Benign endometrial lesions in premenopausal women: three-dimensional power Doppler sonography and cytokines content

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ABSTRACT

Purpose: To investigate the IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IL-1β, IFN-γ at the local and system levels in endometrial polyps and hyperplasia in premenopausal women; to assess whether three-dimensional power Doppler indices can discriminate between hyperplasia and proliferative endometrium.

Materials and methods: The prospective analysis included 80 premenopausal women with the suspicion of endometrial hyperplasia and endometrial polyp according to 2D ultrasonography data. Three groups of patients were analyzed according to histological data. There were 25 women with simple endometrial hyperplasia without atypia (the first study group), 15 patients with hyperplastic endometrial polyps (the second study group) and 40 healthy women with endometrium in the early proliferative phase (control group) in premenopausal age. The levels of some cytokines were determined by enzyme linked to immunosorbent assay in serum and in aspirates from the uterus cavity. Three-dimensional power Doppler indexes of the uterus and endometrium were measured in endometrial hyperplasia and proliferative endometrium.

Results: Significant dysfunctional changes of local immune system of uterine mucosa in endometrial hyperplasia and endometrial polyp are expressed in activation of pro-inflammatory cytokines, with a deficit of anti-inflammatory cytokines. Endometrial three-dimensional power Doppler indices of uterine and endometrium were significantly higher in endometrial hyperplasia.

Conclusions: The local secretion imbalance of pro- and anti-inflammatory cytokines is present in endometrial hyperplasia and polyps in premenopausal age. Endometrial perfusion increases in endometrial hyperplasia by using three-dimensional power Doppler sonography in comparison with healthy women.

Key words: Endometrium, hyperplasia, polyp, cytokines, three-dimensional power Doppler.

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INTRODUCTION

Endometrial hyperplasia is an important medical and social problem. The clinical importance of this pathological entity is the underlying risk of carrying a concomitant genital cancer or risk of progression to endometrial carcinoma during the follow-up. This pathology is a frequent precursor to cases of endometrial carcinoma of the uterus [1]. In addition endometrial hyperplasia remains the most common pathology of the female genital tract. The primary presenting symptom of endometrial hyperplasia and endometrial carcinoma is abnormal uterine bleeding [2].

Great attention to this problem is also linked to increased growth of endometrial cancer in all economically advanced countries. A timely diagnostics of precancerous endometrial diseases is an important step in the prevention of the endometrial cancer.

It should be noted that the classification, diagnosis and management of endometrial lesions are still the subject of vigorous debate [3]. Endometrial biopsy and curettage have become accepted methods of evaluating of endometrial hyperplasia and endometrial carcinoma [4, 5].

The World Health Organization classification is currently the most commonly accepted system of classifying endometrial hyperplasias. According to this classification hyperplasias are classified as simple, complex, with or without atypia [6, 7].

Despite the fact that the basic theory of pathogenesis of endometrial hyperplasia is a hormonal concept (effect of high concentrations of estrogen), many researchers believe that endocrine factor is only part of the picture.

Recent evidence suggests that pro-inflammatory cytokines may play an important role in pathogenesis of different diseases [8 - 11]. Despite the expression of cytokines in pathogenesis of endometrial hyperplasia was studied by many scientists, the published data remain controversial.

Currently the three-dimensional sonography is a new imaging technique that has become available and popular in clinical practice and based on three-dimensional reconstruction of vessels image, received from Power Doppler system [12]. More often in gynecological practice three-dimensional power Doppler sonography is used with the aim of differentiating tumor changes of genitalia [13]. Although the three-dimensional techniques including three-dimensional power Doppler sonography have become available for clinical use, their role in gynecology is currently being studied [14].

Unfortunately, most studies using three-dimensional power Doppler sonography are about diagnosis of malignant endometrial lesions [12, 15, 16, 17]. Alcázar and Galvan 2009, proved that three-dimensional power Doppler might be useful for the prediction of endometrial cancer in women with postmenopausal bleeding [15]. The following study of these authors shows similar results that three-dimensional ultrasound is a new imaging technique that offers unique ways for assessing women with gynecologic cancer [12].

Few studies outline using of this technique in the diagnosis of endometrial hyperplasia. Although 2D study can identify a large number of local endometrial lesions such as benign endometrial polyp, three-dimensional power Doppler sonography can change and improve sensitivity of identifying endometrial hyperplasia.

Despite numerous publications on various aspects of the problem of endometrial hyperplasia, endometrial polyps there is still a place for discussion on many issues of the intrauterine pathology. Issues of pathogenesis of endometrial hyperplasia and polyps remain controversial and are not confined to "traditional" notions.

A better understanding of mechanisms of endometrial pathology could lead to effective therapeutic strategies.

MATERIALS AND METHODS

Prospective analysis included 80 premenopausal women, hospitalized in the Department of Gynecology for diagnostic curettage of the uterine cavity with the suspicion of endometrial hyperplasia and endometrial polyp according to 2D ultrasonography data.

Inclusion criteria were: suspicion of endometrial hyperplasia and endometrial polyp according to 2D ultrasonography data; premenopausal age; the absence of clinical manifestations of the endometrial hyperplasia and endometrial polyp; normal size of uterus according to 2D ultrasonography data; the absence of accompanying diffuse and local pathology of myometrium (uterine fibroids, adenomyosis); the absence of ovarian neoplasms according to 2D ultrasonography data (cysts, cystomas); the absence of sharp or exacerbation of chronic inflammatory diseases of genitals; the absence of oncological diseases of any localization; the absence of exacerbation of chronic extragenital pathology.

Exclusion criteria were: reproductive and postmenopausal ages; the presence of vaginal bleeding during hospitalization; the size of the uterus more than normative parameters of premenopausal age; the presence of accompanying diffuse and local pathology of myometrium (uterine fibroids, adenomyosis); the presence of ovarian neoplasms according to 2D ultrasonography data (cysts, cystomas); the presence of sharp or exacerbation of chronic inflammatory diseases of genitals; the presence of oncological diseases of

any localization; the presence of exacerbation of chronic extragenital pathology

All women were subjected to diagnostic curettage of the uterine cavity. A histopathologic diagnosis was made after diagnostic curettage of the uterine cavity. Endometrial tissue was used for histological examination. Three groups of patients were analyzed according to histological data. There were 25 women with simple endometrial hyperplasia without atypia (the first study group), 15 patients with hyperplastic endometrial polyps (the second study group) and 40 healthy women with endometrium in the early proliferative phase (control group) in premenopausal age.

Study material consisted of serum samples obtained from the patients' blood. Aspirates from the uterine cavity of all women were received by uterine aspiration probe «Yunona» Classic without dilatation of the uterine cervix before the diagnostic curettage of the uterine cavity.

Tumor necrosis factor alpha (TNF-α), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-1β (IL-1β), interferon-γ (IFN-γ) concentrations were determined by enzyme linked to immunosorbent assay in serum and in aspirates from the uterine cavity according to the manufacturer's instructions.

Women from the first study group and control group were preoperatively examined by 3D power Doppler sonography before endometrial sampling to obtain definitive histological diagnosis of endometrial pathology. Ultrasound was performed using Medison Accuvix V10 scanner (Korea). The vascularization index (VI), the flow index (FI), the vascularization-flow index (VFI) of the uterus and endometrium were measured. We used Virtual Organ Computeraided AnaLysis (VOCAL) (SonoView Pro^{TM} Ver. 1.6.0).

All ultrasound data were verified by histology of the endometrial specimens removed during diagnostic curettage of the uterine cavity. These parameters (VI, FI. VFI) were compared between the group of women with normal histology (endometrium in the early proliferative phase) and the pathologic group (simple endometrial hyperplasia without atypia).

The statistical analyses were performed with the commercial statistical package Statistica 6.0 (StatSoft, Inc. 1994-2001). We used parametric and nonparametric methods of statistical analysis. Data are expressed as mean (m) ± standard deviation (SD) in case of normal distribution and Median (Me), 25-th quartile (25), 75-th quartile (75) in case of other-than-normal distribution. For variable with normal distribution Student t-test was used to test the differences between the groups, in other cases Mann-Whitney U-test was used. Spearman correlation coefficient was calculated. Bonferroni correction was used to resolve the problem of multiple comparisons. According to Bonferroni correction $P < 0.025$ was considered significant in the analysis of three groups. $P < 0.05$ was considered significant in the analysis of two groups.

RESULTS

The mean age of the first study group was 49.53 ± 2.65; range 46-55, the mean age of the second study group was 49.82 ± 4.29; range 46-62 years and the mean age of the control group was 49.66 ± 2.70; rang 46-56 years. All data routine clinical and laboratory tests in all groups were within the reference range. The IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IL-1β, IFN-γ concentrations have been identified in aspirates from the uterine cavity to study the role of a possible dysfunction of local immunity (Tab. 1).

Table 1. The IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IL-1β, IFN-γ concentrations in aspirates from the uterine cavity in all groups.

	First study group	**Second study group**	**Control group**
IL-2 pg/mL, Me (25; 75)	111.89 (15.0; 191.18)*	100.15 (78.39; 154.75)*	152.94 (105.74; 287.83)
IL-4 pg/mL, Me (25; 75)	187.21 (101.03; 523.51)	180.17 (66.09; 592.54)	232.28 (47.08; 1087.15)
IL-6 pg/mL, Me (25; 75)	1005.43 (636.14; 1455.33)*	926.15 (545.02; 1705.04)*	264.59 (143.36; 772.47)
IL-8 pg/mL, Me (25; 75)	1332.96 (657.96; 1722.69)*	1362.32 (1165.75; 950.84)*	716.95 (368.44; 1161.33)
IL-10 pg/mL, Me (25; 75)	17.65 (0.00; 19.74)*	38.49 (23.56; 47.92)*	118.91 (72.28; 151.02)
TNF-α pg/mL, Me (25; 75)	125.74 (79.12; 153.19)*	49.54 (43.88; 65.49)*	20.12 (0.00; 52.97)
IL-1β pg/mL, Me (25; 75)	334.156 (105.24; 523.16)*	323.67 (136.72; 498.99)*	128.96 (32.43; 223.86)
IFN-γ pg/mL, Me (25; 75)	60.40 (0.00; 414.56)*	0.00 (0.00; 545.12)	468.74 (300.32; 673.99)

* - statistically significant differences versus the control group ($p < 0.05$)

The received results show that the local IL-2, IL-10 and IFN-γ concentrations were found to be significantly lower in cases of endometrial hyperplasia. On the other hand the local IL-6, IL-8, TNF-α, IL-1β concentrations were significantly higher versus the control group.

The IL-2 and IL-10 concentrations were significantly lower in cases of endometrial polyps on the local level in comparison with the control group. The IL-6, IL-8, IL-1β concentrations in cases of endometrial polyps were significantly higher versus the control group.

Significant differences of the IL-4 levels in aspirates from the uterine cavity were not found between controls versus the group with simple endometrial hyperplasia and endometrial polyps. The IFN-γ and TNF-α value in aspirates from the uterine cavity in patients with endometrial polyps and in normal endometrium did not show significant differences.

We calculated Spearman rank correlation coefficient of the cytokines concentrations with the morphological conclusion (the absence or presence of endometrial hyperplasia or endometrial polyp).

The correlations were between the IL-2 content in aspirates from the uterine cavity and histological data ($R = -0.4$; $p<0.05$), between the IL-6 content in aspirates from the uterine cavity and morphological conclusion ($R = 0.4$; $p<0.05$), between the IL-8 content in aspirates from the uterine cavity and histological data ($R=0.4$; $p<0.05$), between the IFN-γ content in aspirates from the uterine cavity and morphological conclusion ($R = -0.4$; $p<0.05$).

The IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IL-1β, IFN-γ concentrations have been identified in serum too to study their role at the system level.

Cytokines as IL-2, IL-4, IL-6 and TNF-α were not defined in serum. Their concentrations in serum were equal to zero. The IL-8, IL-10, IL-1β, IFN-γ concentrations have been identified in serum (Tab. 2).

Table 2. The IL-8, IL-10, IL-1β, IFN-γ concentrations in serum in all groups.

	First study group	**Second study group**	**Control group**
IL-8 pg/ml, Me (25; 75)	6.45 (4.47; 9.43)	5.46 (0.00; 15.29)	8.02 (1.35; 10.00)
IL-10 pg/ml, Me (25; 75)	3.41 (0.00; 14.19)	13.14 (0.00; 96.49)	3.47 (0.00; 15.32)
IL-1β pg/ml, Me (25; 75)	30.65 (0.00; 135.20)	109.84 (0.00; 163.92)	83.61 (0.00; 114.06)
IFN-γ pg/ml, Me (25; 75)	114.96 (98.14; 152.59)	141.12 (0.00; 220.06)	137.18 (112.02; 186.08)

We found no significant differences of the cytokines value in the serum of patients with endometrial hyperplasia, endometrial polyps and in normal endometrium.

To evaluate uterus and endometrial vascularization using three-dimensional power Doppler ultrasound in women with endometrial hyperplasia, three-dimensional power Doppler indexes (vascularization index, flow index, vascularization-flow index) were measured (Tab. 3).

Because endometrial three-dimensional power Doppler indices and some uterine three-dimensional power Doppler indices were significantly higher in endometrial hyperplasia versus the control group (Tab.3), we also calculated Spearman rank correlation coefficient of three-dimensional power Doppler indices with the morphological conclusion (the presence or absence of endometrial hyperplasia).

Thus, we have identified correlations between morphological conclusion and uterine vascularity index ($R=0.4$; $p<0.05$) and between uterine vascularity-flow index and morphological conclusion ($R=0.3$; $p<0.05$). On the other hand, we found statistically significant strongest correlations between endometrial vascularity index and morphological conclusion ($R=0.8$; $p<0.001$); between endometrial flow index and histological findings ($R=0.7$; $p<0.001$); between endometrial uterine vascularity-flow index and morphological conclusion ($R=0.8$; $p<0.001$).

Table 3. VI, FI, VFI of the uterus and endometrium in women with endometrial hyperplasia and in the control group.

Index	First study group	Control group
Uterine VI. Me (25; 75)	6.82 (4.24; 11.41)*	3.73 (1.83; 6.74)
Endometrial VI. Me (25; 75)	7.33 (4.89; 11.44)**	0.47 (0.01; 1.20)
Uterine FI. Me (25; 75)	14.13 (12.67; 17.76)	13.90 (10.76; 17.18)
Endometrial FI. Me (25; 75)	10.51 (8.79; 14.75)**	4.70 (3.25; 6.52)
Uterine VFI. Me (25; 75)	1.15 (0.53; 1.82)**	0.49 (0.21; 1.21)
Endometrial VFI. Me (25; 75)	0.77 (0.50; 1.67)**	0.02 (0.00; 0.08)

* - statistically significant differences versus the control group ($p < 0.01$); ** - statistically significant differences versus the control group ($p < 0.001$)

DISCUSSION

The available information about the functioning and regulating of local immune system of genitals mucous in norm and pathology is conflicting [8 - 11].

Our findings show significant dysfunctional changes of local immune system of uterine mucosa which are present in endometrial hyperplasia and endometrial polyps. These changes are expressed in activation of pro-inflammatory cytokines, with a deficit of anti-inflammatory cytokines.

There were no significant differences between pro-inflammatory cytokines concentrations and anti-inflammatory cytokines concentrations in the serum in all groups. In addition the cytokines concentrations in blood serum are very small.

These data allow concluding that changes in endometrial hyperplasia and endometrial polyps occur at the local level. Whereas the cytokines content at the system level (serum) does not demonstrate the immune dysfunction in endometrial hyperplasia and endometrial polyps.

In recent years ultrasonic specialists began to use power Doppler sonography increasingly for the evaluation of slow-moving blood flow, the most characteristic for endometrium and myometrium, since this technique increases the number of visible vessels [13, 14].

It is known that the power Doppler indexes may be used for the objective evaluation of blood supply of the investigated tissues. Vascularization index describes the ratio percentage of vessels in a certain volume of tissue. Flow index shows the amount of blood cells in the blood vessels during the study. Vascularization-flow index is an indicator of the organ perfusion [13].

Endometrial three-dimensional power Doppler indices (vascularity index, flow index, and vascularity-flow index) were significantly higher in endometrial hyperplasia versus the control group. These data show increased perfusion of hyperplastic endometrium in comparison with endometrium in the proliferative phase. We were able to register endometrial blood flow in the control group, since the power Doppler ultrasound allows visualizing the blood vessels with a very slow speed of blood flow.

Uterine three-dimensional power Doppler indices (vascularity index and vascularity-flow index) were also significantly higher in endometrial hyperplasia versus the control group.

Application of three-dimensional power Doppler sonography could complement existing routine ultrasonic examination methods and improve the quality of diagnosis of intrauterine pathology.

CONCLUSIONS

The local secretion imbalance of pro- and anti-inflammatory cytokines is present in endometrial hyperplasia and polyps in premenopausal age. The cytokine profile in benign endometrial lesions requires further study.

Three-dimensional power Doppler sonography may be useful as a good diagnostic tool in predicting endometrial hyperplasia in premenopausal women.

Three-dimensional power Doppler indexes may be useful to distinguish benign endometrial lesions such as endometrial hyperplasia and proliferative endometrium.

Conflicts of interest

The author declares that has no competing interests in the publication of the manuscript.

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