Cytokines and endometriosis

J. Markowska[1], *Prof.*; **M. Kowalska**[2], *Ph.D.*; **M. Gogacz**[3], *Ph.D., M.D.*; **J. Lubin**[1], *Ph.D., M.D.*; **M. Markowski**[4], *Ph.D., M.D.*; **J. Kamińska**[2], *Assist. Prof.*

[1]*Karol Marcinkowski University School of Medicine, Poznan*
[2]*Cancer Center and Institute of Oncology, Warsaw*
[3]*32nd Department and Clinics of Gynaecology, Medical Academy, Lublin (Poland)*
[4]*Clarkson University, Potsdam NY (USA)*

Summary

Introduction: Abnormal expression of cytokines is considered to be involved in the pathogenesis of endometriosis.

Material and Methods: In 63 women with various stages of endometriosis preoperative levels of cytokines, including IL-6, IL-8, BVEGF, b-FGF, of TNF RI and TNF RII were analysed employing ELISA tests (R&D kits).

Results: In the sera of endometriosis patients significantly augmented levels of IL-6 and of b-FGF were detected as well as a trend for elevated IL-8 levels.

Conclusion: Elevated serum levels of cytokines may show poor correspondence to the localized pathological process. Endometriosis would find a stricter reflection in cytokine levels in fluids or tissues of the pelvis minor.

Key words: Cytokines; Endometriosis.

Introduction

Endometriosis represents a benign disease of the female reproductive system, related to estrogen-generating function of ovaries. Nevertheless, local spread of the process by infiltration, generation of implants, elevated levels of CA125 at advanced stages of the disease, potential for endometriosis-based carcinogenesis as well as controversies related to its epidemiology, diagnosis and treatment point to serious risks linked to the diagnosis.

Already in 1925 Sampson described the development of endometrioid carcinoma on the background of endometriosis and provided criteria which pointed to malignant transformation of the latter [1]. Later studies demonstrated that endometriosis may provide fertile ground for development of also other types of cancer, e.g., clear cell carcinoma [2, 3]. Such risk of malignant transformation increases mainly in women with an extended history of endometriosis, particularly in postmenopausal women with high serum levels of CA125 [4].

According to Ness and Cottreau development of endometriosis is linked to the local inflammatory reaction, activation of macrophages, elevated levels of cytokines and growth factors [5]. The infiltration and formation of endometriotic implants seem to also be linked to neoangiogenesis.

Cytokines, pleiotropic growth factors, factors of differentiation, migration and apoptosis play key roles in immune and inflammatory reactions, associated with cell proliferation, pro- and anti-angiogenic processes as well as in neoplastic transformation. All the functions seem to be disturbed in endometriosis [6-10]. Therefore, estimation of a broad range of cytokines might be purposeful in studies on pathogenesis as well as on treatment of the disease.

Revised manuscript accepted for publication September 6, 2004

The present study aimed at estimation of various types of cytokines, including pro-angiogenic cytokines, VEGF and b-FGF, the pro-inflammatory cytokine, IL-6, receptors for TNFα, TNF RI and TNF RII and of a chemokine, IL-8, in women with endometriosis. The studies were conducted on 63 women, 17 to 74 years of age (mean 45.5), treated by surgery for endometrioid cysts.

Material and Methods

The patients' blood was sampled before surgery. The cytokines and free cytokine receptors (IL-6, VEGF, bFGF, IL-8, TNF RI, TNF RII) were estimated in serum using kits from R&D Systems (Minneapolis, USA). In the course of surgery the spread of endometriotic pathology was evaluated. The patients were allocated to four groups, corresponding to Stages I to IV according to the verified classification of endometriosis, as required by the American Fertility Society [11].

Statistical calculations were performed using Minitab software. Comparison of mean cytokine levels with reference values was performed using the Student's t-test for a single variable. Comparison of mean levels of individual cytokines in various advanced stages of endometriosis took advantage of the one-way Anova test, accepting the confidence level of $p = 0.05$.

Results

Mean values, standard deviations and p values are listed in Table 1.

As evident in Table 1, levels of IL-6 and b-FGF were significantly elevated in women with endometriosis while levels of IL-8, VEGF, TNF RI and TNF RII were similar in the women with endometriosis and healthy controls. A tendency for elevated levels of IL-8 was noted in patients with endometriosis. Analysis of IL-6 levels in groups of patients with various stages of endometriosis demonstrated significant differences in Stages III and IV, as compared to the reference value ($p = 0.001$ and $p = 0.019$, respectively).

Table 1. — *Concentrations of cytokines in serum: mean values, standard deviations and p values.*

Cytokine	No. of patients	Reference value; pg/ml	Mean value, SD	p
IL-6	63	0.7-2.4	18.54±39.74	0.001*
IL-8	18	10.0-12.5	39.3±76.7	0.078
VEGF	42	< 325	210.4±195.7	0.993
b-FGF	48	< 4.4	14.99±14.32	0.0001*
TNF RI	41	600-1200	1070.6±271.0	0.998
TNF RII	34	< 3500	1918.0±510.0	0.999

Levels of β-FGF in individual stages of endometriosis were higher than the reference value at p = 0.001, p = 0.031, p = 0.001 and p = 0.013 for Stages I to IV, respectively.

Discussion

In view of our results, participation of serum containing cytokines in the development of endometriosis seems to be restricted. The significantly elevated serum levels of IL-6 and of β-FGF and the tendency for augmented serum levels of IL-8 may indicate that both inflammatory processes and angiogenesis may participate in the development of the disease, similarly as they are involved in the development of ovarian cancer [5, 12].

We have not been able to document involvement of TNFα receptors, TNF RI and TNF RII, in the development of the disease, despite the results of Koga *et al.* [8] who documented elevated levels of the receptors in the peritoneal fluid. Possibly, TNF RI and TNF RII, which modulate action of pro-inflammatory cytokine, TNFα, may act only locally, in the peritoneal cavity. It seems probable that the well known reciprocal interactions of cytokines are of local significance. Therefore, determination of the cytokine levels of the same cytokine panel in the serum and in the peritoneal fluid in parallel might permit a more accurate evaluation of cytokine reactions taking place in patients with endometriosis.

References

[1] Sampson J.A.: "Endometrial carcinoma of the ovary arising in endometrial tissue in that organ". *Arch. Surg.*, 1925, *10,* 1.
[2] Lee K.R., Nucci M.R.: "Ovarian mucinous and mixed epithelial carcinomas of mullerian (endocervical like) type: a clinicopathologic analysis of four cases of an uncommon variant associated with endometriosis". *Int. J. Gynecol. Pathol.,* 2003, *22*, 42.
[3] Okuda T., Otsuka J., Sekizawa A., Saito H., Makino R., Kushima M. *et al.*: "p 53 mutations and overexpression affect prognostic of ovarian endometrioid cancer but not clear cell cancer". *Gynecol. Oncol.,* 2003, *88*, 3118.
[4] Tagashira Y., Shimada M., Kigawa J., Iba T., Terakawa N.: "Ovarian endometriosis in a young women". *Gynecol. Oncol.,* 2003, *91*, 643.
[5] Ness R.B., Cottreau C.: "Possible role of ovarian epithelial inflammation in ovarian cancer". *J. Natl. Cancer Inst.*, 1999, *91*, 1459.
[6] Yamamoto S., Kanishi J., Mandai M., Kuroda H., Komatsu T., Nanbu K. *et al.*: "Expression of vascular endothelial growth factor (VEGF) in epithelial ovarian neoplasms: correlation with clinicopathology and patients survival and analysis of serum VEGF levels". *Br. J. Cancer,* 1997, *76,* 1221.
[7] Oehler M.K., Caffier H.: "Diagnostic value of serum VEGF in women with ovarian tumors". *Anticancer Res.*, 1999, *19*, 2519.
[8] Koga K., Osuga Y., Tsutsumi O, Okagaki R., Momoeda M., Yano T. *et al.*: "Increrased concentrations of soluble tumour necrosis factor receptor (S TNFR) I and II in peritoneal fluid from women with endometriosis". *Mol. Hum. Reprod.*, 2000, *10,* 929.
[9] Braun D.P., Gebel H., House R., Rana N., Dmowski W.P.: "Spontaneous and induced synthesis of cytokines by peripheral blood monocytes in patients with endometriosis". *Fertil. Steril.,* 1996, *65*, 1125.
[10] Scappaticci F.A.: "Mechanisms and future directions for angiogenesis-based cancer therapies (review article)". *J. Clin. Oncol.*, 2002, *20,* 3906.
[11] "American Fertility Society. Revised American Fertility Society Classification of Endometriosis". *Fertil. Steril.*, 1985, *43,* 351.
[12] Markowska J., Szala S: "Inhibitors of angiogenesis in therapy of ovarian cancers". *Eur. J. Gynecol. Oncol.*, 2004, *25*, 5, 562.

Address reprint requests to:
J. MARKOWSKA, Prof.
Karol Marcinkowski
University School of Medicine
in Poznan
Lakowa str. 1
61878 Poznan (Poland)