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Age-related relationship between the development of hyperplastic processes and VEGF expression in endometrial cells

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Abstract

The existence of a clear tendency to increase the prevalence of hormone-dependent diseases and endometrial cancer against the background of increasing the frequency of their occurrence and rejuvenation of the age of manifestation leads to the search for new possible markers of diagnosis and prognosis of the development of pathological process. Angiogenesis is one of the forms that lead to the formation of new blood vessels, with an increased metabolic need for perfusion of existing vessels. The vascular endothelial growth factor family (VEGF) is a protein that is the major inducer of angiogenesis. **The objective:** To study the expression of VEGF in endometrial cells in proliferative, hyperplastic, atrophic states at women's different ages. **Materials and methods.** A retrospective analysis of medical records of the pathohistological bureau for the period 2014-2016 was conducted. 2196 pathomorphological findings of endometrial tissue specimens have been examined. Estimation of VEGF expression was performed in 417 endometrial specimens, in the cohorts of the study: in the reproductive, perimenopausal, postmenopausal periods, respectively, in groups with physiological endometrial proliferation phase, hyperplastic, atypical, atrophic endometrium. The results were statistically processed. **Results and discussion.** Analyzing the data presented, higher VEGF expression was detected in atypical hyperplasia in all age categories, but it was likely that higher rates were established in the postmenopausal period,

with atypical endometrial hyperplasia, suggesting physicians' alertness to the process in this category.

Probably low were indexes found at atrophic endometrium in this age category, which confirms the endometrial preservation of its growth factors, and in the presence of processes that stimulate the proliferation of the organ, they can trigger at the molecular-genetic level, neoplastic mechanisms. The data of the retrospective analysis confirm the growth of atypical form of hyperplastic processes and their maximum detection in the age categories 41 - 45 and 46 - 50 years old, and the beginning of detection of endometrial malignancy from the age of 46 - 50 years old, with a gradual increase with age. **Conclusions.** Expression of VEGF level in endometrial tissue cells as an inducer of angiogenesis can be a promising marker for the diagnosis of the risk of proliferative conditions and their prognosis, especially in relation to other markers characterizing immunohistochemical and molecular genetic cellular parameters.

Key words: endometrial hyperplasia; atypical endometrial hyperplasia; angiogenesis; VEGF; malignancy

Introduction

Recently, a clear tendency has been formulated in society for the increase in the prevalence of hormone-dependent diseases related to pre-hyperplastic processes (HPP) and endometrial cancer amid increasing their incidence and rejuvenation of the age of manifestation [3, 5, 9]. The unspecified information on GPE etiopathogenesis limits the possibilities of therapeutic treatment and positive results obtaining. Lack of evidence base, increased list of contraindications to hormone therapy cause ineffectiveness of therapeutic measures and increase of malignancy [1, 6].

A steady scientific search for the concept of the etiology and pathogenesis of HPP provides data that is often subject to revision in the future. The role of unbalanced estrogen stimulation in the development of HPP is well known, but the histogenetic mechanisms of the various lesions development, such as hyperplasia, polyps, adenocarcinoma, have not yet been fully established [1, 4, 7]. From the current standpoint, it should be considered more correct to consider HPP as a result of an imbalance between the processes of proliferation and apoptosis of cells, which are regulated by cellular and extracellular components at the molecular - genetic level [1, 3, 10].

Angiogenesis is one of the forms that lead to the formation of new blood vessels, regardless of the presence of the existing [2, 8] ones. The family of vascular endothelial growth factors (VEGF) is a protein that is the major inducer of angiogenesis, its expression is

regulated, including by hypoxia, hypoglycemia [9], it stimulates the reactions by which endothelial cells migrate, proliferate, form a bound net [2, 9]. Angiogenesis is stimulated if the metabolic needs exceed the perfusion capacity of existing vessels. Under physiological conditions, the processes of angiogenesis are moderately intense and activated for the regeneration of damaged tissues, sewage clots, cyclic changes in the ovaries, proliferation of the endometrium, growth of embryonic and postnatal tissues associated with either hormonal stimulation, or with a response to ischemia. Recent studies have confirmed this adaptation mechanism in hypoxia and hypoglycemia [2, 8, 9].

VEGF expression level progressively decreases after birth and is at low levels in most tissues except for the sites with active angiogenesis: ovaries, uterus, skin. There are VEGF 6 growth factors: VEGF - A, VEGF -B, VEGF-C, VEGF-D, VEGF-E, placental growth factor (PLGF). Of these, VEGF - A is one of the major and it is expressed in vascular smooth muscle cells, macrophages, and tumor cells [9].

The risk of endometrial cancer (EC) development determines the morphofunctional features of different types of HPP, as well as disorders that lead to the inactivation of processes that stimulate oncogenesis, including: increased proliferation, angiogenesis, reduced apoptosis. Due to the knowledge of the molecular-genetic mechanisms of carcinogenesis, the treatment of cancer patients is at a whole new level - the impact on molecular targets responsible for the proliferation of malignant cells [7, 9, 10].

The objective: to study the expression of VEGF in endometrial cells in proliferative, hyperplastic, atrophic states at different ages of a woman.

Materials and methods

Retrospective analysis of the medical records of the pathohistological bureau for 2014 – 2016 was made. 2196 pathomorphological findings of endometrial tissue specimens in women with clinical manifestations which required surgery have been learnt. The results obtained were distributed as follows: endometrial hyperplasia, which included simple and complex non-atypical hyperplasia (NAH), atypical hyperplasia (AH), simple and complex atypical hyperplasia (AH), adenocarcinomas (high-, moderate and low-differentiated), endometrium corresponding to the menstrual cycle phases, atrophic endometrium (Atr E), and non-informative findings (N / inf). The histological conditions were distributed according to the patients age categories with an interval of 5 years.

Evaluation of VEGF expression was performed in endometrial tissue specimens of 417 reproductive, perimenopausal and postmenopausal age women. All women were admitted to gynecological department for treatment and diagnostic scraping of the uterine cavity. The

following clinical groups were formed: I group consisted of 57 reproductive age women with hyperplastic processes without atypia, mean age was 35.25 ± 0.37; group II included 48 patients of reproductive age with atypical endometrial hyperplasia (34.48 ± 0.42); group III (a control one) consisted of 49 reproductive age women with proliferative endometrium who planned pregnancy by assisted reproductive technologies (33.22 ± 0.48); group IV included 62 perimenopausal period women with non-atypical hyperplasia (48.10 ± 0.44); V group - 58 patients of perimenopausal age with atypical hyperplasia (48.53 ± 0.48); VI group - 54 postmenopausal patients with non-atypical hyperplastic processes (52.78±0.42); group VII consisted of 43 postmenopausal patients with atypical endometrial hyperplasia (54.05 ± 0.61); Group VIII (Atr. E) comprised 46 patients of postmenopause age with atrophic endometritis (53.39 ± 0.39).

VEGF expression was carried out at the mRNA level by polymerase chain reaction to DNA obtained by the reverse transcription method. The mRNA level of the gene under study was determined by the number of fluorescent signal units, using the number of c. u. fluorescence signal of gene 36B4 for standardization of RNA initial amount. Expression changes were calculated by the ΔΔCt method. The results were processed by the method of variational statistics with Student's confidence test, using standard computer systems.

Results and their discussion

Data on endometrium morphological status, distributed according to age categories are presented in Fig. 1. The most common pathology that led women to medical care were endometrial hyperplastic processes without cell atypia. This violation accounted for 1334 cases (46.69 ± 0.94%). As can be seen from the diagram, the highest incidence of pathology detection occurred in the period of 46-50 years, the time of menopausal transition onset. The next most frequent detection rate was physiological endometrium (PE) accounting for 434 cases (15.52 ± 0.69%), the distribution period in the chart was almost consistently the same, with the highest detection rate after 61 y. o. which targets physicians to more detailed justification and maximum efficiency of the manipulations.

Hyperplasia with atypical endometrial manifestations was found in 155 cases (5.54 ± 0.44%), with maximal manifestation in 41 - 45 and 46 - 50 y.o. The next most frequent was endometrial tissue malignancy, manifestations of adenocarcinoma were established in 51 cases (1.82 ± 0.23%). The latter pathology was the most pronounced since the age of 46-50 y.o. and further frequency's increase with increasing age of the women under examination.

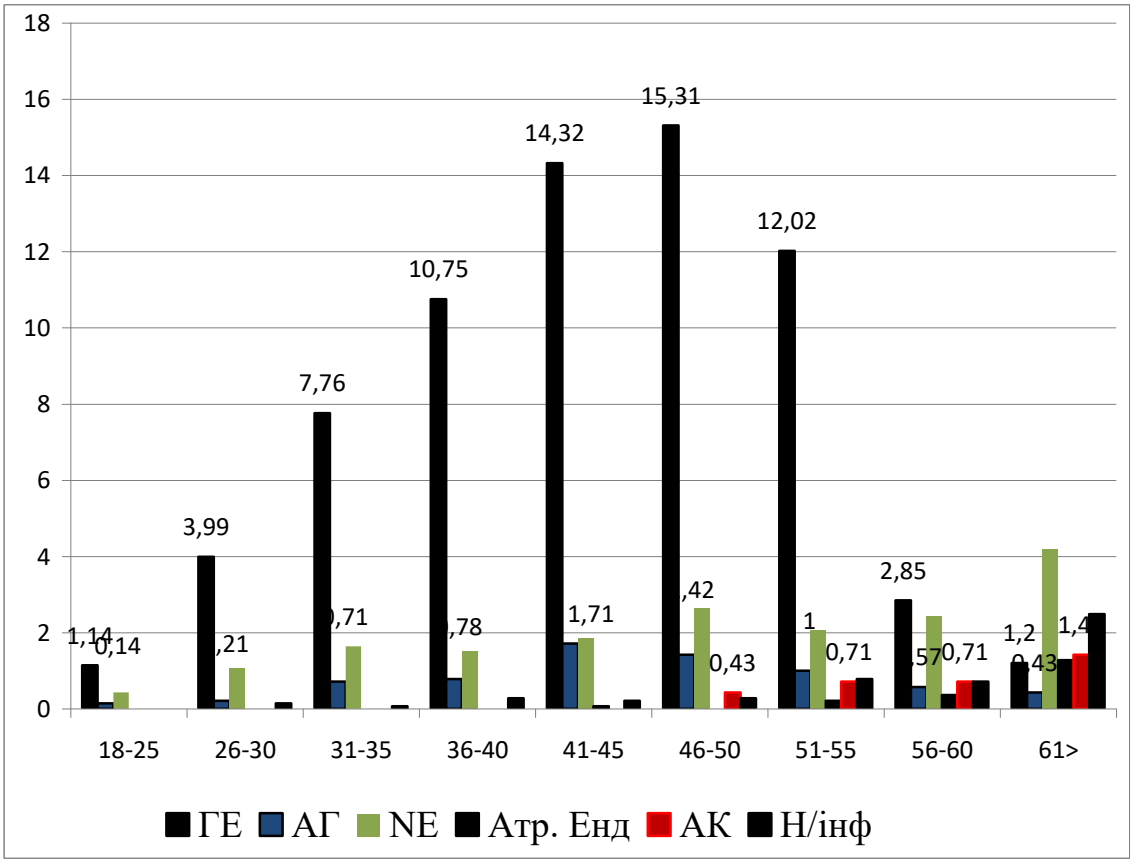

Fig.1. Disturbances of endometrium morphological states by age (%)

Analyzing the diagram's data, it should be taken into account that each age category is characterized by its own morphofunctional features of the endometrial tissue. The frequency of hyperplastic processes, atypical hyperplasia and adenocarcinoma detection indicates that there is a particular concentration of specialists, starting from 40 years, when the mechanisms of initiation and development of disturbances in the self-supply system by mitogenic stimuli, constant proliferation and insensitivity to antimitogenic signals, lost of "Heiflik" limit, decreased ability to apoptosis, susceptibility to tissue invasion and metastasis [1] may be involved.

A further study of VEGF expression (c.u.) in endometrial tissue samples by age categories, it was found that in all reproductive, perimenopausal, and postmenopausal periods, the age group of women in the cohorts was significantly comparable ($p < 0.01$). In all women, no acute or chronic disease was detected before the fractional treatment-diagnostic scraping of the uterine body, according to protocols, the results of clinical and laboratory examinations were within the reference values.

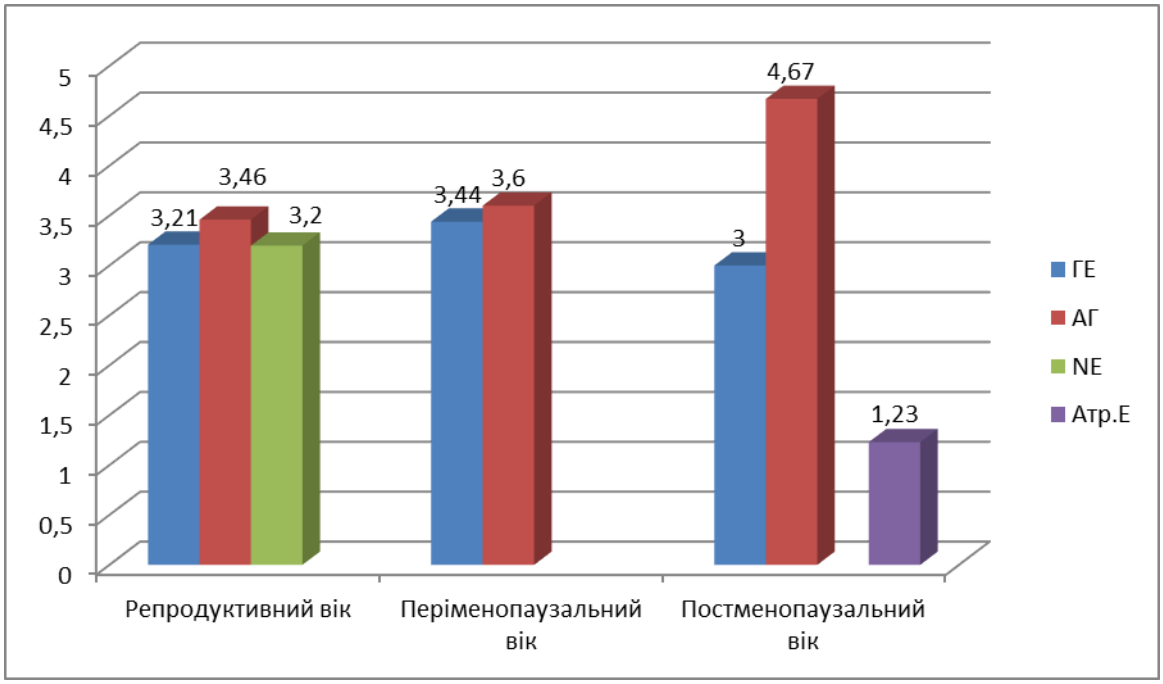

Fig. 2. VEGF expression in endometrium cells at different pathologies and different age groups (c. u.)

Note: $P_I - P_{VII} < 0,01$

$P_I - P_{VIII} < 0,01$

$P_{II} - P_{VII} < 0,01$

$P_V - P_{VII} < 0,01$

$P_V - P_{VIII} < 0,01$

$P_{III} - P_{VIII} < 0,01$

$P_{VI} - P_{VII} < 0,01$

$P_{VI} - P_{VIII} < 0,01$

When analyzing the results of VEGF expression in endometrial tissue cells, a relative indicators increase in atypical endometrial hyperplasia in relation to indicators in hyperplastic processes in this age category was found, as well as relative to the control group of reproductive age. VEGF expression was 3.46 ± 0.10 c.u. in the reproductive period, 3.60 ± 0.09 c.u. in the perimenopausal period, 4.67 ± 0.07 c.u. in the postmenopausal age.

In hyperplastic processes, VEGF indicators were respectively in the reproductive period - 3.21 ± 0.07 c.u., perimenopausal - 3.44 ± 0.09 c.u., postmenopausal - 3.00 ± 0.09 c.u., in the control group, the proliferation phase was 3.20 ± 0.08 c.u.

We used literature data about hormonal effects on angiogenesis processes as a background for control group indexes and analyzed proliferation phase when activity in physiological endometrium is the highest . This was marked in breast cancer studies and those about relationship of VEGF-A expression at estrogens angiogenenis effect. In further studies angiogenesis influence of anti-estrogens including via VEGF-A inhibition was revealed [3, 8].

Angiogenesis in atrophic endometrium was significantly low but kept its activity (1.23±0.04 c.u.). This may prove preservation of activity in endometrial cells in postmenopausal age which at the presence of factors either stimulating proloferative processes or inhibit apoptosis can enter into angiogenic processes and participate in the development of hyperplastic or atypical states in postmenopausal period.

We got significant results at VEGF expression at atypical hyperplasia of endometrium in postmenopausal period which in comparison with epidemiological data (see Fig.1) evidenced high risk of malignisation in postmenopausal women.

Literary data on angiogenic factors role in the development of tumor vascularization are quite mosaic. In most works, it has been established that the processes of tumor angiogenesis differ from physiological one, they provide oxygen and nutrients to the malignant tissue and excrete metabolism products [2, 4, 5]. Tumor larger than 1-2 mm^3 requires its own circulatory system, but the presence of a stable balance between angiogenic and anti-angiogenic factors can leave neoplastic cells inactive for an extended time [7, 9, 10].

Our work contradicts the data that in normal endometrium the synthesis of VEGF is stimulated by estradiol influence, and in case of neoplasia this effect worsens or disappears. Although researchers have studied a highly significant negative relationship between VEGF concentrations and progesterone receptors at endometrial hyperplasia, at the same time, there is no relationship between VEGF expression and progesterone receptors.

However, it should be borne in mind that the etiopathogenetic mechanisms of endometrial tissue malignancy development are not definitively established, the influence of hormonal factors exists, but it is not always associated with cancer risks, since in our work there were more active processes in the postmenopausal period, which is not always related with hormonal changes.

Other studies have demonstrated the value of angiogenic factors and their receptors in tumor tissue vascularization, as well as the relationship with disease prospects and treatment efficacy [2, 3, 4]. The use of anti-angiogenic drugs is now considered one of the most promising areas of anticancer therapy, but further studies are needed as a fully effective cancer therapy has not yet been developed and the number of people in need is increasing.

Conclusions

The level of VEGF concentration in endometrial tissue, as an inducer of angiogenesis, is increased in endometrial hyperplastic processes. Significant growth is observed in atypical hyperplasia, especially in the postmenopausal period.

Determination of VEGF concentration at endometrial proliferative processes may be a perspective diagnostic marker for atypical states and their prognosis especially at the ratio with other markers characterizing molecular-genetic or histochemical cellular indexes.

Researches in this field are rather urgent both in fundamental problems of endometrial cancer pathogenesis and in the field of new drug development directed to molecular growth targets and corresponding components of angiogenic tumor development path.

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