends:

Figure 1: Recruitment, enrolment and study completion

Figure 2: Cumulative common colds adjusted for total person weeks per group in the asthma (open triangles) and control (closed triangles) groups over the course of pregnancy (* IRR 1.77, 95% CI [1.30, 2.42]).

Figure 3: Cumulative PCR positive colds adjusted for total person weeks per group in the asthma (open triangles) and control (closed triangles) groups over the course of pregnancy (IRR 1.18, 95% CI [0.72, 1.94]).