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Elevated serum chemokines are independently associated with both endometriosis and uranium exposure

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Abstract

Endometriosis is a complex disease impacted by the hormonal and immune systems. Cytokines and chemokines are serum biomarkers that maybe useful to develop a noninvasive disease diagnosis. Individuals in the Fernald Community Cohort were exposed to uranium, a heavy metal with radioactive properties and estrogenic potential; therefore, serum samples from women in this cohort with or without uranium and with or without endometriosis were compared for alterations in chemokine, cytokine, and matrix metalloproteinase (MMP) levels. Control women were matched to endometriosis cases by uranium exposure, age, and body mass index. MMP levels were not altered. Five chemokines and one cytokine significantly increased in endometriosis cases versus controls irrespective of uranium exposure. Uranium exposure alone was associated with an increase in inflammatory chemokines. The majority of the elevated chemokines in endometriosis cases play important roles in attracting T helper-2 cells, which may be vital to understanding the immune response in endometriosis.

Keywords

Endometriosis; Cytokine; Chemokine; Uranium

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Declaration of interest

There are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

1. Introduction

Endometriosis has long been considered an estrogen-dependent gynecological disease characterized by the presence and growth of ectopic endometrial tissue in the peritoneal cavity [1]. A growing body of literature is focusing on environmental toxicants that may play a role in the initiation or perpetuation of this disease [2]. Endometriosis can only be confirmed by surgery, definitively by histopathologic analysis on biopsy. The lesions must have at least two of the following features: endometrial epithelium, endometrial glands, endometrial stroma, and hemosiderin-filled macrophages [3]. Endometriosis affects approximately 10% of women and is associated with inflammation, severe and chronic pelvic pain, and infertility [4,5]. No consistent biomarkers exist for a non-invasive diagnosis of endometriosis and the environmental contribution to disease is in its infancy; therefore, these area of research are critically needed.

Endometriosis is a complex disease that is affected by many components. Although estrogen is the primary target for medications to treat endometriosis, the immune system is also being targeted as a major player in the initiation and perpetuation of disease [6–8]. Cells within the immune system secrete cytokines and respond to chemokines secreted by various tissues throughout the body, including immune cells [6,9]. Chemokines, or chemoattractant cytokines, locally mediate leukocyte migration, and can be broken down into two subgroups based upon the position of cysteine residues [10]. Alpha chemokines have an intervening amino acid between the first two cysteines, designated by CXC nomenclature, are potent neutrophil attractors; beta chemokines have two adjacent cysteines, designated by CC nomenclature, attract monocytes, T-cells, natural killer (NK) cells, and eosinophils. Both subgroups of chemokines are produced by the endometrium, and have been implicated in endometriosis [6]. Consistently across the literature, endometriosis cases have increased numbers of neutrophils and macrophages within the peritoneal fluid when compared to control women [11–15]. Many chemokines and cytokines have been examined, with varying results. Some of the most recognized chemokines/cytokines increased with endometriosis include regulated on activation normal T-cell expressed and secreted (RANTES/ CCL5) in endometriosis lesions, interleukin-8 (IL-8)/CXCL8 in endometrium and peritoneal fluid, and monocyte chemoattractant protein (MCP-1/CCL2) in peritoneal macrophages and endometriosis lesions [6,8,16–21]. Conversely, systemic reviews of the literature show few reports of serum chemokines, inconsistent or uncontrolled findings, and the lack of a reliable biomarker for endometriosis [22–24]. Matrix metalloproteinases (MMPs) are proteases important in angiogenesis, and also play a known role in endometriosis [25,26]. For our study, we examined chemokines, cytokines, and MMPs in serum, since they have not been studied or well studied in this biofluid, and some of the reported associations with endometriosis have been inconsistent.

The direct relationship between endometriosis and uranium has not been previously studied. However, one study suggests that uranium may act as an estrogenic compound *in vivo* [27,28]. Decreased number of small primary follicles in the ovary and accelerated vaginal opening was observed in female mice exposed to uranium in drinking water. Additionally, in these mice, an increase in uterine weight and uterine luminal epithelial cell height was seen [27], demonstrating a uterine phenotype. Heavy metals, specifically compounds similar to

uranium such as cadmium, are able to act as metalloestrogens, binding to and stimulating estrogen receptors [29]. Consistent with endometriosis being estrogen-dependent for growth, these studies suggest that women exposed to uranium may have a higher prevalence of this disease.

The Fernald Medical Monitoring Program (FMMP) was established with funds from the settlement of a class action lawsuit against the United States Department of Energy [30]. From 1951–1988, the Feed Materials Production Center (FMPC) in Fernald, Ohio converted uranium ore concentrates and recycled uranium materials to uranium metal; consequently, 310,000 kg of uranium was released into the air, and 99,000 kg was released into the water [31]. The FMMP was established to monitor the health of individuals residing in the Fernald area and to serve as a resource for future epidemiologic studies. At the end of the medical examination program, it was renamed the Fernald Community Cohort (FCC) (<http://med.uc.edu/eh/research/projects/fcc>). Endometriosis was one of the gynecological diseases recorded as part of this program. The goal of this study was twofold: 1. to identify an association between altered serum chemokines or MMPs and endometriosis, using a matched case-control design; women with and without endometriosis and 2. to identify altered serum chemokines or MMPs in women exposed to uranium, with and without endometriosis. We hypothesized that serum chemokines and MMPs will be altered in women with endometriosis compared to women without disease and that uranium exposure may also be associated with chemokine/MMP levels in serum. These differences could then be used to develop a potential panel of altered serum biomarkers, providing a non-invasive and individualized diagnostic or monitoring tool for endometriosis.

2. Materials and methods

2.1. Selecting endometriosis cases and matched controls

We conducted a nested case-control study of endometriosis among women in the FCC, an established longitudinal cohort of persons with and without exposure to uranium. This cohort is composed of voluntarily enrolled individuals who lived within 5 miles of an active uranium ore processing facility in Fernald, Ohio for at least two consecutive years (1/1/1952–12/18/1984) [30,31]. Upon enrollment, all persons signed a consent use of biospecimens and data in future research studies. The FMMP has full Institutional Review Board (IRB) approval from the University of Cincinnati IRB (University of Cincinnati, Study ID: 2012–3745). Due to sample de-identification and the use of banked stored serum samples, the University of Cincinnati IRB determined this study did not require additional IRB oversight (University of Cincinnati, Study ID: 2016–0166). Cases were selected based on a confirmed diagnosis of endometriosis using ICD9 codes 617.0 to 617.9. To be eligible, cases were required to be between the ages of 18 and 45 at the date of diagnosis, and have a banked serum samples collected $\pm$ 5 years from the date of diagnosis (Table 1). If two samples fell within this range, the sample collected before the date of diagnosis was used. We identified 40 cases meeting these criteria among women having a diagnosis of endometriosis.

Cases were further divided into subgroups based on levels of uranium exposure calculated using an algorithm developed by the Centers for Disease Control [32,33]. Exposed females

had a range of cumulative lifetime uranium exposure (beyond background) value of between 0.342 and 4.085 $\mu\text{g}/\text{m}^3$ -years (exposure groups 2 and 3). Unexposed females had values between 0.007 and 0.072 $\mu\text{g}/\text{m}^3$ -years of uranium exposure (exposure group 1).

The remaining women in the cohort served as the pool for selecting matched controls. Women were excluded from this pool if they presented with an endometriosis-associated phenotype. This phenotype was defined as having at least two of the following four categories: pain (pelvic), bleeding/menstrual abnormalities, infertility, and history of hysterectomy/ laparoscopy. Potential control samples also were excluded if they had the following conditions: Crohn's disease, rheumatoid arthritis, uterine fibroids, irritable bowel syndrome, colitis, diverticulitis, ovarian cancer, polycystic ovarian syndrome, or adenomyosis, or if pregnant at the time of serum sample collection. Control women, as well as women with confirmed endometriosis, were excluded if on hormone therapy, birth control, or immunomodulators for 3 months prior to the time of serum sample collection.

Control women were individually matched to cases, using age (± 5 years), body mass index (BMI) at the time of serum collection (± 2 units), and uranium exposure as matching criteria. For unbiased selection, each potential control was given a random number. When two or more potential controls were matches for a case on both age and BMI, the potential control with the lowest random number was selected. When matching on exposure, we reviewed the potential controls by random number order to find the control with an exposure index value closest to that of the case. In total we had four groups: two control groups, one exposed ($n = 20$) and one unexposed ($n = 19$) to uranium, and two endometriosis groups, one exposed ($n = 20$) and one unexposed ($n = 19$) to uranium. Only nineteen unexposed matched pairs were included in the matched pair analyses, since inadequate sample was provided from one unexposed case. A total of 78 samples were analyzed.

2.2. Multiplex cytokine immunoassay

The levels of chemokines and cytokines were measured using Bio-Plex Pro Human Chemokine Panel 40-plex (Bio-Rad Laboratories, Hercules, CA, Cat # 171-AK99MR2). The concentrations of MMPs were measured using Bio-Plex Pro Human MMP Panel 9-plex (Bio-Rad Laboratories, Cat # 171-AM001 M). Based on expected chemokine or MMP values in serum and the known ranges for the Bio-Plex kits, serum samples were diluted for optimal detection. Samples were randomized for analysis using GraphPad Software (GraphPad Software, Inc, La Jolla, CA). For the 40-plex, 25 μl of each serum sample was diluted to 50 μl with provided serum diluent and then used for the immunoassay. For the 9-plex, 12.5 μl of each serum sample was diluted to 50 μl with the provided sample diluent HB. The manufacturer's protocol was followed for the remainder of the protocol.

2.3. Statistical analysis

Three sets of statistical analyses were performed. In order to determine if menstrual cycle phase, defined as follicular or luteal, was a significant factor on the level of the each of the 38 chemokines, we performed linear regression analyses on a set of 41 samples with known menstrual cycle stage. For each chemokine, linear regression was performed on the chemokine using cycle stage, age, and BMI as independent variables. The significant level

was set as 0.15. The second set of 38 analyses investigated whether a difference existed between case and control pairs for each of the 38 chemokines. For each chemokine, a two-sided paired *t*-test on the chemokine measurement was performed. The significant level was set at 0.05. If a chemokine was significantly different between the two groups and for which cycle stage was deemed significant in the prior analysis, a mixed effects modeling was then applied to the chemokine to re-investigate the effect of case/ control status. In the model, we included the effect of case/control status (effect of interest) and effect of cycle information (three categories: unknown, luteal, and follicular) as fixed effects and the pairing effect as the random effect. The third set of 76 analyses was to investigate the uranium effect on the 38 chemokines in the control group and the endometriosis case group separately. For each chemokine in each group, we performed a linear regression on the chemokine measurement, using uranium, age, and BMI as independent variables. The significance level was set at 0.05. If the uranium effect was significant for a chemokine in a group, and for the chemokine, cycle was deemed significant in the prior analysis, the cycle stage was then added as an additional independent variable into the original linear regression to re-investigate the uranium effect on the chemokine in the group.

3. Results

Previous studies have demonstrated a role for elevated chemokines [6,9,17] and MMPs [34] in endometriosis lesions, peritoneal fluid, and peritoneal macrophage gene expression. Having a minimally invasive serum biomarker panel for the detection of endometriosis would greatly aid in noninvasive diagnosis of disease; therefore, we examined serum levels of chemokines and MMPs in case matched samples. Forty chemokines and cytokines, and 9 MMPs were examined. Serum samples for 90% of the cases were obtained prior to or during the year of diagnosis. For the other 10%, the serum sample used in the study was obtained during the year after diagnosis (Table 1). Age and BMI at time of sample were almost identical for cases and controls (Table 1). In the chemokine/cytokine analysis, IL2 and GM-CSF were below the limit of detection for the assay. The remaining 38 chemokines and cytokines were examined comparing control women to women with endometriosis (see Table 1 in Data in Brief [35]). One pair of matched samples in the unexposed case group was removed from analysis due to poor sample read for all chemokines.

Thirty-eight pairs of matched cases and controls were analyzed. Each chemokine was tested for normality; in some chemokines, the data was non-normally distributed, however paired *t*-tests were used because of the robust nature of *t*-tests. Differences between matched cases of endometriosis and controls showed significant increases for CCL1 ($p = 0.021$), CCL11 ($p = 0.023$), CCL22 ($p = 0.044$), CXCL9 ($p = 0.050$), CXCL13 ($p = 0.029$), and IL10 ($p = 0.022$) (Fig. 1).

For the MMPs, 9 MMPs were examined. MMP13 was below the limit of detection. Three pairs of matched samples in the exposed case group were removed from analysis due to poor sample read for all MMPs. The remaining 8 MMPs examined did not show differences when control women were compared to endometriosis cases using paired *t*-tests (Table 2). These data suggest that serum MMP levels are not suitable biomarkers for the detection of endometriosis.

We next examined if the stage of the menstrual cycle, age, or BMI affected chemokine levels. Samples were uncoupled from their matched pair to assess menstrual cycle stage (luteal vs proliferative phase), age, and BMI. Cycle data was known for 41 women. Due to the small sample size in each cycle stage, a p-value was deemed significant if < 0.15 . The cycle stage was shown to significantly affect the levels of CCL15 ($p = 0.015$), CCL20 ($p = 0.140$), CCL21 ($p = 0.078$), CCL24 ($p = 0.127$), CXCL1 ($p = 0.081$), CXCL13 ($p = 0.040$), and CXCL6 ($p = 0.009$) (Table 3). Age at sample was shown to affect the levels of CCL23 ($p = 0.013$). BMI at sample was shown to significantly affect the levels of CCL21 ($p = 0.025$) and CCL3 ($p = 0.043$). No cytokines were affected by cycle, age, and BMI. Of the chemokines that were significantly different in the matched paired analysis, only CXCL13 was also affected by cycle stage. Therefore, we performed a mixed effects model using case/control status and cycle as fixed effects, and pairing effect as random effect. Using this model and taking into account cycle stage, CXCL13 is still significant ($p = 0.035$).

Women in the Fernald Community Cohort were exposed to differing levels of uranium. To determine if uranium had an effect on the level of cytokines, we examined the relationship in control women separately from cases. When examining if uranium affects chemokine levels in control women or if these levels in control women are affected by age or BMI, we find that uranium significantly alters the level of CCL7 ($p_{\text{uranium}} = 0.036$) and CXCL16 ($p_{\text{uranium}} = 0.001$) (see Table 2 in Data in Brief [35]). Additionally, both uranium exposure and age together are significant factors and significantly affect CXCL11 ($p_{\text{uranium}} = 0.028$, $p_{\text{age}} = 0.006$), CXCL6 ($p_{\text{uranium}} = 0.033$, $p_{\text{age}} = 0.021$), IL1b ($p_{\text{uranium}} = 0.0014$, $p_{\text{age}} = 0.032$), IFN γ ($p_{\text{uranium}} = 0.002$, $p_{\text{age}} = 0.031$), and MCP1/CCL2 ($p_{\text{uranium}} = 0.005$, $p_{\text{age}} = 0.035$) (Fig. 2; [see Table 2 in Data in Brief [35]]). BMI and uranium together did not significantly alter chemokine levels.

Of the chemokines that were significantly affected by uranium, cycle stage was added as an independent variable if the cycle stage was significant in the previous analysis. CXCL6 remained significant ($p_{\text{uranium}} = 0.018$). As expected in an environmental exposure cohort, age is correlated with cumulative uranium exposure (data not shown). Therefore, some of the variation due to uranium exposure may be captured by age. Of note, due to small sample size, $p > 0.05$ – 0.1 may be of clinical relevance; therefore, CCL20 ($p_{\text{uranium}} = 0.073$), IL4 ($p_{\text{uranium}} = 0.051$), and TNF α ($p_{\text{uranium}} = 0.077$) may be clinically important. This data suggests that uranium significantly alters inflammation in women.

However, when examining if uranium affects chemokine levels in endometriosis case women or if these levels in endometriosis cases affected by age or BMI, we find that uranium itself does not significantly alter cytokine levels (see Table 3 in Data in Brief [35]). Uranium exposure and age together also do not significantly affect chemokine levels. However, uranium and BMI together affect the level of CXCL2 ($p = 0.007$, Fig. 3). Additionally, in endometriosis cases, age affects CCL21 ($p = 0.037$) and CCL27 ($p = 0.028$), and independently in endometriosis women, BMI significantly affects CCL26 ($p = 0.047$) and CXCL5 ($p = 0.031$). Together these independent findings suggest age and BMI affect the chemokine levels in control women differently than in endometriosis cases.

4. Discussion

Endometriosis has been described since the 1920s yet, we still do not know why greater than 90% of women have retrograde menstruation, but only 10% develop endometriosis [11,36]. The peritoneal milieu of those that develop endometriosis is different than those that do not, and likely this is the key to understanding why endometriosis is perpetuated in certain women. One of the themes in the literature in regards to the milieu is the different immune cell response, including the difference in dominance of helper T cells (Th) and their cytokines. Endometriosis is thought to have a Th2-biased immune response [37–40]. Th1 responses are known to be pro-inflammatory and cytotoxic, whereas Th2 responses are thought to reduce inflammation. The aberrant and suppressed nature of Th1 immune response and dominant Th2 response allows for the dissemination of endometriosis by not properly responding to what should be viewed as inflammatory within the peritoneum. In our study, we similarly find a Th2-biased response with endometriosis.

When performing paired t-tests among matched controls and cases of endometriosis, we found five chemokines (CCL1, CCL11, CCL22, CXCL9, CXCL13) and one cytokine (IL-10) with a significant increase in endometriosis cases versus controls. All have previously been identified in playing a role in the pathogenesis of endometriosis and/or infertility through *in vivo* or *in vitro* research, but their levels have not been examined as a biomarker in serum. CCL1, CCL11, CCL22, and IL-10 are all known to be associated with a Th2 immune response [38,40–42]. Although MMPs have been known to be dysregulated in endometriosis lesions, no differences were found in the serum between the cases of endometriosis and controls within our cohort.

CXCL13 is a B-lymphocyte chemoattractant, and is shown to have increased expression with chronic endometritis, a state of chronic inflammation within the uterus characterized by infiltration of plasma-cytes, among women with unexplained infertility [9,43]. Franasia et al. [44] examined CXCL13 expression in uterine endometrium within humans and rhesus monkeys across the menstrual cycle. This marker increases normally in the endometrium during the secretory phase; however, in women with endometriosis, CXCL13 is aberrantly increased in the proliferative phase of humans with endometriosis compared to control women. Our study similarly finds increased levels of CXCL13 in the serum of women with endometriosis compared to control women. We also find that CXCL13 levels are influenced by cycle stage, and therefore incorporated this into our model. Together these findings further implicate a role for CXCL13 in endometriosis.

We find CXCL9 levels increase in endometriosis cases compared to control women. CXCL9, or monokine induced by interferon-gamma (MIG), is a chemokine thought to have antitumor properties. CXCL9, and other interferon-gamma inducible chemokines, bind to the CXCR3 receptor, which is implicated in endometriosis and ovarian carcinoma cases. CXCR3 expression, shown by immunohistochemistry (IHC), is increased in ovarian tissue of endometriosis cases when compared to ovarian cancer cases co-existing with endometriosis. In contrast, *CXCL9* expression is increased most in cancer cases, followed by endometriosis cases, when compared to controls, and no differences in protein levels were seen using IHC [45]. In a contradictory study, when comparing endometrial samples of

those with deep infiltrating endometriosis versus controls, no differential gene expression of *CXCL9* was seen between groups [46]. Our findings support a role for *CXCL9* in disease diagnosis.

The trend of CCL22, or macrophage derived chemokine, is inconsistent within the literature. Although our study shows an increase in CCL22 levels in the sera of endometriosis cases, a contradictory study looking at endometriosis patient compared to infertile controls not having endometriosis showed no difference in peritoneal fluid levels [47]. Infertile women may not be an ideal control group for women with endometriosis, as so many women with endometriosis have infertility, and therefore may affect results. CCL1, a product of activated T cells, mast cells, and monocytes, is significantly increased in the sera of the endometriosis cases in our study compared to control women. CCL1 expression in a monocyte cell line, as well as in co-culture with a human peritoneal mesothelial cell line, increased with estradiol and a toxicant having estrogenic properties. The receptor for CCL1, CCR8, also increased in human ectopic endometriosis lesions when compared to eutopic endometrium in women with endometriosis [42]. This previous study alludes to a possible effect in the pathogenesis of endometriosis, however it is not conclusive. CCR8 and CCR4, the receptor for CCL22, are both expressed on Th2 cells [41], furthering the theory of Th2 dominant response in endometriosis.

CCL11, or eotaxin-1, is a chemoattractant of eosinophils. In a study comparing severe endometriosis patients versus mild endometriosis and controls, CCL11 is increased in the peritoneal fluid of women with severe disease and increased in the secretory phase of eutopic endometrium when compared to the proliferative phase of women without disease [48]. Their data and ours suggest a role for CCL11 in endometriosis and further exploration into the role of eosinophils are needed.

Our study finds an increase in Interleukin-10 (IL-10), an anti-inflammatory cytokine produced by Th2 cells and activated macrophages, in women with endometriosis. Other studies have similarly shown IL-10 increase in sera of those with endometriosis [15,49–51]. Suen et al. [49] theorize that because IL-10 is anti-inflammatory, it suppresses immunity against endometrial implants, contributing to the development of endometriosis.

In our study, uranium exposure was associated with increased chemokine levels among control women, suggesting that uranium exposure is associated with inflammation. This is similar to previous findings, including one nested case-control study within the same FCC showing that the presence of systemic lupus erythematosus (SLE), an autoimmune disease, was associated with higher levels of uranium exposure, OR 3.92 [52]. An *in vitro* model using a rat alveolar macrophage cell line showed increased TNF α , a well-known pro-inflammatory cytokine, secreted from the macrophages treated with depleted uranium compared to gadolinium, and this increase correlated with prolonged uranium exposure [53]. While we cannot make strong conclusions regarding endometriosis and uranium exposure because we matched cases and controls on uranium exposure in our model, uranium has previously been shown to be associated with inflammation and inflammatory disease states. In our study, among women with endometriosis, uranium exposure was associated with

decreased levels of CXCL2. This is likely due to the disease state of endometriosis masking the uranium effect, since it is a disease associated with inflammation.

To date, no set of biomarkers exist to diagnose endometriosis, leaving clinicians with invasive surgery as the only definitive method to diagnose this disease. Therefore, it is important to continue to report findings of increased expression of chemokines and cytokines with endometriosis to achieve our ultimate goal of noninvasive diagnosis. The large Fernald Community Cohort and the volume of information about cohort members added strength to our study by allowing for closely matched cases and controls. Three chemokines (CCL1, CCL22, and CCL11) and one cytokine (IL-10) elevated in endometriosis cases within our study play an important role in attracting Th2 cells, which may be key to understanding the immune response within the enigmatic disease of endometriosis.

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Altered Chemokine Levels in Endometriosis

Fig. 1.

Serum chemokines are increased in serum of patients with endometriosis. CCL1, CCL11, CCL22, CXCL9, CXCL13, and IL10 are significantly increased in serum of women with endometriosis compared to control women. Dotted line shows 0. The + sign denotes mean for each chemokine. The median is shown as the horizontal black line in each box.

Endometriosis women were case controlled. $p < 0.05$, paired t-tests between endometriosis cases and controls.

Fig. 2.

Uranium Effect on Chemokine Levels in Control Women. Increased uranium exposure is associated with decreased levels of CXCL11 and CXCL16, and increased levels of CCL7, CXCL6, IFN γ , IL1 β , and MCP1/CCL2 in control women. $p < 0.05$, linear regression.

Uranium Effect on Chemokine Levels in Endometriosis Women

Fig. 3.

Uranium Effect on Chemokine Levels in Endometriosis Women. Increased uranium exposure is associated with decreased levels of CXCL2 in women with endometriosis. $p < 0.05$, linear regression.

Table 1

Characteristics of Cases and Controls.

	Cases	Controls
<i>n</i>	39	39
Exposed	20	20
Unexposed	19	19
Mean (sd) age at diagnosis	38.1 (6.2)	–
Mean (sd) age at sample	36.91 (6.59)	37.12 (6.44)
BMI at sample	25.26 (4.69)	25.46 (4.54)
% with sample prior or equal to diagnosis year	90	–
% with sample after diagnosis year	10	–
Exposed - median exposure index	0.67	0.78
Unexposed - median exposure index	0.05	0.03

Table 2

Matrix Metalloproteinase Differences Between Endometriosis Cases Versus Controls.

Matrix Metalloproteinases	Difference (case - control)	
	t-statistics	p value
MMP 1	−0.48	0.634
MMP 2	−0.877	0.387
MMP 3	1.107	0.276
MMP 7	1.303	0.201
MMP 8	1.076	0.289
MMP 9	0.211	0.834
MMP 10	1.406	0.169
MMP 12	0.02	0.984

Table 3

Effect of Cycle Stage, Age, and BMI on Chemokine Level.

Chemokine	Cycle (pg/mL)		Age at sample		BMI at sample	
	β	p value	β	p value	β	p value
CCL1	1.116	0.946	0.070	0.963	-2.939	0.116
CCL11	18.604	0.758	-4.950	0.375	-3.541	0.606
CCL13	13.125	0.498	2.324	0.195	-1.520	0.489
CCL15	-1377.010	0.015	-39.744	0.423	-8.033	0.896
CCL17	-81.002	0.542	-6.136	0.615	-10.498	0.486
CCL19	-119.783	0.307	-12.115	0.254	-3.274	0.802
CCL20	-29.321	0.140	2.695	0.134	-0.980	0.658
CCL21	457.225	0.078	-13.245	0.566	66.616	0.025
CCL22	-25.602	0.825	-4.196	0.693	2.087	0.874
CCL23	209.775	0.445	-64.904	0.013	-21.147	0.497
CCL24	-252.619	0.127	-7.753	0.601	4.567	0.804
CCL25	-25.241	0.881	6.902	0.656	1.577	0.934
CCL26	-11.060	0.648	-2.974	0.187	-2.080	0.451
CCL27	-162.408	0.326	14.731	0.333	-23.920	0.205
CCL3	1.040	0.847	0.214	0.665	1.261	0.043
CCL7	237.311	0.451	31.101	0.284	-35.831	0.317
CCL8	35.625	0.210	4.520	0.081	-2.118	0.501
CX3CL1	-39.335	0.843	22.123	0.222	-25.337	0.258
CXCL1	83.239	0.081	0.475	0.912	-7.504	0.159
CXCL10	19.017	0.604	5.063	0.139	-2.358	0.571
CXCL11	66.071	0.497	10.985	0.222	-13.879	0.208
CXCL12	-191.572	0.746	24.963	0.645	30.832	0.643
CXCL13	-46.188	0.040	0.488	0.807	0.995	0.685
CXCL16	12.333	0.813	3.221	0.503	1.634	0.782
CXCL2	-67.983	0.627	-7.115	0.580	-0.371	0.981
CXCL5	82.846	0.955	-65.198	0.622	173.807	0.294
CXCL6	102.758	0.009	-4.784	0.169	0.484	0.908
CXCL8/IL8	37.283	0.236	0.497	0.862	6.822	0.059
CXCL9	7.852	0.962	4.255	0.780	-2.633	0.889
IFN γ	-1.106	0.494	-0.065	0.656	-0.028	0.878
IL10	-13.062	0.640	-0.746	0.772	0.802	0.800
IL16	175.928	0.218	6.053	0.636	5.334	0.736
IL1B	-5.897	0.786	0.610	0.756	-0.732	0.765
IL4	-7.020	0.482	0.485	0.596	0.646	0.564
IL6	11.765	0.417	1.062	0.419	2.286	0.167
MCPI/CCL2	18.913	0.494	-1.107	0.663	-0.770	0.806
MIF	698.324	0.585	174.502	0.142	-13.307	0.926
TNF α	-1.757	0.824	1.243	0.094	-0.497	0.580

Bold values = the significance for cycle (because it was a complex set of analysis) was set to be 0.15.

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