

VALUE OF HYSTEROSCOPY AND GENETIC RESEARCH OF WOMEN WITH ABNORMAL UTERINE BLEEDING IN PERIMENOPAUSE

Zebo Zafarjonovna Askarova

Independent applicant of PhD Samarkand State Medical Institute, Uzbekistan

Nasiba Raximbayevna Saparbayeva

Urgench branch of Tashkent Medical Academy

Department of Obstetrics and Gynecology, Urgench, Uzbekistan

Madina Zafarjanovna Kurbaniyazova

Urgench branch of Tashkent Medical Academy

Department of Obstetrics and Gynecology, Urgench, Uzbekistan

Dilfuza Abdullayevna Aliyeva

Doctor of Medical Sciences, Professor, Head of the Scientific Advisory Polyclinic Department of RSNPMTSiG

Abstract

The perimenopause period is characterized by a gradual extinction of ovarian function, during which hypoluteinism is replaced by anovulation with relative hyperestrogenia and then hypoestrogenia. At any stage of perimenopause, there is a high probability of the formation of menstrual dysfunction, in particular, abnormal uterine bleeding (AUB). The frequency of AUB in premenopause reaches 60-70% among gynecological diseases [5, 12, 18].

Today, hysteroscopy is becoming increasingly important as a method for detecting intrauterine pathology in patients with abnormal uterine bleeding. [11.13].

The pathology of the endometrium and uterine cavity is represented by hyperplastic processes, uterine body leiomyoma, developmental abnormalities (Müller's abnormalities), inflammatory and immunopathological conditions, tumor processes that are clinically manifested by abnormal bleeding, as well as changes in neighboring organs and systems resulting from tumor damage.

Keywords: hysteroscopy, abnormal uterine bleeding, perimenopausal, women molecular genetics indicators.

Introduction

Diagnostic hysteroscopy and ultrasound in the diagnosis of abnormal uterine bleeding in perimenopausal women showed the advantage of diagnostic hysteroscopy over ultrasonic methods of sensitivity, specificity: 81% and 65%, respectively.

Abnormal uterine bleeding and malignancy determine the morphofunctional features of various types of endometrial hyperplasia (EH), as well as epigenetic and genetic disorders leading to inactivation of tumor suppressor genes, increased proliferation, angiogenesis, and decreased

apoptosis [7, 8, 15, 18]. In recent years, there has been a tendency to study the molecular genetics foundations of the development of AUB, to search for predictors of its formation and progression in EA. The results of the studies were mixed, which did not contribute to solving the problem. In some studies, the following were selected as prognostically significant markers: loss of PTEN expression, TP53 gene mutations, decrease in the apoptotic BCL-2 / BAX index [1, 14], as well as a low level of expression of both progesterone receptor isoforms (PGR).

Many authors evaluate the relationship of molecular genetics indicators of proliferative activity (Ki-67), apoptosis (APAF-1), neoangiogenesis (VEGF), and components of the extracellular matrix (MMP-1, MMP-9, TIMP-1) and the morphological variant of endometrial hyperplasia leading to AUB. [3,5,6].

The purpose of the study:

To determine the diagnostic sensitivity of hysteroscopy with targeted biopsy compared with ultrasound methods for women with abnormal perimenopause bleeding and the role of genetic factors in predicting the course of AUB in perimenopause.

Materials

We observed women in perimenopause suffering from abnormal uterine bleeding at the age of 45-48 years who were divided into 2 groups.

Group I - the main 35 women with AUB in perimenopause who underwent office hysteroscopy for the purpose of diagnosis and treatment.

Comparison group II: 35 women with AUB in perimenopause who underwent ultrasound examination and curettage of the uterine cavity for the purpose of diagnosis.

The survey methods were:

- general clinical - medical history, objective status, laboratory research methods.

Gynecological history and gynecological status. Ultrasound Doppler mapping.

Curettage of the uterine cavity with subsequent histology hysteroscopy with targeted biopsy.

Methods

Modern methods for studying the endometrium include studying the genetic structure of the endometrium in a normal, physiological, and abnormal, pathological condition. To this end, a polymerase chain reaction, immunohistochemistry to detect specific proteins, Western blotting, as well as immune studies of products secreted by endometrial cells and cultured in an artificial environment by endometrial cell cultures are carried out. [3.9.10]

Morphological diagnostics is considered the gold standard for diagnosing the state of the uterine cavity and endometrium. However, changes in the endometrium in different parts of the uterus often have a different character (mixed hyperplasia, focal hyperplasia, endometrial polyps) [5, 14].

This fact dictates the need to conduct intrauterine diagnostic and therapeutic measures under visual control. Hysteroscopy significantly expands the diagnostic capabilities for identifying intrauterine pathology, allows monitoring the effectiveness of treatment and performing manipulations in the uterine cavity [3, 4, 7, 13].

Hysteroscopy combined with targeted endometrial biopsy is predominant, compared with ultrasound, endometrial biopsy with traditional curettage in the uterine cavity. [4]

Discussion Results:

A comparative analysis of the diagnostic value of hysteroscopy with targeted biopsy and standard curettage performed in the diagnosis of uterine cavity pathology in 70 patients confirmed the 100% specificity of the methods in both groups, but showed their different sensitivity: 81 and 65%, respectively.

Color Doppler mapping was performed for all examined women in both groups. After ultrasound studies of the comparison group, it was revealed that in 7 (21.2%) of them endometrial polyps were found, in 8 (23.4%) endometrial hyperplasia, adenomyosis was in 3 (8.5%) women, uterine leiomyoma 5 (16%), a combination of leiomyoma and adenomyosis 4 (14%).

Curettage of the uterine cavity with subsequent histology was performed in 35 examined patients with the aim of diagnosing and stopping bleeding. The histology responses were as follows: glandular endometrial hyperplasia in 5 (13.4) patients, glandular cystic hyperplasia in 3 (7.5%) patients, endometrial polypsis in 4 (12%), leiomyoma in 2 (3%), in the rest 23 (64.1%) revealed the inflammatory process of the endometrium. The diagnosis of leiomyoma and a combination of leiomyoma with adenomyosis was not confirmed by histology in 3 and 4 cases, respectively. The sensitivity of diagnostic methods in the comparative group was 65%.

Hysteroscopy with targeted biopsy was performed in 35 (100%) patients of the main group. : endometrial polyps 14 (40% of cases), endometrial hyperplasia 3 (9.0%), chronic endometritis 2 (7.0%), submucous uterine fibroids 5 (14.0%), uterine cavity synechia 2 (5.5%), endometrial cancer 1 (2.3%), septum in the uterine cavity 1 (2.3%), ligatures in the uterine cavity 2 (5.4%), cervical canal polyps 4 (12.0%). When analyzing the results of hysteroscopy and histological findings, it was found that the number of correct diagnoses was 81%, incorrect - 8%. The following endometrial pathology was not confirmed by histological diagnosis: endometrial polyps (proliferative endometrium) - 1 case, endometrial hyperplasia (atrophic endometrium and proliferative endometrium) - 1, chronic endometritis (focal endometrial hyperplasia) - 1 case.

To achieve the above goal, we studied the role of the genetic marker MMP9 and mutation of the TP53 suppressor gene of women, which were divided into 2 groups: the main and the control. The main group consisted of 75 women with AUB in perimenopause. The control group consisted of 25 healthy women.

Matrix metalloproteinase (MMP) are the family of extracellular zinc-dependent endopeptidases that can break down all types of extracellular matrix proteins. They play a role in tissue remodeling, angiogenesis, cell proliferation, migration and differentiation of cells, apoptosis, and tumor growth inhibition. They involved in the cleavage of membrane receptors, the release of apoptotic ligands, such as FAS, as well as in the activation and deactivation of chemokines and cytokines.

MMP were first described in vertebrates in 1962, and later found in invertebrates and plants. The main differences between MMP and other endopeptidases are their dependence on metal ions, the ability to destroy the structures of the extracellular matrix. [10,12,26].

In women with various pathological conditions, such as AUB and endometrial cancer, the level of MMP-9 is increased. It was shown that in patients with AUB in serum, the concentration of MMP-9 is significantly higher than in practically healthy individuals.

p53 (p53 protein) is a transcription factor that regulates the cell cycle. P53 acts as a suppressor of the formation of malignant tumors, respectively; the TP53 gene is an anti-oncogen. Mutations of

the TP53 gene are found in cells of about 50% of cancers. It is often called the “guardian of the genome” [13,17,23].

For complete impairment of gene function (in particular, anti-oncogen), as a rule, inactivation of both copies of the gene on two chromosomes of the diploid cell genome is necessary. Such a violation may occur as a result of an extended deletion - complete or partial loss of the nucleotide sequence encoding the gene. Substitutions, loss or insertion of single nucleotides in DNA are also possible. Replacing a nucleotide at some point can cause the formation of the so-called termination codon, and protein synthesis at this point will be interrupted. Losses or insertions of single nucleotides lead to a malfunction of the correct framework for the translation of mRNA into protein. After such a mutation, “incorrect” amino acids are included in the growing polypeptide chain and termination codons appear which prematurely terminate the synthesis of the polypeptide chain. Another possibility is the replacement of single nucleotides, which change the meaning of a codon encoding an amino acid residue. This leads to the replacement of one amino acid in the protein by another, and if the replacement occurs on a functionally important part of the protein, then it can change its functionality or even completely lose it. Such substitutions are called missense mutations.

Among the tumor suppressor genes, TP53 is the best known. Its product is phosphoprotein p53, which regulates the transcription of several other genes. In a normal cell, p53 is inactive, but in extreme events the genome guard activates and plays the role of performing various anti-cancer functions: it activates the DNA repair system (the Nobel Prize in Chemistry was awarded to study the mechanisms of this system in 2015); if DNA is damaged, p53 delays mitosis of dividing cells, blocking the transition from the G1 phase to the S phase and giving the repair system time to repair the damage; if DNA damage cannot be repaired, p53 includes a cell death program called apoptosis. At the same time, the TP53 gene itself is very often susceptible to mutations in many types of cancerous tumors. For TP53, all of the above types of mutations are known, and usually there is no relationship between the type of cancer and certain types of mutations [18,22,25].

Group I consisted of 75 women with AUB in perimenopause. Exclusion criteria were women with coagulopathic diseases, cancer patients and women in whom AMA was an iatrogenic complication. The age of the women surveyed ranged from 45-51 years. All women underwent the following examination methods:

- anamnesis and assessment of the nature of bleeding
- clinical blood test
- gynecological examination
- Doppler mapping
- sonohysterography
- curettage of the uterine cavity with subsequent histology
- hysteroscopy with targeted biopsy and histology.
- determination of serum levels of MMP3 and mutation of the TP53 gene by PCR.

In 16 (21.2%) of them, endometrial polyps were found, in 17 (22.6%) endometrial hyperplasia, 13 (17.3%) of uterine fibroids were 5.3% of them submucous, adenomyosis was in 6 (8.23%) women, a combination of endometrial hyperplasia and uterine leiomyoma 12 (16%), a combination of leiomyoma and adenomyosis 11 (14.67%). Pathomorphological causes of abnormal uterine bleeding are shown in the table.

Table 1
Pathomorphological causes of AUB

Pathomorphological causes of AUB	P=75	%
Endometrial polyps	16	21,2
Endometrial hyperplasia	17	22,6
Myoma	5	17,3
Leiomyoma	5	
Submucous node	3	
Adenomyosis	6	8,23
Hyperplasia + adenomyosis	11	14,67
Hyperplasia + leiomyoma	12	16
	75	100

The women with AUB, a significant ($p < 0.05$) increase in serum MMP-9 level was observed when comparing the main group and the control group. In the main group, the concentration of MMP-9 was 423.94 ng / ml (SD = 128.9; the range of values was 237.0–740.0 ng / ml), in the control group, 162.7 ng / ml (SD = 23, 7; 120.0–269.0 ng / ml). In the analysis of MMP-9, based on the data obtained, a concentration of 240 ng / ml should be considered a critical value: when it is exceeded, a statistically significant ($p = 0.063$) correlation with the probability of hyperplastic processes in the endometrium is noted. When taking the above specified value of the concentration of MMP-9 as a threshold isolated assessment of the level of MMP-9 in the blood serum, it has a sensitivity of 86%, specificity of 80%.

Table 2
The content of MMP-9 in the blood serum of examined women

The main group of examined MMP-9 (ng/ml)	Control group MMP-9 (ng/ml)
423,94±17,71*	162,7±3,35

* Compared to the control group at $p < 0.05$.

The tumor suppressor gene and its corresponding p53 protein play a central role in the development of apoptosis [30,38]. P53 mutations are the most common genetic disorder in the development of malignant tumors. The appearance of mutant forms of this gene leads to the accumulation in tumor cells of inactive forms of the protein that cannot fulfill the functions of normal p53 [26, 33, 37]. Mutations of this gene can be associated with the aggressive course of the disease and the resistance of tumor cells to the effects of anticancer drugs and radiation therapy. [21; 29]. In RE, according to various researchers, mutant p53 is found in 44–64% of patients [35].

Endometriosis, leiomyoma, polyps can be attributed to multifactorial diseases, in the genesis of which an important role belongs not to one but to many different genes. Given the proximity of

these diseases to cancer, it can be assumed that the gene network of these diseases and ER should include identical or similar genes. One of these is TP53, whose mutations naturally occur in a wide variety of tumors. The product of this gene is a key element in controlling the cellular response to various types of stress.

By PCR, the blood serum of the main and control group of patients was examined.

Table 3

Gene polymorphism	Main group		Control group	
	N	M \pm M%	N	M \pm M, %
TP53 Arg72Pro	75	30,7 \pm 4, 1	25	4,2 \pm 2,4

Of the 75 women in the main group, the level of MMP9 was increased in 69 (92%). In the control group, the level of MMP9 was increased in 3 (12%) women. Mutated p53 was positive in 23 (31%) in the examined women with AUB; in the control group, an increase in the level of mutated p53 was observed in 1 (4%) patient. Thus, in women with AUB, the level of MMP9 is increased in 92% of cases. Based on this result, it can be judged that this genetic marker is specific for AUB.

A positive result for mutated p53 is a criterion for the formation of an increased cancer risk group, which makes it possible to optimize the management of women with this pathology.

Conclusion:

A comparative study of diagnostic hysteroscopy and transvaginal ultrasound in the diagnosis of abnormal uterine bleeding in perimenopausal women showed the advantage of diagnostic hysteroscopy over ultrasound methods in sensitivity, specificity: 81% and 65%, respectively. Thus, diagnostic hysteroscopy, combined with surgical intervention, in its modern office version, is one of the main methods for the comprehensive diagnosis and treatment of women with abnormal uterine bleeding in perimenopause.

Literature:

1. Ablakulova V.S. About the risk of recurrence of endometrial polyps. Second honey magazine. Of Uzbekistan. 1999; 1; 53-55s.
2. Ailamazyan E.K. Gynecology: from puberty to menopause. — M.: MEDpress, 2017. — 512 p.
3. Zaporozhan V. N., Vikhlyaeva E. M., Zheleznov B. I. Dysfunctional uterine bleeding // Guide to endocrine gynecology / Ed. E. M. Vikhlyaeva. -- Moscow: Honey. inform. Agency, 2015. -- 768 p.
4. Klyucharov I.V., Saveliev E.V. Our experience in evaluating the diagnostic value of hysteroscopy with biopsy in determining endometrial pathology from the perspective of evidence-based medicine // Endoscopic surgery. 2005. No. 1. P. 60–61.
5. Savelyeva G. M., Breusenko V. G., Kaplusheva L. M. Hysteroscopy. M.: GEOTAR, 1999.176 s.
6. Topchiev O. I., Pryanishnikova V. A., Zhemkova Z. P. Endometrial biopsy. M.: Medicine, 1978. 232 p.

7. Chernukha G.E., Ilyina L.M. Inflammation is the biological basis of heavy menstrual bleeding. *Gynecological endocrinology* 2015; 20-7
8. American College of Obstetrics and Gynecology. Practice Bulletin No. 128, Diagnosis of abnormal uterine bleeding in reproductive aged women. *Obstet Gynecol.* 2012;120:197-206.
9. American College of Obstetricians and Gynecologists. ACOG Committee Opinion No. 440: the role of transvaginal ultrasonography in the evaluation of postmenopausal bleeding. *Obstet Gynecol.* 2009;114:409-411.
10. A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women / M. J. Murray [et al.] // *Fertil Steril.* 2004. Vol. 81. P. 1333–1343.
11. Cooper J. M., Brady M. R. Hysteroscopy in the management of abnormal uterine bleeding // *Obstetrics and gynecology clinics.* 1999. Vol. 26, № 1. P. 217–236.
12. Lethaby A, Augood C, Duckitt K. Nonsteroidal anti-inflammatory drugs for heavy menstrual bleeding. *Cochrane Database Syst Rev.* 2007;4:CD000400.
13. Lethaby A, Farquhar C, Cooke I. Antifibrinolytics for heavy menstrual bleeding. *Cochrane Database Syst Rev.* 2000;4:CD000249.
14. Wittkowsky AKA. Systematic review and inventory of supplemental effects on warfarin and other anticoagulants. *Thromb Res.* 2005;117:81-86.
15. Vikhlyaeva E. M., Zheleznov B. Yu., Zaporozhan V. N. A guide to endocrine gynecology. Hyperplastic processes of the endometrium / Ed. E. M. Vikhlyaeva. - M.: Honey. Inform. Agency, 2017.- p. 684–710.
16. Vikhlyaeva E. M., Fanchenko N. D. The state of estrogen receptor systems and the clinical effect of cryosurgery of hyperplastic epithelium. *Obstetrics and Gynecology* 2000 №. 6 9-11
17. Gogaeva E.V. Obesity and menstrual dysfunction. *Gynecological endocrinology.* 2001.-Volume 3 №. 5-174-176.
18. Dubossarskaya Z. M. Reproductive endocrinology: Training method. allowance.— Dnepropetrovsk: Lira LTD, 2018.— 416 p. 26. Panay N., Studd J. Treatment of gestagen intolerance // *Progress in the management of the Menopause.*— N. Y., 1998. — P. 151–167.
19. Zaydieva Y.Z. Abnormal uterine bleeding in perimenopause // *Russian Bulletin of the Obstetrician-Gynecologist* 5, 2018 p. 92-99.
20. Zaporozhan V.N., Vikhlyaeva E. M., Zheleznov B. I. Dysfunctional uterine bleeding // *Guide to endocrine gynecology / Ed. E. M. Vikhlyaeva. - M.: Honey. inform. Agency, 2015.- 768 p.*
21. Kustarov V.N., Chernichenko I.I. Dysfunctional uterine bleeding.- SPb .: SPbMAPO, 2017.— 163 p.
22. Smetnik V. P., Tumilovich L. G. Non-operative gynecology. - M.: Honey. inform. Agency, 2016.— 632 p.
23. Tarasova M. A., Yarmolinskaya M. I. Dysfunctional uterine bleeding // *Journal. Obstetrics and female diseases.* — 2018. — №1.— p. 77–81.
24. Chernukha G.E., Ilyina L.M. Inflammation is the biological basis of heavy menstrual bleeding. *Gynecological endocrinology* 2015; 20-7
25. Scherbina N. A., Tanko O. P., Rakov A. V. Treatment of dysfunctional uterine bleeding // *Experiment. I Clinical Medicine.* - 2001.— №. 1.— S. 135–136. . Scherbina N.A.,

Tanko O.P., Rakov A.V. Treatment of dysfunctional uterine bleeding // Experiment. I Clinical Medicine. — 2015. — №. 1.— S. 135–136.

26. American College of Obstetrics and Gynecology. Practice Bulletin №. 128, Diagnosis of abnormal uterine bleeding in reproductive aged women. *Obstet Gynecol.* 2012;120:197-206.

27. American College of Obstetricians and Gynecologists. ACOG Committee Opinion №. 440: the role of transvaginal ultrasonography in the evaluation of postmenopausal bleeding. *Obstet Gynecol.* 2009;114:409-411.

28. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 121. Long-acting reversible contraception: implants and intrauterine devices. *Obstet Gynecol.* 2011;118:184-196.

29. AlHilli MM, Hopkins MR, Famuyide AO. Endometrial cancer after endometrial ablation: systematic review of medical literature. *J Minim Invasive Gynecol.* 2011;18:393-400.

30. Barbieri RL. A new (to the US) first-line agent for heavy menstrual bleeding (Editorial). *OBG Management.* 2010;22:9-12.

31. Basila D, Yuan CS. Effects of dietary supplements on coagulation and platelet function. *Thromb Res.* 2015;117:49-53.

32. Bosteels J, Kasius J, Weyers S. Hysteroscopy for treating subfertility associated with suspected major uterine cavity abnormalities. *Cochrane Database Syst Rev.* 2015;2:CD009461.

33. Donnez J, Tatarchuk TF, Bouchard P. Ulipristal acetate versus placebo for fibroid treatment before surgery. *N Engl J Med.* 2012;366: 409-420.

34. Ferry J, Farnsworth A, Webster M, Wren B. The efficacy of the pipelle endometrial biopsy in detecting endometrial carcinoma. *Aust N Z J Obstet Gynaecol.* 1993;33:76-78.

35. Grady D. Clinical practice management of menopausal symptoms. *N Engl J Med.* 2018;355:2338-2347

36. Goldstein SR. Modern evaluation of the endometrium. *Obstet Gynecol.* 2010;116:168-176

37. Goldstein SR, Lumsden M.A. Abnormal uterine bleeding in perimenopause. *Climacteric.* 2017;414-420.

38. Goldstein SR. Pregnancy. 1. Embryo. *Endovaginal Ultrasound*, 2nd ed. New York (NY): Wiley-Liss, Inc. 1991.

39. Goldstein SR. Use of ultrasonohysterography for triage of perimenopausal patients with unexplained uterine bleeding. *Am J Obstet Gynecol.* 1994;170:565-570.