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Non-invasive prediction of endometriosis revisited; 3 biomarkers as Angiopoietin-2, Interleukin-1*β* and Vascular Endothelial Growth Factor

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ABSTRACT

Introduction: Endometriosis affects up to 1 every 5 women at their reproductive age, with variable and complex symptomatology. Patients may be asymptomatic but may have pain episodes or subfertility. Its negative impact is on patients' health and quality of life. Objective: it was to investigate the serum and peritoneal fluid (PF) concentrations of Angiopoietin-2, Interleukin-1*β*, and Vascular Endothelial Growth Factor, aiming to evaluate their diagnostic performance in endometriosis. Methods: Serum and peritoneal fluid samples were taken from 112 women undergoing laparoscopy for infertility, pelvic pain or adnexal masses. 61 diagnosed with endometriosis and 51 controlled. Primary outcome was to estimate serum and PF concentrations of Angio-2, IL-1*β* and VEGF and secondarily correlate these concentrations to disease stages thus assuming their diagnostic potential. Results: Significant differences were found between patients and control as regards serum and PF concentration of all studied markers except serum IL-1*β*. Serum Angio-2 and PF VEGF showed a significantly higher level in more advanced stages of endometriosis. PF VEGF showed a positively significant correlation with the stage of the disease, spearman coefficient t = 0.442 p = 0.014. PF concentrations of Angio-2 and Serum VEGF did not show significant pattern changes with stage-related levels. Diagnostic potential of serum and PF concentrations of the 3 markers were assessed by the ROC curve. Angio-2 proved an excellent diagnostic ability for endometriosis. PF and serum VEGF proved an equal diagnostic performance, whereas, PF IL-1*β* was the least efficient. Based on the results, we suggested preliminary serum threshold values for these markers to be used as diagnostic or follow-up landmarks with relatively acceptable sensitivity, specificity, positive and negative predictive values. Conclusion: Non-invasive predictive biomarkers for endometriosis were Serum Angio-2, IL-1*β*, and VEGF independently or in combination with the estimated threshold values. Serum Angio-2 merit is considered as a novel marker for endometriosis due to its diagnostic power.

Keywords: Endometriosis; Angiopoietin-2; VEGF; IL-1*β*

1. INTRODUCTION

Endometriosis affects up to 1 every 5 women at their reproductive age group, with variable and often complex symptomatology. Patients may be asymptomatic but may have pain episodes, and/or subfertility [1]. Unlike its aetiopathogenesis, its negative impact is on patients' health and quality of life is clear [2,3]. The stage of endometriosis can be morphologically classified by using the revised American Fertility Society (rAFS) staging system according to the localization and the size of nodes in the peritoneum and ovaries, and the presence of adhesions in the ovary and the fallopian tube [3]. This rAFS classification includes the following stages: I = Minimal (1 - 5 points); II = Mild (6 - 15 points); III = Moderate (16 - 40 points); IV = Severe (<40 points) [4].

Effective treatment could be achieved; however, diagnosis of the disease remains a problem, simply, because accurate diagnosis can only be attained via laparoscopic inspection, preferably with histopathologic confirmation of suspected lesions. This remains an invasive technique, with possible complications and health care

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costs [5]. However, ovarian endometriomas, and deep infiltrating endometriotic nodules can be detected adequately by vaginal ultrasound, but it does not rule out peritoneal endometriosis or endometriosis-associated adhesions [1]. This situation has led the researchers recently to refocus on the possible mechanism by which endometriosis occurs. The fact is that an altered immune mechanism might be involved in the development of the condition is now widely accepted, which explains why some women are more prone than others to develop the disease. The concentration of some immunological markers in the peritoneal fluid largely correlates with the disease occurrence, progression, and symptoms [6].

There is substantial evidence that immunologic factors play a role in the pathogenesis of endometriosis and endometriosis-associated infertility. Decreased natural killer cell cytotoxicity leads to an increased likelihood of implantation of endometriotic tissue and modulation of growth and inflammatory behavior of ectopic endometrial implants.

The alteration in cytokines level and other angiogenic factors as Vascular Endothelial Growth Factor (VEGF) may explain the adhesion, implantation and the progression of the transported fragment of endometrium in the different site of endometriosis. Many cytokines have been studied to prove the immunological theory of endometriosis as Interleukin-1β (IL-1β). It has been generally accepted that the establishment of new biomarker as angiopoietin-2 (Angio-2) played a key part in the progression of endometriosis, however this role still needs further confirmation to suggest a relation to endometriosis already proven for many pivotal angiogenesis stimulators such as VEGF [7,8].

Setting a non-invasive biochemical diagnostic and prognostic test for endometriosis with an acceptable level of accuracy and sensitivity, would fundamentally change those patients' quality of life, save their costs and risks of the more invasive tools, and eventually make it smoother for health care providers to manage the condition with ease.

The current study was designed to evaluate the serum and peritoneal fluid concentrations of Angiopoietin-2, Interleukin-1β, and Vascular Endothelial Growth Factor in patients with endometriosis as well as controls, thus adding some insight to the formulation of a non-invasive test to screen, detect, or follow up patients with endometriosis.

The ultimate objective was to prove any of these markers independently or in combination as non-invasive diagnostic biomarker in patients with endometriosis.

2. PATIENTS AND METHODS

Blood and (PF) samples were taken from 112 women undergoing laparoscopy for infertility, pelvic pain, and/or adnexal masses in their first half of the menstrual cycle. Procedures were done at a specialized unit of Gynecologic endoscopy at a University hospital.

Sixty one were histologically confirmed to have endometriosis and 51 were controls. No cases were excluded. A full informed consent was taken from each subject. Enrolment took place after formal approval of the Ethical committee of the Faculty of Medicine, Alexandria University was obtained.

PF samples were collected by aspiration from the peritoneal cavity during laparoscopy.

Serum samples: Five ml of venous blood were aseptically collected and the clear serum was stored at –70˚C until analysis.

Measurement of IL-1β, VEGF, and Angio-2 in serum and PF were done using a commercially available, enzyme-linked immunosorbent assays (ELISA) using the reagents and protocol supplied with the ELISA kit (R&D Systems, Minneapolis, Minnesota, United States) Frozen serum and PF samples were thawed and then analyzed.

3. STATISTICAL ANALYSIS

Data distribution was analyzed by using the Statistical Packages for the Social Sciences (SPSS version 10.0, Chicago, IL) and the Sigma-Stat software package (version 3.5; SPSS, Chicago, IL). Normally distributed data were presented as mean, standard deviation, whereas skewed data were expressed as median and range. Pearson's correlation was also used for evaluation of the linear relationship between different clinical variables. Pairwise comparisons between groups were performed using the Wilcoxon-Mann-Whitney test. In order to determine which serum marker could best represent patients with endometriosis, sensitivity and specificity were assessed with receiver operating characteristic ROC curves. The area under the curve was approximately the percentage correctly classified if the test was used as a diagnostic tool. The same analyses were performed with the PF measures and statistical significance was assessed using two-tailed tests and an alpha level of $p < 0.05$.

4. RESULTS

A total of 112 women underwent laparoscopy for infertility (n = 49), pelvic pain (n = 22), and/or adnexal masses (n = 41). Sixty one were diagnosed with endometriosis and 51 were controls. No cases were excluded. Out of the 61 patients with endometriosis, 25 (40.98%) had early disease (stages I and II) and 36 (59.02%) had late disease (stages III and IV).

The median age of the study group was 33.3 years, median body mass index (BMI) was 27.0, and median parity was 1.

No significant differences were found as regards age, parity and BMI among the studied groups.

5. CLINICAL DATA

Patients were presented with pain, discharge, and/or infertility, those who presented with the latter were at advanced stages only (**Table 1**).

A significant difference was found between patients and controls' serum and PF concentration of all studied markers except serum IL-1β (**Table 2**).

Meanwhile, significant difference was elicited between early and late stages in terms of serum Angio-2 and PF VEGF concentrations with significant increase in more advanced stages (Z, p = 3.311 (0.042*), 2.188* 0.034 respectively), but the other studied markers with no specific pattern of stage-related level changes (**Table 3**).

Table 1. The relation between the serum and peritoneal fluid concentration of the 3 biomarkers and the stage of endometriosis among women.

(pg/ml)	Pain		Discharge		Infertility	
	Early	Late	Early	Late	Early	Late
Serum Angio-2						
Min - Max	3400 - 5320	3800 - 6240	3735 - 4300	3900 - 6240	-	3635 - 6530
Mean ± SD	4278.5 ± 618.6	4341.3 ± 692.1	5401.5 ± 399.5	4653.8 ± 1090.5	-	4302.5 ± 550.5
Median	4115.00	4230.00	4017.50	4237.50	-	4280.00
Z (p)	0.272 (0.786)		0.355 (0.533)			-
PF Angio-2						
Min - Max	2980 - 3990	3120 - 9000	2980 - 3990	3280 - 4840	-	3120 - 9500
Mean ± SD	3524.2 ± 372.4	4653.9 ± 2128.3	3575.0 ± 435.5	4060.0 ± 1103.1	-	4524.6 ± 1969.2
Median	3605.00	3805.00	3665.00	3842.50	-	4060.00
Z (p)	1.414 (0.157)		0.643 (0.800)			-
Serum VEGF						
Min - Max	600 - 3600	800 - 3750	650 - 1950	1210 - 3540	-	600 - 4020
Mean ± SD	1975.5 ± 971.2	2229.2 ± 961.3	1300.0 ± 919.2	2512.5 ± 1005.8	-	2060.1 ± 1128.8
Median	2008.00	2150.00	1300.00	2650.00	-	2054.00
Z (p)	0.571 (0.568)		0.165 (0.267)			-
PF VEGF						
Min - Max	650 - 3600	1100 - 14000	850 - 3600	2400 - 3900	-	1100 - 14000
Mean ± SD	2047.5 ± 1059.6	4233.1 ± 3967.8	2187.5 ± 1141.9	3150.0 ± 1060.7	-	4596.7 ± 4029.3
Median	2375.00	3000.00	2150.00	3150.00	-	3085.00
Z (p)	1.687 (0.092)		0.240 (0.267)			-
Serum IL-1β						
Min - Max	33 - 254	56 - 260	65 - 201	198 - 215	-	33 - 254
Mean ± SD	138.9 ± 74.5	147.8 ± 65.6	143.5 ± 62.2	206.5 ± 12.0	-	142.2 ± 71.0
Median	122.00	142.00	154.00	206.50	-	132.50
Z (p)	0.381 (0.703)		0.165 (0.267)			-
PF IL-1β						
Min - Max	39 - 456	46 - 490	101 - 355	278 - 378	-	39 - 480
Mean ± SD	238.9 ± 126.2	289.3 ± 131.9	257.5 ± 114.2	328.0 ± 70.7	-	293.9 ± 147.4
Median	245.00	316.50	287.00	328.00	-	331.50
Z (p)	1.142 (0.253)		0.355 (0.533)			-

Z: Z for Mann Whitney test. *Significant at $p < 0.05$.

Table 2. The relation between endometriosis and serum and PF levels of the studied biomarkers.

	Endometriosis		Z (p)
	Cases n = 61	Control n = 51	
Serum Angio II (pg/ml)			
Min - Max	3400.00 - 6240.00	2566.00 - 5211.00	
Mean ± SD	4307.83 ± 775.30	3765.30 ± 604.94	2.794* (0.005)
Median	4232.50	3642.50	
PF Angio II (pg/ml)			
Min - Max	2985.00 - 9500.00	2980.00 - 6169.00	
Mean ± SD	4843.00 ± 1682.46	4211.83 ± 873.00	3.622* (<0.001)
Median	4803.50	3700.00	
Serum VEGF (pg/ml)			
Min - Max	600.00 - 4020.00	160.00 - 402.00	
Mean ± SD	2158.37 ± 1026.87	286.83 ± 77.07	6.653* (<0.001)
Median	2024.00	291.50	
PF VEGF (pg/ml)			
Min - Max	650.00 - 14000.00	96.00 - 322.00	
Mean ± SD	3174.67 ± 2857.60	214.30 ± 68.74	6.654* (<0.001)
Median	2700.00	207.00	
Serum IL-1β (pg/ml)			
Min - Max	33.00 - 260.00	25.00 - 240.00	
Mean ± SD	142.73 ± 67.29	129.73 ± 66.24	0.673 (0.501)
Median	135.50	108.00	
PF IL-1β (pg/ml)			
Min - Max	39.00 - 490.00	35.00 - 302.00	
Mean ± SD	277.63 ± 134.27	174.43 ± 84.04	3.149* (0.002)
Median	282.50	191.00	

Z: Z for Mann Whitney test. *Statistically significant at $p < 0.05$.

In addition, a positive correlation of PF VEGF with the stages of the disease was found, (spearman coefficient = 0.442*) (**Table 4**).

Non-invasive prediction of endometriosis and the diagnostic potential of the serum and PF concentrations of the 3 markers were assessed by the Receiver-operating characteristic (ROC) curves. Serum Angio-2 proved best diagnostic performance of 100% with an outstanding area under the curve AUC 1.000 followed by serum VEGF AUC 0.920 and the PF AUC 0.810, then serum IL-1β AUC 0.737 and PF IL-1β AUC 0.515 was the least efficient.

Preliminary threshold values for the 3 studied markers in serum were assumed to serve as diagnostic or follow-up landmarks with relatively acceptable sensitivity, specificity, positive and negative predictive values (**Table 5**).

6. DISCUSSION

Our results confirmed that serum VEGF, Angio-2, and IL-1β respectively have an excellent diagnostic ability to screen cases with endometriosis with a good level of sensitivity and specificity. This was not related to the stage of the disease which makes these markers, preferably in combination, more suitable to construct a non-surgical, screening system prototype rather than a staging module. All samples were taken during the first half of

Table 3. Comparison between the two stages of endometriosis according to serum and PF of the 3 biomarkers.

	Stages of endometriosis		Z (p)
	Early	Late	
Serum Angio-2 (pg/ml)			
Min - Max	3400 - 5320	3800 - 6240	
Mean ± SD	4285.6 ± 559.5	4341.3 ± 692.1	3.311 (0.042*)
Median	4115.00	4550.00	
PF Angio-2 (pg/ml)			
Min-max	2980 - 3990	3120 - 9500	
Mean ± SD	3524.2 ± 372.4	4670.3 ± 2045.2	1.566 (0.117)
Median	3605.00	3842.50	
Serum VEGF (pg/ml)			
Min - Max	600 - 3750	800 - 4020	
Mean ± SD	2111.2 ± 109.31	2229.2 ± 961.3	0.233 (0.816)
Median	2024	2150	
PF VEGF (pg/ml)			
Min - Max	650 - 1400	1100 - 3600	
Mean ± SD	2047.5 ± 1059.6	3926.1 ± 3421.8	2.188 (0.034*)
Median	2375.00	3005.0	
Serum IL-1β (pg/ml)			
Min - Max	33 - 254	56 - 260	
Mean ± SD	139.4 ± 65.3	147.8 ± 68.6	0.381 (0.703)
Median	132.50	142.00	
PF IL-1β (pg/ml)			
Min - Max	39 - 480	46 - 490	
Mean ± SD	269.8 ± 131.1	289.3 ± 139.9	0.381 (0.703)
Median	273.00	316.50	

*Statistically significant at $p < 0.05$.

Table 4. Correlation between serum and PF levels of Ang-2, VEGF and IL-1β with the stages of the disease.

		Serum			PF		
		Angio-2	VEGF	IL-1B	Angio-2	VEGF	IL-1B
Angio-2	r		0.152	0.112		0.011	0.062
	p		0.422	0.557		0.954	0.744
VEGF	r			0.241			0.032
	p			0.199			0.865
IL-1β	r						
	p						
Stages Early & Late	rho	0.102	0.034	0.036	0.296	0.442*	0.034
	p	0.592	0.858	0.850	0.112	0.014	0.859

r: Pearson coefficient. rho: Spearman coefficient. *Statistically significant at $p \leq 0.05$.

Table 5. Diagonstic performance of the studied biomarkers with suggested threshold values.

	Serum Ang-2 (pg/ml)	Serum VEGF (pg/ml)	Serum IL-1β (pg/ml)
Threshold (cut of values)	3700	500	120
Sensitivity	100.00	90.00	60.00
Specificity	100.00	60.00	53.33
Positive predictive value (PPV)	100.00	69.23	56.25
Negative predictive value (NPV)	100.00	85.71	57.14
Accuracy	100.00	75.00	56.67

the menstrual cycle to neglect any effect of the sampling time on the results. Bedaiwy *et al.* [5] made the sampling time all through the menstrual cycle and found differences related to sampling time that they claimed not significant, so we preferred to avoid those differences.

PF laparoscopic sampling; especially for VEGF, and IL-1β can be more supportive in establishing diagnostic tests in subtle or atypically-presenting cases, where evident endometriosis lesions would be hardly present to assess histologically. We did not, however find any significant correlation between symptoms such as pain with the levels of PF studied markers including the cytokine; IL-1β. This contradicts what Scholl *et al.* [9] stated about an increased level of PF cytokine with the severe pain and dysmenorrhea in endometriosis.

We were also able to estimate a preliminary cut-off values, based on the number of patients studied, for the serum markers in this cohort of patients, where Angio-2 showed the best sensitivity, and specificity followed by VEGF and IL-1β respectively. There were relatively acceptable positive and negative predictive values, as well, at the estimated cut-off levels. We assumed a promising role for these makers that could be extended to similar substances as diagnostic or follow-up landmarks in cases destinated to develop endometriosis. Similarly, Mihalyi *et al*. [10] concluded a diagnostic role of a panel of six selected plasma biomarkers in the diagnosis of different stages of endometriosis with good sensitivity and specificity. They studied, however, different biomarkers than those in the present study, including some cytokines, and obtained serum samples during the secretory phase or during menstruation. Bedaiwy *et al.* [5] also assumed a non- surgical diagnosis of endometriosis be possible by measuring serum IL-6 and PF TNF-α.

VEGF is a heparin-binding glycoprotein with potent angiogenic, endothelial cell-specific mitogenic and vascular permeability activities.

Studies have demonstrated that VEGF is involved in both the etiology and maintenance of peritoneal endometriosis [11]. Moreover, in normal endometrium the expression of VEGF is potentiated by a variety of cytokines, especially Interleukin-1β (IL-1β) [12].

In this study we confirmed a good diagnostic performance of VEGF both in serum and PF.

We found significant differences of PF VEGF and serum Angio-2 levels related to the stage of endometriosis, this was in accordance with Meresman *et al.* [13] who reported same results stating that VEGF-A levels in the serum and PF of patients with severe endometriosis (stages III-IV) were significantly higher than in those with minimal endometriosis owing to the modulation of this marker by the disease itself.

Angio-2 is a cytokine that can amplify the effects of other angiogenic factors, mainly VEGF but it can not independently activate angiogenesis. Angio-2 is the endogenous antagonist of Angiopoietin-1, binding the same receptor. Angio-1 has been demonstrated in endometrial and periendothelial cells and in glandular and stromal cells of endometriotic lesions.

Zhang *et al.* [14] suggested overexpression of Angio-2 gene responsible for development and/or progression of endometriosis.

Data on Angio-1 and Angio-2 remain too scarce to clarify any role or mechanism in the aetiopathogenesis of endometriosis. We assumed a role for serum Angio-2 in non-invasive prediction of endometriosis based on our results which demonstrated an out-standing diagnostic ability for serum Angio-2 of 100% with (AUC 1.000), however It was much less efficient for PF Angio-2 (AUC = 0.937), this results were in accordance with Di Carlo *et al*. [15] who stated that patients with endometriosis had higher levels of angiogenic factors including Angio-2 compared with normal controls. Meanwhile, those angiogenic factors' concentrations in serum and PF, as reported by Bourlev *et al.* [16] normalize within a week after surgical removal of endometriotic lesions in women with advanced endometriosis.

IL-1β, a proinflammaory cytokine, was produced mainly by monocytes macrophages, and dendritic cells. In health, circulating human blood monocytes or bone marrow aspirate do not constitutively express IL-1β, Stimulants such as the complement component C5a, hypoxia, and adherence to surfaces or clotting of blood induce the synthesis of large amounts of IL-1 mRNA in monocytic

cells [17-19].

A predictive performance of IL-1β was also suggested in the light of this work. Based on our analysis, the PF IL-1β was significantly higher in patients than controls wit—h better diagnostic potential compared to serum IL-1β. This could be assumed to the fact that, being a proinflammatory cytokine, locally released in the vicinity of active endometriotic lesions. Menstrual pain scores with endometriosis were assessed in relation to PF cytokine concentration in a study by Scholl *et al.* [9] where a significant difference was found according to the pain score. Cytokine profiles were also tackled in a study by Kalu *et al.* [20] where an elevated level of PF interleukins but not serum confirmed a role in the pathogenesis of endometriosis.

Hou *et al.* [21] suggested a possible clinical strategy for the treatment of endometriosis by neutralization of IL-1β, thus counteracting its effect on endometrial stromal cells.

Serum Angio-2 and VEGF markers with the estimated peritoneal fluid levels would be an addition to the diagnostic potential in endometriosis.

7. CONCLUSION

Non-invasive predictive biomarkers for endometriosis as Serum Angio-2, VEGF and IL-1β independently or in combination were estimated with threshold values. Serum angio-2 merit is considered as a novel marker for endometriosis due to its diagnostic power.

REFERENCES

[1] Kennedy, S., Bergqvist, A., Chapron, C., D'Hooghe, T., Dunselman, G., Greb, R., Hummelshoj, L., Prentice, A., Saridogan, E. on Behalf of the ESHRE Special Interest Group for Endometriosis Endometrium Guideline Development Group (2005) ESHRE guideline for the diagnosis and treatment of endometriosis. *Human Reproduction*, **20**, 2698-2704. doi:10.1093/humrep/dei135

[2] Vercellini, P., Trespidi, L., De Giorgi, O., Cortesi, I., Parazzini, F. and Crosignani, P.G. (1996) Endometriosis and pelvic pain: Relation to disease stage and localization. *Fertility and Sterility*, **65**, 299-304.

[3] Leng, J.H., Lang, J.H., Dai, Y., Li, H.J. and Li, X.Y. (2007) Relationship between pain symptoms and clinicopathological features of pelvic endometriosis. *Zhonghua Fu Chan*, **42**, 165-168.

[4] American Society for Reproductive Medicine (1997) The revised American Society of endometriosis: 1996. *Fertility and Sterility*, **67**, 817-821. doi:10.1016/S0015-0282(97)81391-X

[5] Bedaiwy, M.A., Falcone, T., Sharma, R.K., Goldberg, J.M., Attaran, M., Nelson, D.R. and Agarwal, A. (2008) Prediction of endometriosis with serum and peritoneal fluid markers: A prospective controlled trial. *Human Reproduction*, **17**, 426-431. doi:10.1093/humrep/17.2.426

[6] Ramey, J.W. and Archer, D.F. (1993) Fertil Steril. Peritoneal fluid: its relevance to the development of endometriosis. *Fertility and Sterility*, **60**, 1-14.

[7] Cai, J.T., Zhang, Y.D., Zhang, Y., Yu, R., Li, M.X., Zhang, Y., Peng, G.Q. and Peng, L.X. (2008) Expression of heparanase and angiopoietin-2 in patients with endometriosis. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, **136**, 199-209. doi:10.1016/j.ejogrb.2006.09.018

[8] Wang, H., Gorpudolo, N., Li, Y., Feng, D., Wang, Z. and Zhang, Y. (2009) Elevated vascular endothelial growth factor-A in the serum and peritoneal fluid of patients with endometriosis. *Journal of Huazhong University of Science and Technology—Medical Sciences*, **29**, 637-641. doi:10.1007/s11596-009-0520-7

[9] Scholl, B., Bersinger, N.A., Kuhn, A. and Mueller, M.D. (2009) Correlation between symptoms of pain and peritoneal fluid inflammatory cytokine concentrations in endometriosis. *Gynecological Endocrinology*, **25**, 701-706. doi:10.3109/09513590903159680

[10] Mihalyi, A., Gevaert, O., Kyama, C.M., Simsa, P., Pochet, N., De Smet, F., De Moor, B., Meuleman, C., Billen, J., Blanckaert, N., Vodolazkaia, A., Fulop, V. and D'Hooghe, T.M. (2010) Non-invasive diagnosis of endometriosis based on a combined analysis of six plasma biomarkers. *Human Reproduction*, **25**, 654-664. doi:10.1093/humrep/dep425

[11] Donnez, J., Smoes, P., Gillerot, S., Casanas-Roux, F. and Nisolle, M. (1998) Vascular endothelial growth factor (VEGF) in endometriosis. *Human Reproduction*, **13**, 1686-1690. doi:10.1093/humrep/13.6.1686

[12] Lebovic, D.I., Shifren, J.L., Ryan, I.P., Mueller, M.D., Korn, A.P., Darney, P.D. and Taylor, R.N. (2000) Ovarian steroid and cytokine modulation of human endometrial angiogenesis. *Human Reproduction*, **15**, 67-77. doi:10.1093/humrep/15.suppl_3.67

[13] Meresman, G., Buquet, R., Bilotas, M., Sueldo, C. and Baranao, R.I. (2010) Effect of vascular endothelial growth factor and interleukin-1 on apoptosis in endometrial cell cultures from patients with endometriosis and controls. *Journal of Reproductive Immunology*, **84**, 193-198. doi:10.1016/j.jri.2009.12.002

[14] Zhang, Y., Cai, J.T., Li, M.X., Zhang, Y., Liu, H.N. and Yu, R. (2006) Expression of heparanase and angiopoietin-2 mRNA in endometriosis. *Zhonghua Fu Chan Ke Za Zhi*, **41**, 291-294.

[15] Di Carlo, C., Bonifacio, M., Tommaselli, G.A., Bifulco, G., Guerra, G. and Nappi, C. (2009) Metalloproteinases, vascular endothelial growth factor, and angiopoietin 1 and 2 in eutopic and ectopic endometrium. *Fertility and Sterility*, **91**, 2315-2323. doi:10.1016/j.fertnstert.2008.03.079

[16] Bourlev, V., Iljasova, N., Adamyan, L., Larsson, A. and Olovsson, M. (2010) Signs of reduced angiogenic activity after surgical removal of deeply infiltrating endometriosis. *Fertility and Sterility*, **94**, 52-57. doi:10.1016/j.fertnstert.2009.02.019

[17] Rein, D.T., Schmidt, T., Bauerschmitz, G., Hampl, M., Beyer, I.M., Paupoo, A.V., Curiel, D.T. and Breidenbach, M. (2010) Vascular Endothelial Growth Factor (VEGF)-targeted gene therapy for the treatment of endometriosis. *Fertility and Sterility*, **93**, 2687-2694.

[18] Plank, M.J., Sleeman, B.D. and Jones, P.F. (2004) The role of the angiopoietins in tumour angiogenesis. *Growth Factors*, **22**, 1-11. doi:10.1080/08977190310001643218

[19] Gescher, D.M., Berndorrf, U., Meyhoefer-Malik, A., Moubayed, P. and Malik, E. (2004) Immunolocalization of angiopoietin1 in human peritoneal endometriotic lesions. *Fertility and Sterility*, **81**, 857-862. doi:10.1016/j.fertnstert.2003.09.032

[20] Kalu, E., Sumar, N., Giannopoulos, T., Patel, P., Croucher, C., Sherriff, E. and Bansal, A. (2007) Cytokine profiles in serum and peritoneal fluid from infertile women with and without endometriosis. *Journal of Obstetrics and Gynaecology Research*, **33**, 490-495. doi:10.1111/j.1447-0756.2007.00569.x

[21] Hou, Z., Zhou, J., Ma, X., Fan, L., Liao, L. and Liu, J. (2008) Role of interleukin-1 receptor type II in the pathogenesis of endometriosis. *Fertility and Sterility*, **89**, 42-51. doi:10.1016/j.fertnstert.2007.01.044

