CONCISE REPORT

Rheumatoid factor seropositivity is inversely associated with oral contraceptive use in women without rheumatoid arthritis

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Objectives: To examine whether oral contraceptive use is associated with the presence of serum rheumatoid factor in women of reproductive age without rheumatoid arthritis.

Methods: 304 women selected from parents of children who were at increased risk of developing type 1 diabetes were studied, because they were enriched with the human leucocyte antigen-DR4 allele, a susceptibility marker for both type 1 diabetes and rheumatoid arthritis. Participants visited a clinic where blood was drawn for rheumatoid factor testing, and exposure data were collected via questionnaires. A medical history and joint examination were performed to rule out rheumatoid arthritis. Participants and examiners were unaware of the participants' rheumatoid factor status at the time of examination and questionnaire.

Results: Use of oral contraceptives at any time was inversely associated with rheumatoid factor positivity (adjusted odds ratio (OR) 0.2, 95% confidence interval (CI) 0.07 to 0.52) independent of age, education and smoking. Smoking ≥ 20 pack-years was also associated with rheumatoid factor positivity (adjusted OR 56.38, 95% CI 4.31 to 736.98) compared with never smoking. Smoking 1–19 pack-years was not associated with a positive rheumatoid factor.

Conclusions: Our results suggest that oral contraceptive use, and possibly cigarette smoking, act early in the development of the immune dysregulation that occurs in rheumatoid arthritis.

Rheumatoid factor is a well-established test used in the diagnosis and prognosis of rheumatoid arthritis. In addition, rheumatoid factor precedes rheumatoid arthritis, and individuals with stable increases in rheumatoid factor have an increased incidence of rheumatoid arthritis,¹ suggesting that factors regulating rheumatoid factor production may have an important early role in the pathogenesis of rheumatoid arthritis.

Specific major histocompatibility complex class II molecule DRB1 alleles encoding the shared epitope are the most important genetic risk factors for the development of rheumatoid arthritis.² However, most individuals carrying the shared epitope do not develop rheumatoid arthritis, suggesting that a genetic predisposition is necessary but not sufficient for disease.

Several epidemiological studies have investigated the association between oral contraceptives and rheumatoid arthritis.^{3–7} Although most studies have shown a protective effect, other studies have failed to find such an association. Methodological issues and potential biases inherent in case-control studies may have resulted in these varying results.^{3–4}

Cigarette smoking has been associated with rheumatoid factor seropositivity and other measures of disease severity in rheumatoid arthritis,⁸ and with the risk of seropositive rheumatoid arthritis.⁹ However, the association between

cigarette smoking and rheumatoid factor positivity in participants without rheumatoid arthritis has not been definitively established.^{10–11}

We conducted a cross-sectional study of healthy women and examined whether exposure to sex hormones and smoking were associated with the presence of rheumatoid factor.

METHODS

Study population

The study population comprised 304 women selected from parents of children enrolled in the Diabetes Autoimmunity Study in the Young (DAISY), a birth cohort of children with an increased risk of type 1 diabetes.¹² We selected the DAISY parental population because it is enriched for the human leucocyte antigen (HLA)-DR4 allele, a susceptibility marker for both type 1 diabetes and rheumatoid arthritis. The DAISY parent cohort was assembled as follows: newborns at increased risk for type 1 diabetes were identified at St Joseph's Hospital, Denver, Colorado, USA, by screening umbilical cord blood samples for the diabetes susceptibility HLA alleles, DR4 and DR3. Newborns possessing either allele were asked to participate in the follow-up study. Additionally, children having a first-degree relative with type 1 diabetes also enrolled in the DAISY cohort. All parents of children currently enrolled in the DAISY study and who had their blood previously drawn and stored were eligible to participate in this study. The Colorado Multiple Institutional Review Board approved this study, and informed consent was obtained from all study participants.

Data collection

Six women self-reported having rheumatoid arthritis. After confirmation via reviews of medical records, based on either the diagnosis of a board-certified rheumatologist or classification by the 1987 American College of Rheumatology (ACR) criteria, they were excluded from this cohort. The remaining 298 women without self-reported rheumatoid arthritis were interviewed and examined to assess for symptoms or signs consistent with rheumatoid arthritis (according to the 1987 ACR criteria, during a single study visit). Participants and examiners were unaware of the participants' rheumatoid factor status at the time of examination and questionnaire. The interview included questions about morning stiffness, joint swelling, joint pain, subcutaneous nodules and past rheumatoid factor serology. Participants underwent a 68-joint count examination by a study rheumatologist or a study nurse practitioner trained in joint examinations. None of the 298 women examined met the criteria for rheumatoid arthritis based on the 1987 ACR classification criteria.

History and current data regarding reproductive, hormonal, smoking and coffee consumption exposures were obtained at

Abbreviations: ACR, American College of Rheumatology; DAISY, Diabetes Autoimmunity Study in the Young; HLA, human leucocyte antigen

Table 1 Risk factors for rheumatoid factor seropositivity in the study cohort of 298 women

Characteristic	RF present (n=31)	RF absent (n=267)	p Value	Unadjusted OR* (95% CI)	Final model adjusted OR† (95% CI)
Mean (SD) age (years)	37.7 (6.2)	38.2 (6)	0.70‡	0.99 (0.93 to 1.05)§	0.98 (0.91 to 1.05)
Ethnicity, n (%)			0.51¶		
White non-Hispanic	26 (83.9)	235 (88)		1	
Other**	5 (16.1)	32 (12)		1.41 (0.51 to 3.94)	
Education ≤ 12 years, n (%)††	2/29 (6.9)	36/252 (14.3)	0.28¶	0.44 (0.10 to 1.95)	0.18 (0.02 to 1.52)
Shared epitope positive, n (%)‡‡	11 (35.5)	121 (45.3)	0.30¶	0.66 (0.31 to 1.44)	
History of T1DM, n (%)	6 (19.4)	30 (11.2)	0.19¶	1.90 (0.72 to 5)	
History of T1DM in FDR, n (%)	7 (22.6)	60 (22.5)	0.99¶	1.01 (0.41 to 2.45)	
History of RA in FDR, n (%)	4 (12.9)	23 (8.6)	0.43¶	1.57 (0.51 to 4.88)	
Ever use of OC, n (%)	22 (71)	246 (92.1)	0.0002¶	0.21 (0.09 to 0.51)	0.20 (0.07 to 0.52)
Cigarette smoking, n (%)			0.0007¶		
Non-smoker	22 (71)	183 (68.5)		1	1
1–19 pack-years	6 (19.4)	82 (30.7)		0.61 (0.24 to 1.56)	0.85 (0.32 to 2.28)
≥20 pack-years	3 (9.7)	2 (0.8)		12.48 (1.98 to 78.80)	56.38 (4.31 to 736.98)
Mean (SD) duration of OC use (years)§§	6.7 (4.5)	7.4 (5.5)	0.58‡	0.98 (0.90 to 1.06)§	
Mean (SD) number of pregnancies	2.9 (1.2)	3.1 (1.7)	0.44‡	0.91 (0.71 to 1.16)§	
History of breast feeding, n (%)	28 (90.3)	243 (91)	0.90¶	0.92 (0.26 to 3.26)	
Mean (SD) duration of breastfeeding (months)¶¶	17.9 (13.1)	21.9 (18.1)	0.15‡	0.99 (0.96 to 1.01)§	
Ever use of injectable hormones, n (%)	5 (16.1)	50 (18.7)	0.72¶	0.84 (0.31 to 2.28)	
Ever use of hormone replacement therapy, n (%)***	3 (9.7)	20 (7.5)	0.67¶	1.32 (0.37 to 4.72)	
Mean (SD) consumption of caffeinated coffee (cups/day)	0.89 (1.52)	1.19 (2.28)	0.33‡	0.91 (0.70 to 1.18)§	
Mean (SD) consumption of decaffeinated coffee (cups/day)	0.23 (0.67)	0.14 (0.47)	0.48‡	1.34 (0.73 to 2.46)§	

FDR, first-degree relative; OC, oral contraceptive; RA, rheumatoid arthritis; T1DM, type 1 diabetes mellitus.

*OR calculated using univariate logistic regression; †each variable was adjusted for the other variables in the final model; ‡p Value calculated using Student's t test; §OR was calculated for any 1-unit increase in the variable (ie, year, for age and oral contraceptive duration, and month, for breast-feeding duration); ¶p value calculated using χ^2 test of heterogeneity for a 2×X comparison, with X being the number of categories of the risk factor; **includes Hispanic, black, Asian and biracial women; ††data on education available for 281 subjects; ‡‡shared epitope-positive subjects have an HLA-DR4 allele known to encode the shared epitope (DRB1*0401, DRB1*0404, DRB1*0405 or DRB1*0408); §§mean of 268 women who have ever taken oral contraceptives; ¶¶mean of 271 women who have ever breast-fed; ***data on hormone replacement therapy were missing for one individual.

the time of the clinic visit by a self-administered questionnaire. Race/ethnicity was categorised as non-Hispanic white or other, which included Hispanic, black, Asian and biracial women. Exposure to oral contraceptives was defined as a positive history for “ever” use of oral contraceptives. Duration of oral contraceptive use was the total number of years of use of oral contraceptives over the participant's lifetime. Women were considered “never smokers” if they smoked <100 cigarettes in their lifetime. For women who reported that they smoked >100 cigarettes, we further quantified exposure into 1–19 and ≥20 pack-years.

Measurement of autoantibodies

Rheumatoid factor was measured in sera of study participants using nephelometry according to the manufacturer's specifications (Dade Behring, Newark, Delaware, USA). A concentration of ≥15 IU/ml was identified as a positive test based on results from controls consisting of blood bank donors showing 5% positivity (Dade Behring N Latex RF package insert). Only positive results confirmed with a blinded duplicate were considered positive for these analyses.

HLA-DR4 subtyping

HLA-DR4 typing was performed at Roche Molecular, California, USA, using methods described previously.¹² All HLA-DR4-positive samples were evaluated at the Benaroya Research Institute, Virginia Mason, Seattle, Washington, USA, for screening for subtypes that contain the shared epitope. For determination of DR4 subtypes, the polymerase chain reaction product was treated with shrimp alkaline phosphatase (Roche Applied Science, Indianapolis, Indiana, USA) and Exonuclease I (Epicentre), followed by DNA sequencing using Big Dye

Terminator V.1.1 Cycle Sequencing (Applied Biosystems, Foster City, California, USA). Participants with the following alleles were considered to be shared epitope positive: DRB1*0401, DRB1*0404, DRB1*0405 or DRB1*0408.

Statistical analyses

All univariate and multivariate analyses were performed with SAS V.9.1. Using multivariate logistic regression analyses, we examined the independent effect of oral contraceptive use and cigarette smoking on rheumatoid factor status, considering the variables listed in table 1 as potential confounders and covariates. Age and education were retained in the final model to adjust for any effect they had on rheumatoid factor positivity, oral contraceptive use and smoking.

RESULTS

Of the 298 women, 31 (10.4%) had a positive rheumatoid factor. Women who ever used oral contraceptives were less likely to be rheumatoid factor positive than women who never used oral contraceptives (odds ratio (OR) 0.21, 95% confidence interval (CI) 0.09 to 0.51; table 1). Women who smoked ≥20 pack-years were more likely to be rheumatoid factor positive than women who never smoked (OR 12.48, 95% CI 1.98 to 78.8).

After adjusting for age, education and smoking, ever use of oral contraceptives was significantly inversely associated with the presence of rheumatoid factor (adjusted OR 0.2, 95% CI 0.07 to 0.52; table 1). Smoking ≥20 pack-years was also independently associated with a positive rheumatoid factor compared with never smoking (adjusted OR 56.38, 95% CI 4.31 to 736.98).

DISCUSSION

We evaluated associations with the presence of rheumatoid factor, rather than clinical disease. Studies showing rheumatoid factor antecedent to the onset of clinical disease support this approach.¹ Our findings are consistent with previous studies that found a protective effect of oral contraceptive use on the development of rheumatoid arthritis.^{3–7} Our unique finding that oral contraceptives are inversely associated with the presence of rheumatoid factor in healthy women suggests that these exogenous hormones may act very early in the development of the immune dysregulation that occurs in rheumatoid arthritis.

We did not find a significant “dose–response” effect with the duration of oral contraceptive use in our population, although in oral contraceptive users, rheumatoid factor-positive women had used oral contraceptives for a slightly shorter duration than rheumatoid factor-negative women. This may suggest a threshold effect, whereby the longer durations reported by the women carried little influence beyond the threshold duration.

The precise mechanism behind the protective effect of oral contraceptives on rheumatoid arthritis is unknown, but it may be that oral contraceptives, which are largely synthetic hormones, drive the immune system towards T helper 2 cytokine responses, and decreased production of proinflammatory and other cytokines leading to T helper 1-associated rheumatoid arthritis-specific cellular autoimmunity.¹³

We observed an association between rheumatoid factor positivity and smoking only in the heaviest smokers. Previous studies that have examined an association between rheumatoid factor and cigarette smoking in individuals without rheumatoid arthritis are conflicting.^{10–11} However, studies on seropositive rheumatoid arthritis and smoking reported associations between pack-years smoked and rheumatoid factor levels¹⁴; and another study found an association between smoking and seropositive rheumatoid arthritis only in subjects who had smoked ≥ 20 years at an intensity of 6–9 cigarettes/day.¹⁵ Although our study was limited by the small number of heavy smokers, our results, considered in combination with the findings from previous studies, indicate that there might be a cumulative or threshold dose effect of smoking.

Our study population was selected through children at increased risk for type 1 diabetes, and thus enriched with HLA-DR4. However, because the DAISY cohort was identified and assembled during a population-based screening, it was therefore established without prior bias relative to rheumatoid arthritis. Also, rheumatoid factor-positive and rheumatoid factor-negative women for this study were drawn from the same healthy cohort, thus minimising selection bias. Additionally, our study was not susceptible to recall and interviewer bias because the exposure data were collected without knowledge of the autoantibody status by the individual or the interviewer.

Given that all the women in this study were selected from a cohort of largely Caucasian parents who had given birth to at least one child, wider applicability of these results may be limited. Although these results are consistent with the findings of previous investigators, studies showing a protective effect of oral contraceptive use on rheumatoid factor status in a more ethnically mixed population and in nulliparous women would further support our findings. Given that our study was cross sectional, we cannot discern the temporal sequence between exposure and outcome needed to establish causality. Prospective studies designed to follow-up individuals for the development of rheumatoid factor and rheumatoid arthritis are necessary to evaluate these hypotheses further. Nevertheless, our results support the hypotheses that oral contraceptive use is

protective, and that cigarette smoking may confer increased risk very early in the rheumatoid arthritis disease course.

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