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Endometriosis-associated changes in serum levels of interferons and chemokines

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Background/aim: The aim of the study was to evaluate the serum concentration of main chemokines and interferons in patients with diagnosed endometriosis.

Materials and methods: A total of 160 women were divided in two study groups (group 1 – endometriosis; group 2 – healthy women). Serum levels of IFN-α, IFN-γ, MCP-1, MIP-1α, MIP-1β, RANTES, eotaxin, IL-8, MIG, IP-10, and IL-17A were measured with Human Multiplex Cytokine Panels.

Results. Serum levels of IFN-γ, MCP-1, and IL-8 were significantly higher (mean 14.03, 57.24, and 534.24, respectively, compared to 0.58, 20.51, and 259.82, respectively), and serum levels of IP-10 and eotaxin were significantly lower in women with endometriosis compared to the controls (mean 1.15 and 1.01, respectively, compared to 3.90 and 3.22, respectively).
Conclusions. According to our results women with endometriosis have elevated levels IFN-γ, MCP-1, and IL-8, and lower serum levels of IP-10 and eotaxin, indicating unbalanced immune activity in endometriosis.

Key words: Chemokine, inflammation, endometriosis, interferon

1. Introduction

Endometriosis is a gynecological condition characterized by the presence of tissue implants resembling endometrial glands and stroma in areas outside of the uterus (1). The ectopic implants are found most commonly in the ovaries and on the visceral and peritoneal surfaces within the pelvis. As many as 10% of women aged 30-40 years can be affected, although many more can have asymptomatic disease (2). Symptomatic cases have been associated with deregulated cytokine and chemokine productions in both ectopic and eutopic endometrium (3,4).

Chemokines are a superfamily of small polypeptides that induce chemotaxis, activate specific leukocyte subpopulations in vitro, and regulate leukocyte traffic in vivo by binding to cell-surface receptors. They have been classified into two major subfamilies, CXC and CC, based on the arrangement of the first two of the four conserved cysteine residues. CXC chemokines are further subdivided into two classes, ELR and non-ELR, depending on the presence or absence of a glutamate–leucine–arginine (ELR) sequence preceding the first two cysteines (5).

Over the last decade, a new protein, interferon-g–inducible protein-10 (IP-10) or CXCL10, belonging to the non-ELR CXC chemokine family, has been investigated. IP-10 expression has been shown to be involved in the recruitment of lymphocytes in chronic inflammation of the kidney, liver, and thyroid (5). Endometrial stromal cells express IP-10 mRNA and secrete its protein. IP-10 protein has been detected in the peritoneal fluid of healthy women and women with endometriosis; thus it could be useful as a marker of the disease (5,6).

On the other hand, the immunological balance seems to be disturbed in endometriosis; therefore, it is useful to describe the variations in immune factors that contribute to this imbalance. Interleukin (IL)-8 and other pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) are factors considered to play an important role in endometriosis pathogenesis. It has been shown that concentrations of IL-8 and TNF-α in the peritoneal fluid are significantly correlated with the stage of the disease (7–9), but there is little evidence about serum concentrations of these cytokines and the severity of the disease. IL-8 is a macrophage-produced chemokine exhibiting strong angiogenic, pro-inflammatory, and growth-promoting effects that induce both chemotaxis of neutrophils and the expression of several cell adhesion molecules. The adherence of endometrial cells induces further IL-8 expression by an integrin-dependent mechanism,

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suggesting that this cytokine might act as an autocrine growth factor in the pathogenesis of endometriosis (6,10). IL-8 might also facilitate the initial attachment of endometrial cells to the peritoneal surfaces because it stimulates adhesion of endometrial stromal cells to fibronectin (6,11).

Interferon (IFN)-γ, IP-10, and monocyte chemotactic protein (MCP)-1 have also been involved in the pathogenesis of endometriosis; previous studies showed that IFN-γ and MCP-1 synergistically increase monocyte activation (12). Elevated levels of MCP-1 in peritoneal fluid and endometrial glands of endometriosis patients have been recently reported (12). Thus, a higher concentration of MCP-1 might be partly responsible for higher IFN-γ production by monocytes and macrophages.

Given the relationships between the serum concentration of IFN-gamma, MCP-1, IL-8, IP-10, and the pathogenesis of endometriosis, our study aimed to evaluate the serum profile of the main chemokines (IFN-α, IFN-γ, MCP-1, MIP-1α, MIP-1β, RANTES, eotaxin, IL-8, MIG, IP-10, and IL-17A) in patients with endometriosis and their usefulness as specific disease markers.

2. Materials and methods

2.1. Study population and design

We conducted a case-control study that included 160 patients, recruited in consecutive order, divided into two groups. Group 1 (endometriosis group) contained 80 women with regular menses, and with no history of pelvic infections, autoimmune and neoplastic diseases who were undergoing laparoscopy or laparotomy for suspected endometriosis. The evidence of endometriosis was verified by histopathological analysis. The severity of endometriosis was staged according to the revised American Society for Reproductive Medicine (rASRM) classification. Group 2 (control group) contained 80 healthy nonpregnant women aged between 18 and 40 years old without clinical and para-clinical evidence of endometriosis who were undergoing laparoscopy for unexplained infertility. Patients with previous pelvic surgeries, history of cancer, suspected malignancy, adenomyosis or leiomyoma, pre-surgical suspicion of evidence of premature ovarian failure, or the use of ovarian suppressive drug, such as oral contraceptives, GnRH agonists, progestins, or danazol in the preceding 6 months were excluded from the study. None of the patients had taken anti-inflammatory medications or had been diagnosed with an inflammatory or infectious condition for ≥6 months before the study.

The study protocol was approved by the Local Ethics Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, and signed informed consent was received from each woman before sample collection. The study was conducted under the tenets of the Helsinki Declaration. First 5 mL of venous blood was collected from each patient before breakfast and before any surgical procedure, and it was centrifuged and the serum obtained was stored at –70 °C for future determinations.

2.2. Cytokine evaluation

We used multiplex cytokine kits (Invitrogen Human Cytokine 30-Plex Panel, LHC6003) in order to measure serum levels of IFN-α, IFN-γ, MCP-1 (CCL-2), MIP-1α (CCL-3), MIP-1β (CCL-4), RANTES (CCL-5), eotaxin (CCL-11), IL-8 (CXCL8), MIG (CXCL9), IP-10 (CXCL10), and IL-17A. Measurements were performed with a Luminex 200 system (Luminex Corporation, Austin, TX, USA) in accordance with the manufacturer's specifications (Invitrogen Corporation, Carlsbad, CA, USA). The sensitivity of the test was specified by the manufacturer (Invitrogen Corporation).

Average sensitivity of the test for IFN-α was <5 pg/mL with an interassay variation coefficient of 7.9%. For IFN-γ, the average sensitivity of the test was <0.5 pg/mL, with an interassay variation coefficient of 9.0%. The average sensitivity of the test for MCP-1 was <5 pg/mL, with an interassay variation coefficient of 5.8%. In the case of MIP-1α, the average sensitivity of the test was <5 pg/mL, with an interassay variation coefficient of 9.5%. The sensitivity of the test for MIP-1β was <5 pg/mL, and the interassay variation coefficient of 3.6%. The test for RANTES revealed an average sensitivity of <10 pg/mL, with an interassay variation coefficient of 8.9%. For CCL-11, the average sensitivity of the test was <0.5 pg/mL, with an interassay variation coefficient of 9.0%. In the case of IL-8, the average sensitivity of the test was <5 pg/mL, with an interassay variation coefficient of 9.8%. For MIG, the average sensitivity of the test was <5 pg/mL, with an interassay variation coefficient of 9.4%. The average sensitivity of the test for IP-10 was <0.5 pg/mL, with an interassay variation coefficient of 3.7%, and for IL-17A, the average sensitivity of the test was <1 pg/mL, with an interassay variation coefficient of 6.0%.

2.3. Statistical analysis

Statistical analyses were performed using Microsoft Excel and IBM SPSS software (version 21.0). The data are presented as mean ± standard deviation (SD) and standard error (SE) for the groups. The Kolmogorov–Smirnov test for normality, Levene's test for equality of variances, and t-test were used as statistical tests. P values less than 0.05 were regarded as significant.

3. Results

Tables 1–3 present the biometry data, the markers' distribution among the studied groups, and the detection rate. Almost all our cases from the endometriosis group were staged as III (30%–37.50%) or IV (47%–58.75%)

Table 1. Descriptive statistics of the endometriosis and control groups.

Group	Age (years) (SD)	Weight (kg) (SD)	Height (cm) (SD)	BMI (kg/cm^2) (SD)
Endometriosis	30.60 (5.48)	62.05 (9.06)	164.72 (5.11)	22.91 (3.52)
Control	26.35 (2.13)	56.92 (8.09)	167.22 (6.77)	20.30 (2.12)
Average	28.47 (4.65)	59.48 (8.92)	165.97 (6.09)	21.60 (3.17)

SD, Standard deviation; BMI, Body mass index.

Table 2. Descriptive statistics of the studied markers.

	Detection rate % (n)	Mean (pg/mL) (SE) ± SD	Skewness (SE)	Kurtosis (SE)
IFN-γ	89.37 (143)	7.31 (2.03) ± 17.27	5.474 (0.28)	36.64 (0.55)
RANTES	99.37 (159)	25.21 (0.171) ± 1.53	–0.372 (0.26)	–0.43 (0.53)
CCL-11	82.5 (132)	2.153 (0.25) ± 2.07	1.957 (0.29)	4.71 (0.58)
MCP-1	95 (152)	37.91 (3.49) ± 30.47	1.804 (0.27)	3.90 (0.54)
IL-8	96.25 (154)	398.81 (63.15) ± 554.19	2.573 (0.27)	6.71 (0.54)
IP-10	76.87 (123)	2.48 (0.37) ± 2.97	2.031 (0.30)	5.12 (0.59)
IL-17	77.5 (124)	1.32 (0.19) ± 1.56	1.641 (0.30)	2.37 (0.59)

SE, Standard error; SD, Standard deviation; IFN, Interferon; RANTES, regulated on activation, normal T cell expressed and secreted; CCL-11, Eotaxin; MCP, Monocyte chemotactic protein; IL, Interleukin; IP, Interferon gamma-induced protein.

Table 3. Studied markers group distribution and distribution normality of the studied markers between groups.

	Group (n)	Mean (pg/mL) (SE) ± SD	KS with Lilliefors significance correction	
			Statistic	Probability
IFN-γ	E (72)	14.03 (3.76) ± 22.61	0.310	< 0.001
	C (71)	0.58 (0.16) ± 0.97	0.230	0.032
RANTES	E (80)	25.46 (0.22) ± 1.42	0.168	0.200*
	C (79)	24.96 (0.25) ± 1.62	0.177	0.200*
CCL-11	E (64)	1.01 (0.14) ± 0.82	0.260	0.005
	C (68)	3.22 (0.39) ± 2.31	0.228	0.035
MCP-1	E (72)	57.24 (5.71) ± 34.29	0.159	0.200*
	C (80)	20.51 (1.36) ± 8.64	0.148	0.200*
IL-8	E (78)	534.24 (101.74) ± 635.42	0.333	<0.001
	C (76)	259.82 (68.25) ± 420.73	0.115	0.200*
IP-10	E (63)	1.15 (0.28) ± 1.61	0.257	0.006*
	C (60)	3.90 (0.62) ± 3.43	0.362	<0.001*
IL-17	E (62)	1.41 (0.30) ± 1.70	0.240	0.015
	C (62)	1.24 (0.25) ± 1.43	0.237	0.023

*A lower bound of the true significance; E, Endometriosis; C, Control; KS, Kolmogorov–Smirnov; SE, Standard error; SD, Standard deviation; IFN, Interferon; RANTES, regulated on activation, normal T cell expressed and secreted; CCL-11, Eotaxin; MCP, Monocyte chemotactic protein; IL, Interleukin; IP, Interferon gamma-induced protein.

and only 3 cases (3.75%) were in stage II of endometriosis according to the rASRM staging criteria. The detection rate for IFN-α, MIP-1α, MIP-1β, and MIG, respectively, in the studied groups was 11.25%, 5.0%, 7.5%, and 16.25%, respectively, without the possibility of establishing statistical significance. To be able to use parametric tests, we verified the distribution normality in the studied groups. Table 3 also presents the obtained data with the normality test.

Table 4 presents the results obtained with the t-test for independent samples, which shows that mean serum levels of IFN-γ, MCP-1, and IL-8 were significantly higher in patients with endometriosis compared to the healthy controls (mean 14.03, 57.24, and 534.24, respectively, compared to 0.58, 20.51, and 259.82, respectively; $P = 0.001$, $P < 0.001$, and $P = 0.028$, respectively). At the same time we can observe a significant lower level of CCL-11 and IP-10 in patients with endometriosis compared to the healthy controls (mean 1.01 and 1.15, respectively, compared to 3.22 and 3.90, respectively; $P < 0.001$, and $P < 0.001$).

Figures 1–3 show the mean serum levels of the studied markers between the groups with a significantly lower serum level of IP-10 and eotaxin (CCL-11), and a significantly higher serum level of IFN-γ, MCP-1, and IL-8 in the endometriosis group. No significant differences were observed in the serum levels of IL-17A and RANTES between the studied groups.

4. Discussion

Despite the long years of research, the pathophysiology of endometriosis is still uncertain. A large number of studies suggest that immune system alterations play critical roles in the development and progression of endometriosis, along with hormonal, genetic, and environmental factors.

Our study focused particularly on serum concentration of IFN-α, IFN-γ, MCP-1, MIP-1α, MIP-1β, RANTES, eotaxin, IL-8, MIG, IP-10, and IL-17A in patients with diagnosed endometriosis. We found significantly higher serum levels of IFN-γ, MCP-1, and IL-8 in women with endometriosis compared to the healthy controls, and a significantly lower serum concentration of IP-10 and eotaxin between the studied groups. We also found that IL-17A and RANTES had similar serum concentrations between women with endometriosis and women free of the disease. On the other hand, IFN-α, MIP-1α, MIP-1β, and MIG had a very low detection rate in the studied groups, and so we were unable to draw any conclusions regarding their significance.

IFNs are cytokines involved in cellular communication and immune responses. IFN-γ along with other cytokines (IL-1, TNF-α) has been previously implicated to play an important role in the pathogenesis of endometriosis (13). At the same time, its role in endometriosis is not completely understood. Some authors have described increased IFN-γ mRNA expression in endometriosis compared to

Table 4. Statistical significance of the studied markers between the groups.

		Levene's test for equality of variances	t-test for equality of means (2-tailed)
IFN-γ	Equal variances assumed	0.000	0.001
	Equal variances not assumed		0.001*
RANTES	Equal variances assumed	0.228	0.152
	Equal variances not assumed		0.152
CCL-11	Equal variances assumed	0.001	0.000
	Equal variances not assumed		0.000*
MCP-1	Equal variances assumed	0.000	0.000
	Equal variances not assumed		0.000*
IL-8	Equal variances assumed	0.240	0.029*
	Equal variances not assumed		0.028
IP-10	Equal variances assumed	0.002	0.000
	Equal variances not assumed		0.000*
IL-17	Equal variances assumed	0.946	0.680
	Equal variances not assumed		0.680

* Significant differences; IFN, Interferon; RANTES, regulated on activation, normal T cell expressed and secreted; CCL-11, Eotaxin; MCP, Monocyte chemotactic protein; IL, Interleukin; IP, Interferon gamma-induced protein.

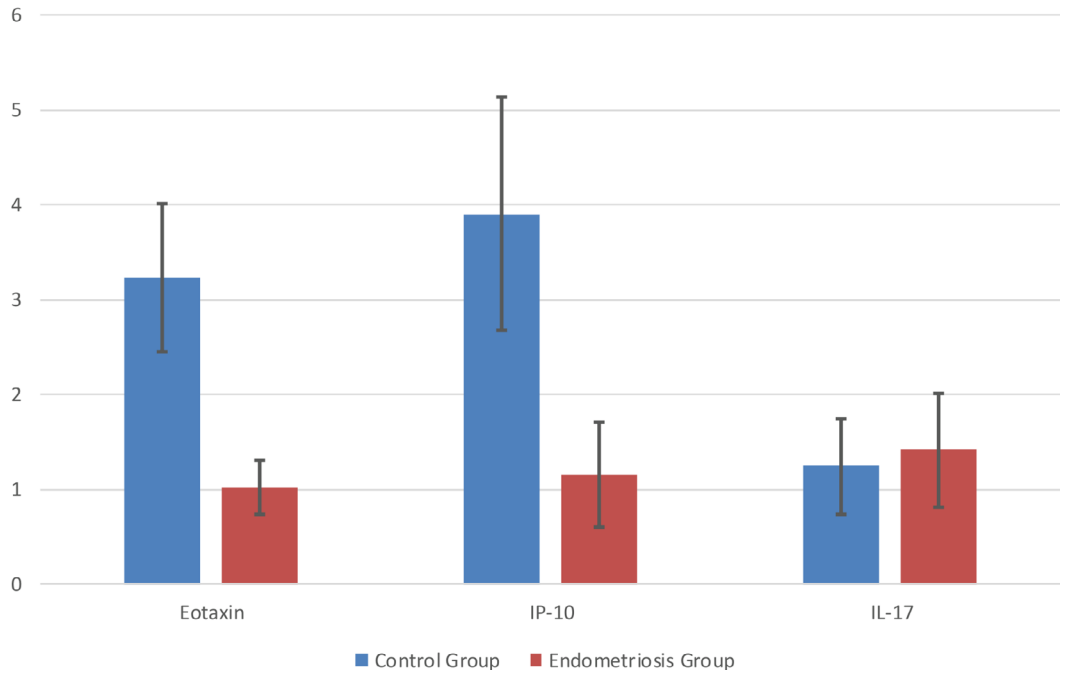

Figure 1. Mean serum levels of eotaxin, IP-10, and IL-17 between the groups.

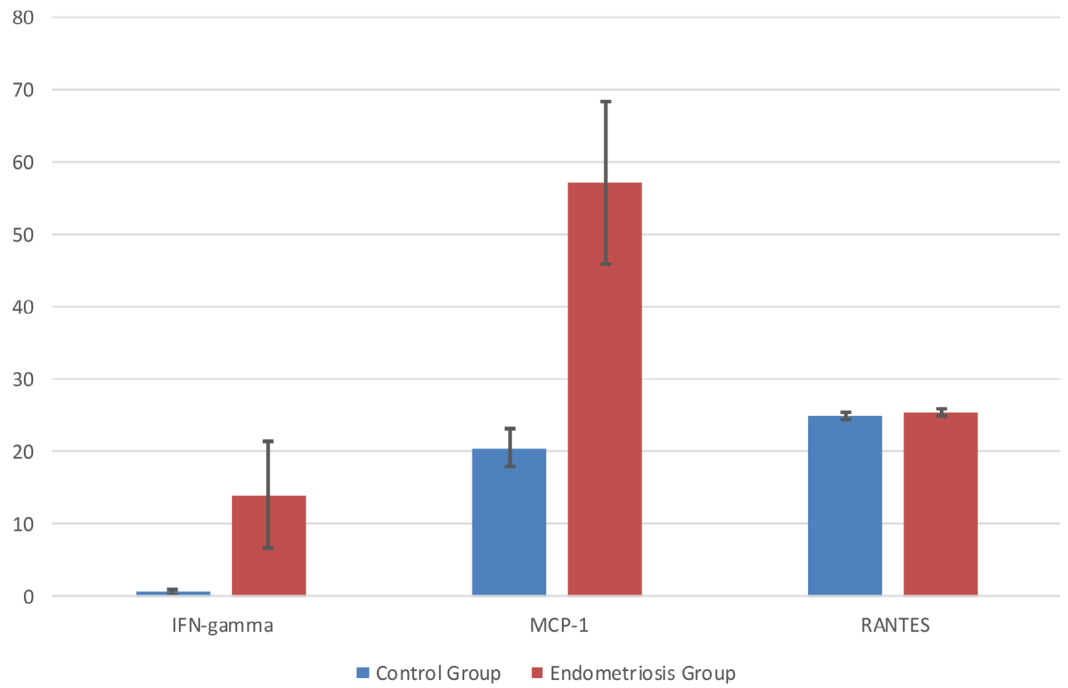

Figure 2. Mean serum levels of IFN-γ, MCP-1, and RANTES between the groups.

eutopic endometrium (12). It is known that IFN-γ inhibits the proliferation of human endometrial epithelium (14), but the resistance of endometriotic cells to IFN-γ-induced cell growth inhibition and apoptosis has been found to be a possible mechanism in endometriosis pathogenesis, and, at the same time, it seems that IFN-γ peritoneal fluid (PF) levels could be suppressed in endometriosis patients (15). Different studies have reported contradictory results regarding peripheral blood (PB) and peritoneal fluid (PF) concentrations of IFN-γ, with decreases, increases, or

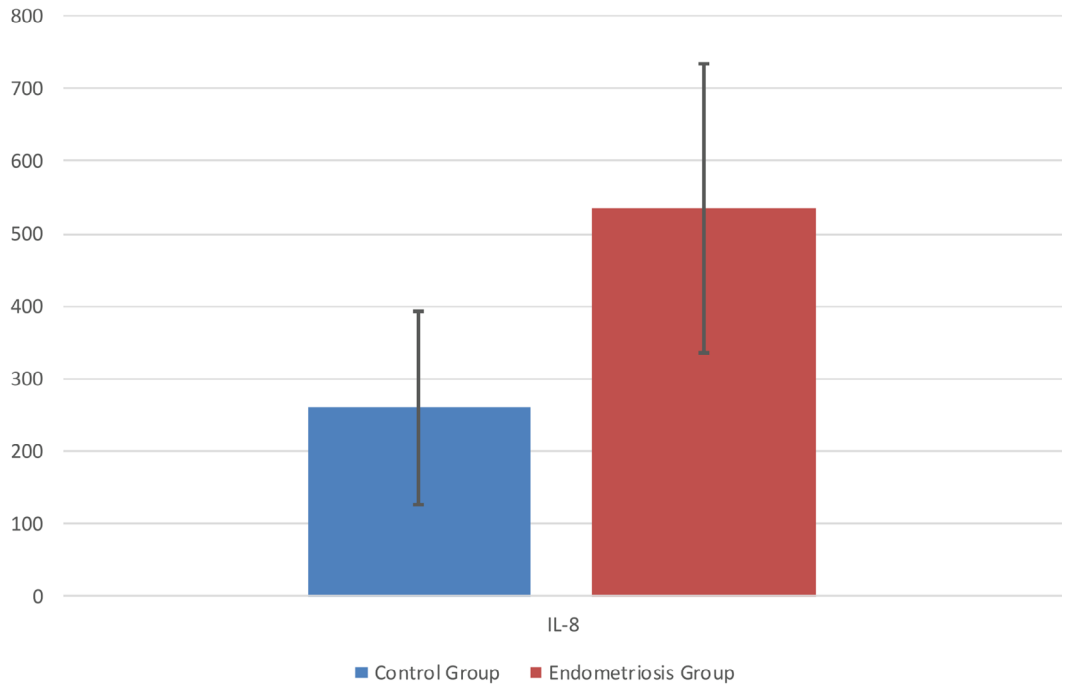

Figure 3. Mean serum levels of IL-8 between the groups.

lack of differences between women with endometriosis and controls (5,16,17). Our results are somewhat in contradiction with the local inhibitory effect of IFN-γ, as we found an increased level in endometriosis patients compared with controls, but at the same time INF-γ serum levels could be influenced by other factors and thus not a true reflection of the local endometriosis environment.

MCP-1 is a small cytokine belonging to the CC family. It is secreted by a number of cell types, including macrophages, endothelial cells, fibroblasts, and mesothelial and endometrial cells and attracts monocytes, eosinophils, T lymphocytes, and NK cells (18). Expression of MCP-1 is induced in human endometrial stromal cells by adhesion of these cells to extracellular matrix proteins. Recent studies have found that MCP-1 serum concentration was significantly increased in endometriosis rat models than in those without, suggesting a possible diagnostic value (18). On the other hand, PF concentrations of MCP-1 have been shown to increase with endometriosis severity (19). Our results are in accordance with those reported by Othman-Eel et al. (17), which found increased serum levels of IFN-γ, MCP-1, and IL-6 in patients with endometriosis compared to controls, suggesting a possible implication of these cytokines in endometriosis and also a possible diagnostic role.

IL-8 is a pro-inflammatory chemokine secreted by several cell types, especially immune cells, with possible involvement in endometriosis. IL-8, along with other chemokines, plays important roles in angiogenesis associated with endometriosis. Arici et al. reported that IL-8 is produced in healthy endometrium as well as in endometriotic tissue and it induces proliferation of endometrial stromal cells (6). At the same time, Iwabe et al. demonstrated that IL-8 exerts growth actions in normal endometrial cells and in endometriotic cells (20). A very recent review emphasized IL-8 as the best biomarker for endometriosis, among other chemokines (21). Moreover, a case-control study showed increased serum levels of IL-8 and IL-6 in patients with ovarian endometriomas (OEs) (22).

IP-10 is produced by activated lymphocytes, endothelial cells, fibroblasts, etc., and possesses antiangiogenic properties. Some authors have shown that IP-10 is also produced by eutopic endometrial cells (14). At the same time, different authors have reported significant decreases in IP-10 PF concentrations in women with advanced-stage endometriosis as compared with early-stage disease (23,24). CCL-11 is a selective chemoattractant for eosinophils implicated in allergic responses. Hornung et al. indicated that CCL-11 is produced in epithelial cells of normal endometrium and endometriosis tissues, varies across the menstrual cycle, and is elevated in women with endometriosis (25). Recently, CCL-11 has been implicated with a critical role in the development of endometriosis as an angiogenic factor (26). Moreover, another recent study showed that CCL-11 induced by IL-4 might promote angiogenesis and the subsequent development of endometriosis (27).

Regarding IL-17A, a very recent study showed differential expression of IL-17A in human ectopic endometriotic lesions and matched eutopic endometrium from women with endometriosis. Moreover, surgical removal of lesions resulted in significantly reduced plasma IL-17A concentrations, thus showing that endometriotic lesions produce IL-17A and that the removal of the lesion via laparoscopic surgery leads to a significant reduction in systemic levels of IL-17A (28). Our results show similar levels between endometriosis patients and controls, thus adding to the controversy around the role of IL-17A in the context of endometriosis.

In this context, our results are partially in accordance with previous studies, showing a decrease in serum concentration of both IP-10 and CCL-11 and a higher level of IL-8. These results are obtained in cases of advanced endometriosis, most of the patients presenting for large OEs suggesting a possible correlation between PF concentrations and serum levels of IL-8 and IP-10 as in the study conducted by Yoshino et al., and also confirming the findings reported by Carmona et al.

Limitations of the present study could arise from the study population. On one hand, patients included in the study were only Caucasian women. Most of the literature studies had mainly Asian origin subjects, this being considered a possible bias mark. On the other hand, most of the patients included in our study were stage III or IV. As the study was conducted in a university clinic, the patients presenting for treatment and included in the study had late stages of endometriosis, thus the low rate of early stages. Probably future studies could include patients presenting for unexplained infertility undergoing laparoscopy, thus including patients with early stages discovered incidentally. We could also take in consideration as a limitation the detection sensitivity of multiplexed immunoassays. It was shown that while multiplexed immunoassays have sensitivity comparable to conventional ELISA, it is possible that the robustness may vary among different multiplex bead arrays (29).

In conclusion, the present study evaluated the possible differences in main chemokines serum concentration from patients with endometriosis compared to healthy controls using a multiplexed cytokine assay. We have shown that chemokines with a pro-inflammatory profile, IL-8, MCP-1, and IFN-γ have significantly higher serum levels in women with endometriosis compared to controls, and that eotaxin and IP-10, a chemokine secreted in response to IFN, have significantly lower levels in women with endometriosis compared to women free of disease. No difference was found in the serum levels of IL-17A and RANTES between the groups. All of these changes, associated with changes in cytokine profile, indicate an unbalanced immune activity in endometriosis, which could be responsible for the occurrence and progression of the disease. Further studies are necessary to confirm the involvement of these chemokines in the pathogenesis of endometriosis, and moreover to establish their possible role in diagnosis or treatment of endometriosis.

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