DETERMINAÇÃO DE INDICADORES DE ESTADO DE CITOCINA E FATORES DE ANGIOGÊNESE EM PACIENTES COM ENDOMETRIOSE GENITAL EXTERNA E DEFICIÊNCIA DE VITAMINA D

DETERMINATION OF CYTOKINE STATUS INDICATORS AND ANGIOGENESIS FACTORS IN PATIENTS WITH EXTERNAL GENITAL ENDOMETRIOSIS AND VITAMIN D DEFICIENCY

ОПРЕДЕЛЕНИЕ ПОКАЗАТЕЛЕЙ ЦИТОКИНОВОГО СТАТУСА И ФАКТОРОВ АНГИОГЕНЕЗА У ПАЦИЕНТОК С НАРУЖНЫМ ГЕНИТАЛЬНЫМ ЭНДОМЕТРИОЗОМ И ДЕФИЦИТОМ ВИТАМИНА D

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RESUMO

O estudo foi realizado para encontrar uma associação entre alguns indicadores de status de citocinas, nível de fatores de crescimento endotelial vascular e vitamina D, em mulheres com infertilidade e endometriose genital externa (EGE), a fim de aumentar a efetividade do tratamento dessa patologia. O baixo nível de vitamina D na dinâmica foi determinado em 240 pacientes com idades entre 25 e 35 anos com EGE planejando a gravidez, determinando o nível de 25 (OH) D no soro sanguíneo pelo método quimioluminescente. O status de interleucina (IL-1 β , IL-6, IL-4), TNF- α , VEGF-R1 no soro sanguíneo foi determinado usando imunoensaio enzimático múltiplo. Os resultados dos estudos revelaram um nível aumentado de IL-6, IL-1 β e TNF- α em grupos com baixo teor de vitamina D. Na vitamina D normal, foram registrados níveis significativamente mais baixos de fator de crescimento endotelial vascular (VEGF-R1) no soro sanguíneo. As taxas de gravidez foram maiores nos grupos com níveis normais de 25 (OH) D no soro sanguíneo. O nível sérico médio de VEGF-R1 em mulheres grávidas que engravidaram sozinhas foi 1,3-1,5 vezes menor.

Palavras-chave: *endometriose genital externa, citocinas, VEGF, vitamina D.*

ABSTRACT

The study was performed to find an association between some cytokine status indicators, level of vascular endothelial growth factors, and vitamin D in women with infertility and external genital endometriosis (EGE) in order to increase the effectiveness of treatment of this pathology. The low vitamin D status in the dynamics was determined in 240 patients aged 25 to 35 years with EGE planning pregnancy by determining the level of 25 (OH) D in the blood serum using the chemiluminescent method. Interleukin status (IL-1 β , IL-6, IL-4), TNF- α , VEGF-R1 in blood serum were determined using enzyme-multiple immunoassay. The results of the studies revealed an increased level of IL-6, IL-1 β , and TNF- α in groups with low vitamin D content. In normal vitamin D significantly lower levels of vascular endothelial growth factor (VEGF-R1) in the blood serum were registered. Pregnancy rates were higher in groups with normal 25 (OH) D levels in the blood serum. The mean serum VEGF-R1 level in pregnant women who became pregnant on their own was 1.3-1.5 times lower.

Keywords: *external genital endometriosis, cytokines, VEGF, vitamin D.*

АННОТАЦИЯ

Исследованы цитокиновые показатели (IL-1 β , IL-4, IL-6, ФНО- α) и VEGF-R1 у 240 пациенток с наружным генитальным эндометриозом (НГЭ), проживающих в Республике Дагестан и планирующих беременность, с выявленным дефицитом витамина D. В результате проведенных исследований установлено повышение уровня IL-6, IL-1 β и ФНО- α в группах с низким содержанием витамина D. Низкие показатели фактора роста эндотелия сосудов (VEGF) в сыворотке крови достоверно коррелировали с

Ключевые слова: наружный генитальный эндометриоз, цитокины, фактор роста эндотелия сосудов, витамин D.

1. INTRODUCTION

Endometriosis is a multifactorial pathology that occurs in 7-17% of women, leading in 30-50% of cases to impaired reproductive function (Adamyán and Aznaurova, 2015; Yarmolinskaya and Ailamazyan, 2017; Giudice, 2010). Its pathogenesis results in the disturbance of steroid hormones, the immune imbalance caused by inflammation and / or infection, increased the peritoneal activity of vascular growth factors (Adamyán and Aznaurova, 2015; Demir *et al.*, 2010; Huang *et al.*, 2014; Laschke *et al.*, 2011; Orazov, 2015; Radzinskiy *et al.*, 2016). The anti-inflammatory, antiproliferative and immunomodulatory vitamin D effects are known, thus allowing to consider it as an effective targeted endometriosis therapy (RAoE, 2015; Almassinokiani *et al.*, 2016; Ciavattini *et al.*, 2017; Lerchbaum and Rabe, 2014; Pike and Meyer, 2012). There is evidence that the expression of a large number of genes encoding proteins involved in proliferation, differentiation, and apoptosis are regulated by vitamin D (Denisova and Yarmolinskaya, 2017; Gysemans *et al.*, 2014; Irani *et al.*, 2015; Merhi *et al.*, 2014). Studies proving vitamin D effect on the immune system (Delvin *et al.*, 2014; Gysemans *et al.*, 2014; Irani *et al.*, 2015; Orazov, 2015) suggest that correction of its deficiency positively affects the fertility of patients with external genital endometriosis (EGE) (Paffoni *et al.*, 2014).

Objective: to assess the association of cytokine status, the level of angiogenesis factors, and vitamin D status in women with infertility and EGE. An analysis of the association between immunological parameters in infertility on the background of endometriosis and vitamin D status will clarify further the understanding of the pathogenesis of the disease, predict and realize its rational prevention and treatment.

2. MATERIALS AND METHODS

A prospective comparative analysis of the results of 240 patients of reproductive age management, who had a histologically confirmed diagnosis of external genital endometriosis and infertility depending on the treatment methods

from 2016 to 2019, was made. The study was approved by the ethics committee protocol number has no conflict of interest and was performed without financial support from pharmaceutical companies.

All patients were examined and treated in the departments of operative gynecology No. 1 and No. 2 of the Republic Clinical Hospital in Makhachkala, and in the Family Planning and Reproduction Room. All patients, depending on management tactics, were divided into groups of 60 persons: group 1 - after surgical treatment (ST), but without correction of vitamin D deficiency, group 2 - ST + vitamin D, 3- group - ST + dienogest (without correction of vitamin D deficiency), 4 - ST + dienogest + vitamin D. The mean age of patients from the 1st group was 30.7 ± 0.5 years and did not statistically differ from the mean age of patients from the 2nd group (31.4 ± 1.1 years), 3rd group (28.4 ± 1.2 years) and 4th group (30.1 ± 0.7 years). Inclusion criteria were age 25-35 years, 25 (OH) D blood serum level below 30 ng/ml, surgical removal of foci of endometriosis, the patient's desire to become pregnant, written consent of women to participate in the study. Exclusion criteria were pregnancy, varicose veins, systemic autoimmune diseases, mental disorders, malignant diseases, concomitant genital and endocrine pathologies, male infertility factors.

Low vitamin D status in dynamics was revealed by determining 25 (OH) D level in blood serum 3 days after taking vitamin D preparations by chemiluminescent method using kits and calibrators from Roche Diagnostics (Germany) for Architect 2000 analyzer (USA) according to the international standardization program for the determination of vitamin D - DEQAS. Vitamin D deficiency was determined as 25 (OH) D blood serum level less than 20 ng / ml, vitamin D deficiency was revealed at 25 (OH) D > 21 < 29 ng / ml blood serum concentration, normal vitamin D level at 25 (OH) D blood serum concentration more than 30 ng / ml. Severe vitamin D deficiency is classified as a condition in which the concentration of 25-hydroxycalciferol in the blood is less than 10 ng / ml (RAoE, 2015). It should be noted that none of the patients included in the study had a normal vitamin D concentration D in the body during the initial examination. The interleukin status (IL-1 β , IL-6, IL-4), TNF- α ,

VEGF-R1 in the blood serum of the examined women was determined using enzyme-multiplied immunoassay using the appropriate standard reagents (Cloud-Clone Corp., China; R&D Systems Inc., USA).

Cholecalciferol dosage of was determined according to the degree of vitamin D deficiency detected during the initial examination (1300 U per 10 ng / ml), and the course dose according to the recommendations (RAoE, 2015) was 200 thousand MU for therapy of insufficiency and 400 thousand MU - vitamin D deficiency. Therapy with dienogest in a dose of 2 mg once a day was carried out continuously for 3-6 months (Adamyan and Aznaurova, 2015).

Statistical data processing was performed by Statistica v.6.0 program using Student's criterion for groups with parametric values, and Spearman's method for rank, nonparametric values for assessing the correlation. The results of the study are presented as mean $\pm$ standard deviation ($M \pm SD$). Considering that the number of compared groups is four, a critical significance level was $p < 0.0085$.

3. RESULTS AND DISCUSSION

Vitamin D concentration level in the body during the initial examination in the 1st group was 18.2 ± 0.4 [6.7; 27.3] ng / ml, in the 2nd group - 17.6 ± 0.6 [5.2; 27.9] ng / ml, in the 3rd group - 16.1 ± 0.8 [4.6; 25.8] ng / ml and in the 4th group 17.01 ± 0.8 [5.1; 27.3] ng / ml.

Moreover, the low vitamin D status was found with equal frequency and severity in seasonality and region of permanent residence of the examined patients. Besides, there was no dependence on the vitamin D level on age and social conditions.

In this study, obese women were not included. However, overweight patients (BMI 25-29.9 kg / m²) with almost the same frequency as normal growth and weight indicators were observed. It should be noted that there were no patients with insufficient body weight (BMI <18.5 kg / m²).

The duration of infertility averaged 6.4 ± 2.8 years in the 1st group, 5.8 ± 3.1 years in the 2nd group, 6.1 ± 2.4 in the 3rd group and 6.5 ± 3.7 years in the 4th group with fluctuations from 2 to 16 years in groups. Moreover, in the examined groups, primary infertility significantly prevailed: in the 1st group - in 81.7% of cases, in the 2nd

group - in 86.7%, in the 3rd group - in 80.0% and in the 4th - in 83.3% ($p < 0.0085$). The duration of primary infertility averaged 6.9 ± 1.2 years in the 1st group, 6.3 ± 0.5 years in the 2nd group, 6.7 ± 0.7 years in the 3rd group and 6.9 ± 0.8 years in the 4th group ($p < 0.0085$), and the secondary - 3.7 ± 0.8 years in the 1st group, 4.0 ± 0.3 years in the 2nd group, 3.2 ± 0.4 in the 3rd group and 3.8 ± 0.7 years in the 4th group ($p < 0.0085$).

During surgery, unilateral salpingo-ovariolysis was performed in 33.3% of cases in the 1st group, in 48.3% in the 2nd group, in 51.7% in the 3rd group and in 38.3% in the 4th group, ($p > 0.05$); in 20.0% of patients in the 1st group, in 18.3% in the 2nd group, in 16.7% in the 3rd group and in 33.3% in 4 group 2 - bilateral salpingo-ovariolysis was made ($p < 0.05$). When ovarian cysts were detected, cystectomy within healthy tissues was performed in 100% of cases using bipolar energy. Left-sided cystectomy was performed in 13.3-26.7% of patients in the groups, right-sided - in 21.7-31.7%, respectively. Bilateral cystectomy was performed in 8.3-18.3% of patients, respectively.

Analysis of the ratio of different color heterotopies revealed that violet and red dominated in the frequency of occurrence and size in all groups. Most often, they were located in the Douglas space, much less often (in 6.7-11.7% of cases in groups ($p < 0.05$)) in the area of the vesicoureteral fold, and (in 5.0-8.3% of cases in groups ($p < 0.05$)) were dispersed along the peritoneum of the pelvis.

Therapy of low vitamin D status was effective, and it was possible to reach the level of 30 ng / ml, which is characterized as normal, within 4-8 weeks. Subsequently, maintenance therapy was carried out.

A 3 months after colecalciferol appointment in order to restore vitamin D status, indicators were compared. Blood serum content of 25 (OH) D was 17.3 ± 0.7 [6.9; 24.8] ng / ml in the 1st group, 38.6 ± 0.7 [32.7; 57.7] ng / ml in the 2nd, 17.2 ± 0.7 [5.4; 24.8] ng / ml in the 3rd group and 35.6 ± 0.5 [31.9; 61.9] ng / ml in the 4th ($p < 0.0085$). Moreover, in the 2nd group, 25-hydroxyvitamin D level over 50.0 ng / ml was observed in 41.7% of the examined, and in the 4th group - in 38.3% ($p < 0.0085$). The patients did not follow any strict or special diets.

One month after the surgical treatment, no complaints of pain were in the 2nd and 4th groups of patients. In the first and third groups, where no correction of vitamin D deficiency was performed, several patients complaints of pain in

the absence and during menstruation remained, but their intensity decreased significantly. Three months after laparoscopy, the pain syndrome recurred in 5 patients in the 1st group, in 3 patients in the 2nd and third groups, and in 2 women in the 4th group. After 6 months, the recurrence of pain syndrome was in frequency quite different in the groups. Thus, in groups that did not receive dienogest, the recurrence rate of pain was 1.6 times higher in the group without vitamin D deficiency correction. In groups (in the 3rd and 4th), where the hormonal treatment was used in complex therapy, the frequency of pain was 2.2 times more rear comparing the 3rd group with the 1st one, 1.7 times when comparing the 4th group with the 2nd group, and 2.7 times when comparing the 4th groups with the 1st group ($p < 0.0085$). After a year of observation, the maximum number of patients with pain in the 1st group increased (by $15.0 \pm 7.8\%$ compared with 6 months), in the 2nd group - by $10.0 \pm 1.7\%$, in the 3rd group - by $6.7 \pm 1.8\%$ and in the 4th group - by $5.0 \pm 0.7\%$ ($p < 0.0085$). As a result, the best indicators in pain relief were noted in the 4th group, which received combined therapy with dienogest on the background of a normal vitamin D level in the body.

In this group of patients, complaints of pain a year after surgical treatment were 2.8 times less than in the 1st group, 1.8 times less than in the 2nd group, and 1.3 times less than in the 3rd group. The intensity of pelvic pain, estimated by VAS in women with pain syndrome, corresponded to an average of 3.9 ± 0.7 points in the 1st group, 3.1 ± 0.3 points in the 2nd, 3.5 ± 0.6 points in the 3rd group and 2.8 ± 0.4 points in the 4th group and was 1.3 times weaker in groups with normal vitamin D levels. Thus, the correction of vitamin D status positively affects pain syndrome treatment, as well as dienogest appointment in the postoperative period, however, the effectiveness of complex interaction is evident.

Complaints of spotting before and after menstruation disappeared in all patients after surgical treatment, but 12 months later these symptoms recurred in 25.0% of women of the 1st group, 13.3% of women of the 2nd group, 11.7% of patients of the 3 group and 6.7% of the 4th group ($p < 0.0085$).

One month after the treatment and diagnostic laparoscopy, menstrual irregularities were observed in 3 patients in the 1st group and 2 in the 2nd group. After 3 months, the frequency of complaints of menstrual dysfunction in these groups increased to 8.3% in the 1st group and

remained at the same level of 3.3% in the 2nd group ($p = 0.04$). After complex therapy, during 6 months this complication occurred in all patients - in 10.0% of cases in the 1st group, in 6.7% in the second group, in 3.3% in the 3rd group and in 1, 7% in the 4th group ($p < 0.0085$).

The year of observation yielded the following results - vitamin D administration allowed to reduce the frequency of menstrual dysfunction by 1.6 times in cases where no hormone therapy was carried out, and by 1.7 times when the restoration and monitoring of vitamin D status was accompanied by dienogest intake of. A low vitamin D level and lack of hormonal correction resulted in this indicator decrease by 4 times when comparing the 1st and 4th groups.

After therapeutic measures aimed at restoring an adequate vitamin D level, some indicators of cytokine status were performed, which showed that IL-6, IL-1 β and TNF- α concentration in the groups with a normal vitamin D level was lower compared with group 1 and 3, where vitamin D status was not corrected, their higher level was registered in the group with only surgical treatment. The level of pro-inflammatory IL-4 was higher in the groups in which the normal vitamin D status was restored, however, the difference between the groups was not as pronounced as when comparing other interleukins in groups. IL-4 blocks the stimulated and spontaneous production of other pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α), but its activity in these studies was not very pronounced. Probably, the high IL-6 level in the 1st group is compensatory and could indicate antitumor processes activation on the background of increased inflammatory processes and restoration of neoangiogenesis (Radzinskiy *et al.*, 2016), confirmed by high VEGF-R1 level of. The lowest VEGF-R1 level was observed in the group of women receiving the correction of vitamin D deficiency together with hormone therapy. However, the assessment of the plasma level of the vascular endothelial factor was ambiguous. In general, it was found that cytokine status indicators significantly differed in groups depending on the saturation of the body with vitamin D.

Pregnancy rates varied markedly in groups. In the first group, the fact of spontaneous pregnancy was recorded in 21.7% (13) of the observations, in the second - in 35.0% (21) of the patient, in the third - in 48.3% (29) and in 4th - 51.7% (31). At the same time, the frequency of undeveloped pregnancy in the groups, in general,

was higher in 13.3% (8) in the 1st group and in the 2nd group - 10.0% (6), and much less frequently in the 3rd group 6.7 % (4) and in the 4th group - 5.0% (2) ($p = 0.94$). Repeated determination of VEGF-R1 level in these patients showed a sharp increase by 1.3-2.1 times at the time of pregnancy termination. Thus, in the 1st group it was 257.4 ± 21.4 ng / ml, in the 2nd group - 203.2 ± 14.2 ng / ml, in the 3rd group - 246.4 ± 17.7 ng / ml and in the 4th group - 221.4 ± 19.2 ng / ml ($p < 0.0085$). According to Cöl-Madendag *et al.* (2014), early abortion at the early sate of pregnancy termination is a result of a disturbance in the interaction of VEGF with its receptors in the mother-fetus system (VEGFR1 and VEGFR2): a significant decrease in receptor expression occurs (Volkova and Alyautdina, 2011).

Mean VEGF-R1 level in the blood serum of spontaneously pregnant women was 1.3-1.5 times lower than in the groups in general and amounted to 134.9 ± 35.5 ng / ml in the 1st group and 94.7 ± 11.9 ng / ml in the 2nd group, 114.4 ± 25.9 ng / ml in the 3 group and 95.1 ± 15.5 ng / ml in the 4 group, which significantly correlated with the pregnancy rate in groups ($r_1 = - 0.45$; $r_2 = - 0.55$; $r_3 = - 0.48$; $r_4 = - 0.41$; $p 0.40.0085$). The IVF procedure was completed in 28.3% of the patients in the 1st group, 25.0% in the 2nd group, 21.7% in the 3rd group, and 21.7% in the 4th group. As a result of IVF, pregnancy occurred in 8.3% in the 1st group, 8.3% in the 2nd group, 10.0% in the 3rd group, and 11.7% in the 4th group. It was found that in women who became pregnant as a result of IVF, the level of the vascular endothelial factor was 1.2 - 1.7 times higher and amounted to 227.1 ± 13.4 ng / ml, 215.3 ± 32.3 ng / ml, 228.5 ± 27 , 175.4 ± 38.1 ng / ml in groups, respectively.

The observed total vitamin D deficiency in women with EGE is consistent with the results of studies by other authors. For example, a prospective cohort study in the USA that included 70,566 patients showed that there is an inverse correlation between 25 (OH) D level and the incidence of endometriosis. In women with normal vitamin D levels (located in the upper quartile), the incidence of endometriosis was 25% lower than in those in whom the vitamin D plasma level was within the lower quartile (RR = 0.76; 95% CI: 0.60- 0.96; $p = 0.004$) (Merhi *et al.*, 2014). The absence of difference in vitamin D saturation of the body of the examined women by seasons is most likely due to the fact that the study was performed in a region without marked fluctuation in the length of day and night in

seasons, which can be observed in more northern regions, the number of sunny days does not depend on the time of the year, and there are also cultural characteristics of nutrition, with prevalence of flour foods, and wearing clothes covering most of the body, including the head.

Modern ideas on endometriosis pathogenesis of show that this disease meets all the criteria for autoimmune diseases, because inflammation is observed on the background of impaired immune regulation. Studies by C.A. Gysemans *et al.* (2014) prove the anti-inflammatory, antiproliferative, and immunomodulatory features of vitamin D the effect on the body by affecting the synthesis of certain pro-inflammatory cytokines, preventing transcription of a number of their genes (Gysemans *et al.*, 2014). IL-6, IL-1 β , and TNF- α activation, registered in our study in women with vitamin D lack or deficiency, shows systemic inflammation leading to changes in the peritoneal fluid, which, in turn, affects the quality and activity of sperm , slows down the acrosomal interaction and association of sperm with oocyte, decreased fertility (Delvin *et al.*, 2014; Gysemans *et al.*, 2014; Irani *et al.*, 2015; Orazov, 2015; Paffoni *et al.*, 2014). Normalization of vitamin D status leads to changes in the cytokine ratio, which is confirmed by epy increases number of pregnancies in the examined groups by 1.6 times only with the correction of vitamin D deficiency and 2.4 times with correction of insufficiency on the background of hormonal treatment with dienogest.

Modern literature indicates the special role of neovascularization in endometriosis pathogenesis (Radzinskiy *et al.*, 2016). The processes of significant neovascularization and neurogenesis activation in ectopic foci in external genital endometriosis were revealed. Already in the early stage of endometrioid heterotopia, an increased density of blood vessels, widening of their lumen, and increase in the number of "immature vessels" are observed (Burlev, 2016; Huang *et al.*, 2014). The main trigger factor for angiogenesis and vasculogenesis in physiological conditions and pathology is the vascular endothelial growth factor. Besides, VEGF as a strong factor in vascular permeability and leukocyte mobilization is involved in the development of the inflammatory process (Cöl-Madendag *et al.*, 2014; Demir *et al.*, 2010; Laschke *et al.*, 2011; Radzinskiy *et al.*, 2016). The decreased parameters of vascular endothelial growth factor in the groups with normal vitamin D levels and the inverse

relationship between the moderate rate of pregnancy and VEGF level in the blood can confirm the effectiveness of the indirect vitamin D effect on neovascularization processes. However, the literature data is ambiguous. Thus, in non-pregnant patients with tubal-peritoneal infertility factor, a 1.5-fold increase in serum VEGF in comparison with women who have positive ECF treatment results (Gerilovich *et al.*, 2013). In our study, in women who became pregnant as a result of ECF, the level of the vascular endothelial factor was 1.2 - 1.7 times higher. Such multidirectional parameters may indicate that in infertility on the background of endometriosis, pregnancy can only occur on the background of pronounced stimulation of angiogenesis.

4. CONCLUSIONS

The data obtained show that correction of vitamin D insufficiency on the background of dienogest therapy allows increasing efficiency of EGE symptom treatment and favors impregnation.

In vitamin D status correction in women with EGE and infertility, the best indicators of immune (cytokine) status were noted. IL-6, IL-1 β and TNF- α high level in groups with a low vitamin D content indicates a high immune tension in the body, which negatively affects impregnation.

The deficiency of vitamin D correction is accompanied by the increased the number of pregnancies in the examined groups by 1.6 times only with the correction of vitamin D deficiency and 2.4 times with the correction of insufficiency on the background of hormonal treatment with dienogest.

A significantly moderate inverse correlative relationship was found between VEGF-R1 serum level and the probability of pregnancy in women with EGE after surgical treatment (from -0.41 to -0.55).

Thus, the obtained data indicate the need for further study, the generalization of data on vitamin D role in the pathogenesis of external genital endometriosis, infertility associated with it, and its therapeutic effects.

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Table 1. The cytokine composition in the blood serum of patients with EGE in groups with and without vitamin D deficiency

Parameter	Group 1	Group 2	Group 3	Group 4
IL-1 β , pg/ml	15.94 $\pm$ 2.41	8.27 $\pm$ 2.75	11,82 $\pm$ 1.24	6.36 $\pm$ 1.87
Significance of differences	p1-2=0.0041	p2-4=0.0076	p3-4=0.0061	p1-4=0.0015
IL-6, pg/ml	7.51 $\pm$ 2.18	4.17 $\pm$ 2.74	5.93 $\pm$ 2.27	4,35 $\pm$ 1,71
Significance of differences	p1-2=0.0001	p2-4=0.0087	p3-4=0.0035	p1-4=0.0019
TNF- α , pg/ml	15.49 $\pm$ 2.73	9.23 $\pm$ 3.51	11.01 $\pm$ 3.68	7.75 $\pm$ 2.79
Significance of differences	p1-2=0.0052	p2-4=0.0071	p3-4=0.0078	p1-4=0.0024
IL-4, pg/ml	14.35 $\pm$ 4.17	18.73 $\pm$ 5.77	13.48 $\pm$ 4.83	17.87 $\pm$ 4.92
Significance of differences	p1-2=0.0075	p2-4=0.0094	p3-4=0.0043	p1-4=0.0076
VEGF-R1, ng/ml	198.1 $\pm$ 72.1	127.2 $\pm$ 69.1	176.3 $\pm$ 74.7	103.4 $\pm$ 61.2
Significance of differences	p1-2=0.0055	p2-4=0.0074	p3-4=0.0028	p1-4=0.0021

Figure 1. The level of concentration of vitamin D in the examined groups during the initial examination (ng / ml)

Figure 2. Pain syndrome dynamics in the examined patients depending on the treatment methods during one year after surgery.

Figure 3. Dynamics of menstrual cycle disturbance in the examined patients depending on treatment methods during one year after surgery