Chumak Z. V. Expression of the VEGF marker in endometrial cells in hyperplastic processes. Journal of Education, Health and Sport. 2020;10(10):82-89. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2020.10.10.008
https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2020.10.10.008
https://zenodo.org/record/4110745

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.

Received: 09.09.2020. Revised: 22.09.2020. Accepted: 20.10.2020.

EXPRESSION OF THE VEGF MARKER IN ENDOMETRIAL CELLS IN HYPERPLASTIC PROCESSES

Z. V. Chumak

Odessa National Medical University; City Center for Menopause, Odessa

Abstract

Hormone-dependent diseases related to preproliferative processes and endometrial cancer have a clear tendency to increase and rejuvenate. **The purpose:** to study the expression of VEGF marker in endometrial cells under physiological, hyperplastic, atypical conditions in women's different age periods. 2196 pathomorphological findings of endometrial tissue samples in women with clinical manifestations requiring surgical treatment have been analyzed. VEGF expression was performed in endometrial tissue samples of 458 women of late reproductive, perimenopausal and postmenopausal age. Each age category is characterized by its morphofunctional features of the endometrial tissue. **Results.** The frequency of detection of hyperplastic processes, atypical hyperplasia and adenocarcinoma indicates that starting from the period after 40 y.o. the mechanisms of initiation and development of factors in the system of self-sufficiency in mitogenic stimuli, constant proliferation and insensitivity to antimytogenic signals", loss of "Hayflick's limit", reduced ability to apoptosis, propensity to immortalization take place. **Conclusion:** The expression of VEGF concentration in endometrial tissue as an inducer of angiogenesis is increased in hyperplastic processes. Significant growth is observed in the processes of atypical hyperplasia, especially in the postmenopausal period. Determination of VEGF concentration in endometrial proliferative processes can be a promising marker for diagnosing the risk of atypical conditions and their prognosis. Research in this direction is quite relevant.

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

Introduction

A clear tendency to increase the prevalence of hormone-dependent diseases related to preproliferative processes and endometrial cancer are observed against the background of increasing their incidence and rejuvenation of the age of manifestation [1, 3, 13]. Unspecified information about the etiopathogenesis of hyperplastic processes limits the possibilities of therapeutic treatment and obtaining positive results. The lack of evidence base, the increased list of contraindications to hormone therapy cause the ineffectiveness of therapeutic measures and the growth of malignancy [3, 4, 15, 18].

Microcirculatory-tissue theory, as a new paradigm of carcinogenesis, currently occupies one of the leading places in clinical and scientific research [1, 3, 4, 6]. It has a certain role in molecular mechanisms of oxygen homeostasis regulation control. It is proved that hypoxia disrupts energy metabolism, inhibits the synthesis of biologically active substances, stimulates angiogenesis (vascular endothelial growth factor -VEGF), angiogenin and growth factors [2, 7, 8, 9, 10].

Angiogenesis is one of the forms that lead to the formation of new blood vessels, regardless of the presence of existing ones [2, 11, 12]. VEGF are proteins that are the main inducers of angiogenesis, their expression is regulated, including hypoxia, hypoglycemia [8, 13, 14], they stimulate the reactions by which endothelial cells migrate, proliferate gather in tubes and form a connected grid [2, 12, 16]. Angiogenesis is stimulated if metabolic needs exceed the perfusion capacity of existing vessels. Under physiological conditions, the processes of angiogenesis are moderate in intensity and are activated for regeneration of damaged tissues, thrombus drainage, cyclic changes in the ovaries, endometrial proliferation, growth of embryonic and postnatal tissues, which is associated with either hormonal stimulation or response against ischemia. The results of recent studies have confirmed this adaptation mechanism in hypoxia and hypoglycemia [1, 2, 5, 17].

The level of VEGF expression progressively decreases after birth, and is at low levels in most tissues except the places with active angiogenesis: ovaries, uterus, skin [2, 3]. Simple disorders of cell respiration do not lead to malignancy, it is necessary that the disorders were irreversible and passed on generation of cells, but did not cause their death [1, 11, 12]. There is an assumption that hypoxia contributes to the violation of tumor cell differentiation, the latter turn into "aggressive" forms that easily grow into tissues [12, 14, 15].

Activation of angiogenesis is carried out by transcription of such factors as VEGF-A, stromal growth factor (SDF1), stem cell factor (SCF), angiopoietin [2, 12,15].

The purpose: to study the expression of VEGF marker in endometrial cells under physiological, hyperplastic, atypical conditions in women's different age periods.

Materials and methods: a retrospective analysis of archival data of medical documentation of the Odessa Regional Pathological Bureau of Pediatric and Gynecological Departments for 2016-2019 was done. We analyzed 2196 pathomorphological findings of endometrial tissue samples in women with clinical manifestations requiring surgical treatment. VEGF expression was performed in endometrial tissue samples of 458 women of late reproductive, perimenopausal and postmenopausal age. The following clinical groups were formed: I group (control) consisted of 49 late reproductive period women, their average age was 38.67 ± 0.35 y. o.; in the II group were included 57 women of reproductive age with hyperplastic processes without atypia, and average age 41.04 ± 0.36 y. o.; group III consisted of 48 patients of late reproductive age (39.08 ± 0.37) with atypical endometrial hyperplasia, group IV (control) consisted of 43 women of perimenopausal age in the proliferation phase (47.74 ± 0.38.); group V numbered 62 perimenopausal women with atypical hyperplasia (48.10 ± 0.44), group VI included 58 patients of perimenopausal age with atypical hyperplasia (48.53 ± 0.48); group VII consisted of 41 postmenopausal age (53.46 ± 0.69) patients, group VIII included 54 women with atypical hyperplastic processes (52.78 ± 0.42); group IX consisted of 46 postmenopausal patients with atypical endometrial hyperplasia (53.39 ± 0.39).

VEGF expression was performed at the mRNA level by PCR to DNA obtained by reverse transcription. The mRNA level of the gene under study was determined by the number of conventional units of the fluorescent signal using the number of RU fluorescent signal of the *36B4* gene to standardize the original (outlet) amount of RNA. Changes in expression were calculated by the ΔΔCt method.

The results were processed by the method of variation statistics with the assessment of reliability according to the Student's criterion using standard computer systems.

Results and discussion

As a result of retrospective analysis, the data on the endometrium morphological state were obtained. They were distributed according to age categories (Fig. 1). The most common pathology that led women to medical care were endometrial hyperplastic processes without cell atypia. This violation amounted to a total of 1334 cases (46.69 ± 0.94%). As can be seen from the diagram, the highest frequency of detection took place at the age group 46-50 y. o., the time of menopausal transition. Next in frequency of detection was physiological

endometrium (NE), it constituted 434 cases (15.52 ± 0.69%), the period of distribution in the chart was almost consistently the same, with the highest percentage of pathology in the period after 61 y.o., which targets doctors to carrying out manipulations with more detailed substantiation and maximum efficiency. Hyperplasia with atypical manifestations of endometrium was detected in 155 cases (5.54 ± 0.44%), with the maximum manifestation in the age categories 41-45, 46-50 y.o. The next most frequent detection was malignancy of endometrial tissue, manifestations of adenocarcinoma were found in 51 cases (1.82 ± 0.23%), this pathology was most pronounced starting from 46-50 y.o. and further increase in frequency with increasing age of women in the groups under examination.

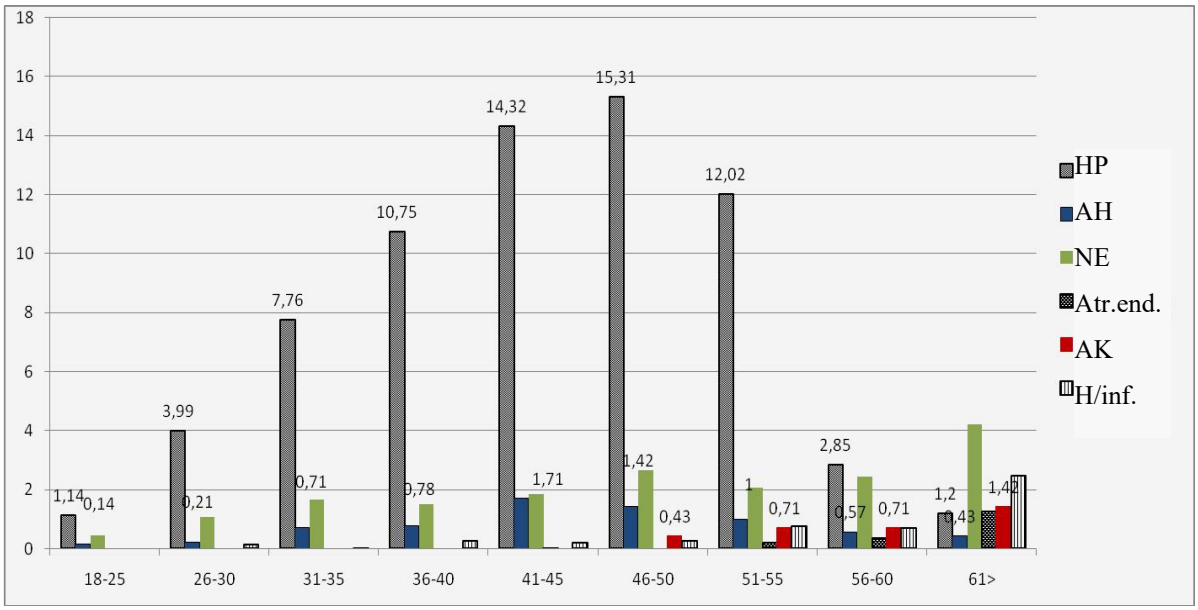

Fig. 1. Distribution of morphological states of the endometrium by age (%)

Analyzing the data shown in the diagram, it should be borne in mind that each age category is characterized by its morphofunctional features of the endometrial tissue. The frequency of detection of hyperplastic processes, atypical hyperplasia and adenocarcinoma indicates the presence of specialists concentration, starting from the period after 40 y.o., when the mechanisms of initiation and development of factors in the system of self-sufficiency in mitogenic stimuli, constant proliferation and insensitivity to antimytogenic signals", loss of “Hayflick’s limit”, reduced ability to apoptosis, propensity to immortalization [1].

Further study of VEGF (RU) expression in endometrial tissue samples, according to age categories, found that in all groups of the reproductive, perimenopausal and

postmenopausal periods, the age category of women in cohorts was significantly compared ($p < 0.01$). No acute or chronic diseases were detected in all women before fractional medical-diagnostic scraping of the uterine body cavity, according to the protocols, the results of clinical and laboratory examinations were within the reference values.

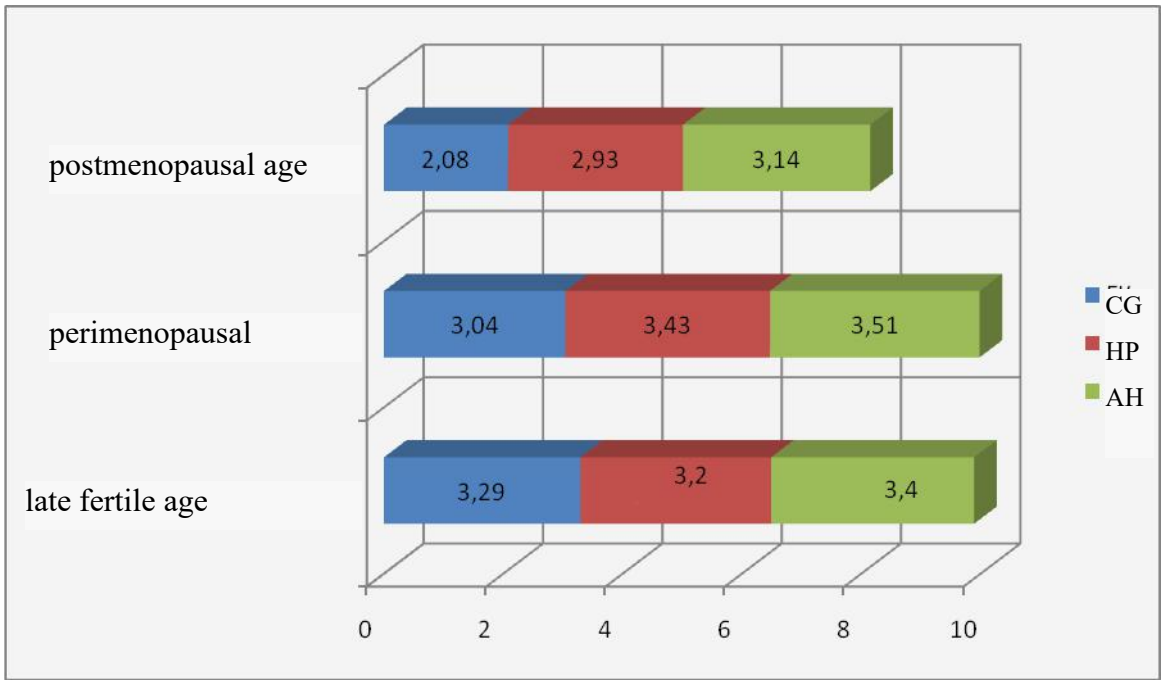

Fig. 2. Expression of VEGF in endometrial cells in different pathological conditions in different age categories (RU)

The analysis of the results of VEGF expression data in endometrial tissue cells revealed a relative increase in the numbers of indicators in the presence of atypical endometrial hyperplasia, relative to indicators in hyperplastic processes in this age group, as well as relative to the control group. The expression of VEGF, respectively, in atypical hyperplasia in the late reproductive period was 3.46 ± 0.67 RU, in the perimenopausal period - 3.60 ± 0.66 RU, in the postmenopausal age - 2.99 ± 0.94 RU with a significant difference in p_{III} -p_{IX} <0.05; p_{VI}-p_{IX} <0.05, which may indicate at atypical pathological endometrial changes enhanced tissue angiogenesis.

In hyperplastic processes, VEGF values were, respectively, in group II - 3.21 ± 0.56 RU, in group V - 3.44 ± 0.67 RU, and in group VIII - 3.00 ± 0.63 RU (p_{II}-p_V <0.05; p_V -p_{VIII} <0.05). Analyzing the data presented, it has been revealed that at proliferative processes in endometrial tissues angiogenesis processes, strengthening blood supply of tissues, prevailed in most cases.

Mosaic of literature data about hormonal influence on angiogenesis, for the control group, in the late reproductive and perimenopausal periods is evident. We chose to study the phase of proliferation, when angiogenic activity in the physiological endometrium is highest, which was analyzed in the study of breast cancer. A close relation between expression of VEGF-A at angiogenic effect of estrogens has been established, while in other researches antiangiogenic influence of antiestrogens, including, through inhibition of VEGF-A was received [2, 12].

Angiogenesis in atrophic endometrium was statistically low, but it retained its activity in most tissues - 1.23 ± 0.04 RU. This may indicate the preservation of potential capacity in endometrial cells in postmenopausal age, which in the presence of stimulating proliferative processes factors, or inhibit apoptosis, are able to enter into angiogenic processes and participate in the development of proliferative conditions in the postmenopausal age. However, it is interesting to study endometrial tissue, changes in VEGF expression depending on the duration of the postmenopausal period.

Reliable results were obtained about the expression of VEGF in atypical endometrial hyperplasia in the postmenopausal period, which, compared with epidemiological data, indicates a high risk of malignancy in postmenopausal women. The latter may be triggered by the factors stimulating oncological processes and their frequency increases in all organs and systems with age.

The results of our work contradict the data that in normal endometrium the synthesis of VEGF is stimulated by the influence of estradiol, in neoplasia this effect worsens or disappears. Although researchers have studied a highly significant negative relationship between VEGF and progesterone receptor concentrations in endometrial hyperplasia, there is no correlation between VEGF and progesterone receptor expression [2, 3, 9]. However, it should be borne in mind that the etiopathogenetic mechanisms of development of endometrial tissue malignancy are not definitively established, the influence of hormonal factors exists, but it is not always associated with cancer risks, because in our work were more active processes in the postmenopausal period. hormonal changes.

Literature data on the results of angiogenic factors in the development of tumor vascularization are quite mosaic. In most studies it was found that the processes of tumor angiogenesis differ from physiological, they provide oxygen and nutrients to malignant tissue and remove metabolic products [1, 3, 13]. Tumors larger than 1 - 2 mm^3 require their own blood supply, but the presence of a stable balance between angiogenic and antiangiogenic factors can leave neoplastic cells in an inactive state for a long time [15, 16].

The results of our work contradict the data that in normal endometrium the synthesis of VEGF is stimulated by the influence of estradiol, in neoplasia this effect worsens or disappears. Although the researchers studied a highly significant negative relationship between VEGF and progesterone receptor concentrations in endometrial hyperplasia, there is no relationship between VEGF and progesterone receptor expression at all. However, it should be borne in mind that the etiopathogenetic mechanisms of endometrial tissue malignancy are not definitively established, the influence of hormonal factors exists, but it is not always associated with cancer risks and in our work more active were processes in the postmenopausal period, which is not always associated with hormonal changes. .

Other studies have demonstrated the importance of angiogenic factors and their receptors in tumor vascularization, as well as the relationship with disease prospects and treatment efficacy [2, 3]. The use of antiangiogenic drugs is currently considered one of the most promising areas of anticancer therapy, but further research is needed, as absolutely effective cancer therapy has not yet been developed, and the number of people who need it is growing.

Conclusions:

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

Determination of VEGF concentration in endometrial proliferative processes can be a promising marker for diagnosing the risk of atypical conditions and their prognosis, especially in relation to other markers that characterize the molecular genetic or immunohistochemical cellular parameters.

Research in this direction is quite relevant both in the development of fundamental aspects of the pathogenesis of endometrial cancer, and in the direction of creating new therapies aimed at molecular growth targets and the corresponding components of the angiogenic pathway.

References:

1. Blokhin D.Yu. "Post-genomic view" on the problems of oncogenesis / Clinical oncohematology .- 2009.- T2.- No. 3.- P.277-283.

2. Gershtein E.S., Kushlinsky D.N., Tereshkina I.V. and other Growth factor of vascular endothelium and tumors of the female reproductive system. Part 2. Ovarian and endometrial cancer. / Fundamental Oncology. - 2015. - No. 2. - P.2-11.

3. Levakov S.A., Sheshukova N.A., Kedrova A.G. et al. Molecular biological profiles of endometrial hyperplasia and endometrial intrinsic epithelial neoplasia. / 2018.- T.14.- No. 2.- P.76-81.

4. Orazov M.R. Discussion issues of management of patients with endometrial hyperplasia. / Akusherst. I Ginek. News, opinions, training.- 2015.- No. 3.- P. 46-58

5. Chernukha G.E., Asaturova A.V., Ivanov I.A. et al. The structure of endometrial pathology in different age periods / Akush. iginek.- 2018.- No. 8.- P.129-134.

6. Chumak ZV, Zelinsky AA, Shapoval NV Immunohistochemical and molecular genetic markers of hyperplastic and unoplastic endometrium.- Bulletin of morphology.- 2015.- No. 2 (T.21) .- P.547- 552.

7. Anna M. Mahecha, Hongbo Wang. The influence of vascular endothelial growth factor-A and matrix metalloproteinase-2 and-9 in angiogenesis, metastasis, and prognosis of endometrial cancer.-Onco Targets Ther.- 2017.- 10, 4617-4624.

8. Chumak Z.V., Shapoval M.V., Artyomenko V.V. Age-related relationship between the development of hyperplastic processes and VEGF expression in endometrial cells. Journal of Education, Health and Sport. 2020;10(4):209-217. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2020.10.04.023

9. Enhanced expression of vascular endothelial growth factor and increased microvascular density in women with endometrial hyperplasia: a possible relationship with uterine natural killer cells. Elfayomy A.K., Almasry S.M., Attia G.M. et al. // Rom J MorpholEmbryol.- 2015;56(2 Suppl):725-34.

10. Expression profiling and significance of VEGF-A, VEGFR2, VEGFR3 and related proteins in endometrial carcinoma. Wang J., Taylor A., Showeil R. et al. // Cytokine.- 2014.- Aug; 68(2): 94-100.

11. Xu N., Sun X., Sun W-J. Expresion and clinical correlation of NGAL and VEGF in endometrial carcinoma.-Eur Rev Med Pharmacoi Sci. 2018. Feb. - 22 (3), 632-636.

12. Expression profiling and significance of VEGF-A, VEGFR2, VEGFR3 and related proteins in endometrial carcinoma. Wang J., Taylor A., Showeil R. et al. // Cytokine.- 2014.- Aug; 68(2): 94-100.

13. Vascular endothelial growth factor (VEGF) regulation by hypoxia inducible factor-1 alpha (HIF1A) starts and peaks during endometrial breakdown, not repair, in a mouse menstrual-like model. Xihua Chen, Jianbing Liu, Bin He et al. // Human Reproduction June 2015.- 30(9).-DOI: 10.1093/humrep/dev156

14. New concepts for an old problem: the diagnosis of endometrial hyperplasia. Peter A. Sanderson, Hilary O.D. Critchley, Alistair R.W. Williams.// Hum Reprod Update.-2017.- Mar.23 (2).- P.232-254

15. James V. Lacey Jr., Victoria M. Chia. Endometrial hyperplasia and the risk of progression to carcinoma. Maturitas.- 63.-2009.-P.39–44.

16. Preoperative predictors of endometrial cancer at time of hysterectomy for endometrial intraepithelial neoplasia or complex atypical hyperplasia.Monica Hagan Vetter, Blair Smith, Jason Benedict et al. Original Research Gynecology.- 2020.-V.222. Yan/ 01.-P.60/ https://doi.org/10.1016/j.ajog.2019.08.002

17. ShivajiNeelgund, P. B. Hiremath. Abnormal uterine bleeding in perimenopause.// Gale academic onefile.- June 27, 2016. P.375-381.

18. The significance of sonographically thickened endometrium in asymptomatic postmenopausal women. Runa Ozelci, Berna Dilbaz, Funda Akpınar et al.// Obstet Gynecol Sci. 2019;62(4):273-279. Published online June 24, 2019.- DOI: https://doi.org/10.5468/ogs.2019.62.4.273