Plasma Neutrophil Extracellular Trap Remnant Levels Are Lower in Premenopausal Healthy Women Using Oral Contraceptive Pills

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Background

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by RA-specific autoantibodies with a 3-fold higher prevalence in females. Of interest, oral contraceptive pills (OCPs) have been shown to have a protective effect on the development of RA, but the specific mechanism by which this occurs has yet to be elucidated. RA-associated inflammation includes the recruitment of neutrophils that undergo neutrophil extracellular trap (NET) formation which can lead to the externalization of citrullinated proteins that can trigger the development of anti-citrullinated protein antibodies (ACPA). Therefore, we investigated the relationship between OCP use, NETs and ACPA in pre-menopausal women without RA.

Methods

Utilizing samples from the Denver site of the Studies of the Etiologies of RA cohort, we included all premenopausal women without RA and without pre-RA (i.e. serum anti-CCP-IgG positive) and split them into two groups: 1) current OCP users without prior use of other hormonal contraception, and 2) never/prior OCP users without current/prior use of other hormonal contraception. Plasma samples were tested for NET remnants using immunofluorescence for cell free DNA (cfDNA, Quanti-iT PicoGreen dsDNA) and ELISA for calprotectin (Werfen). Serum was tested by ELISA for anti-CCP-IgA (research modification of CCP3.1, Werfen) as a measure of mucosal-based ACPA. ACPA positivity via anti-CCP-IgA detection between groups was determined by chi-squared testing. NET levels between groups were determined by

Mann-Whitney U. Median levels of ACPA and NET complexes between groups compared by Wilcoxon rank sum test. Statistical analysis was performed using SPSS software.

Results

In women without RA, plasma NET remnants cfDNA and calprotectin were significantly lower in current OCP users compared to never/prior OCP users. Serum anti-CCP-IgA levels were also significantly lower in current OCP users in healthy controls. Using linear regression adjusting for age, history of pregnancy and ever smoking, cfDNA levels remained significantly associated with current OCP use [B=(-)0.48, 95% CI: (-)142.2-(-)49.6, $p<0.001$)], while lower calprotectin maintained a trend toward an association with current OCP use in multivariable analyses ($p=0.09$). In FDRs, calprotectin but not cfDNA levels were lower in current OCP users. There was no difference in anti-CCP-IgA levels based on OCP status and no difference in anti-CCP-IgG levels based on OCP status in either group ($p>0.05$). There was no difference in cfDNA, calprotectin or anti-CCP-IgA levels between prior and never users in either group ($p>0.05$ for all). In current OCP users, there was no correlation between cfDNA, calprotectin or anti-CCP levels and duration of OCP use ($p>0.05$).

Conclusion

This study found lower levels of plasma NET remnants and anti-CCP antibody levels in premenopausal women without RA who were current OCP users. The finding was more pronounced in women without an FDR with RA. These findings suggest that reduced NETosis thereby reducing anti-CCP antibodies could be a novel mechanism by which OCPs contribute to a reduced risk of RA development in women.