

The Role of the Angiogenic Factor VEGF-A and Ultrasound Examination in the Diagnosis of Adenomyosis

Raximova Z. A. *, Muminova Z. A.

Tashkent Medical Academy, Tashkent, Uzbekistan

Abstract According to the World Health Organization, approximately 10% of women and girls of reproductive age worldwide suffer from endometriosis. This number is continuously increasing, and this condition has been classified as a modern-day epidemic. (WHO 2022). The pathogenesis and etiology of adenomyosis remain unidentified. The causes of adenomyosis development are reliably reflected in two theories: invagination and metaplasia. Determining the pathogenesis of this condition requires complex morphometric research.

Keywords Endometriosis, Adenomyosis, “Junction zone”, Ultrasound examination (UTT), Magnetic resonance examination (MRT), Transvaginal sonography, Uterine artery dopplerometry

1. Introduction

According to the World Health Organization, approximately 10% of women and girls of reproductive age worldwide suffer from endometriosis. This number is continuously increasing, and this condition has been classified as a modern-day epidemic. (WHO 2022).

As a result, the prevalence of adenomyosis reaches 70-90% as the most common form of genital endometriosis. (L.V. Adamyan, Russia 2022). Adenomyosis is a common cause of dysmenorrhea, infertility, menometrorrhagia, and chronic pelvic pain of varying intensity. It often leads to psychosomatic and autonomic disorders. (Sidorova I.S. and others).

The pathogenesis and etiology of adenomyosis remain unidentified. The causes of adenomyosis development are reliably reflected in two theories: invagination and metaplasia. Determining the pathogenesis of this condition requires complex morphometric research.

2. Research Objective

To determine the degree of adenomyosis by identifying the “transitional zone” in ultrasound examination, conducting functional testing using Doppler and analyzing the level of expression of VEGF-A in cervical tissue.

3. Research Methods

* Corresponding author:

zulfia6232@gmail.com (Raximova Z. A.)

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The researches were conducted on women at the Gynecology Department of Tashkent City Maternity Complex No. 9 from 2021 to 2023.

To identify adenomyosis at its initial stage, ultrasound data from 117 patients were analyzed, in whom varying degrees of diffuse adenomyosis were diagnosed based on clinical, instrumental, and biochemical examinations.

Classification of patients into groups based on ultrasound criteria for the degree of adenomyosis.

Group I - n=25, Grade I adenomyosis
Group II - n=23, Grade II adenomyosis
Group III - n=24, Grade III adenomyosis
Group IV (Control) - n=25, healthy women

4. Research Results

The main reasons for patients with adenomyosis to visit the doctor included: dysmenorrhea (pain in the lower abdomen and painful menstruation starting 2-5 days before menstruation), irregular menstrual cycles prone to hypermenorrhea (prolonged bleeding lasting more than 7 days), a shortened cycle interval (menstruation occurring every 23-25 days), bleeding before and after menstruation, lack of ovulation during expected ovulation periods, varying intensity of uterine bleeding, and infertility lasting 2 to 6 years.

At the initial stage of non-invasive instrumental diagnosis for adenomyosis, all patients in this study underwent ultrasound examination utilizing color Doppler imaging. The sonographic examination includes assessment of the size of the uterus, structural changes (such as the endometrium, “transition zone” and myometrium), as well as characteristics of blood supply based on qualitative and quantitative

parameters of blood flow. Dynamic monitoring throughout the menstrual cycle is also included. The research was conducted during the first and second phases of the cycle.

In patients with first-degree adenomyosis, the size of the uterus does not practically differ from that in group 4 (control-1). However, these patients already have an increase in the thickness, width, and volume of their uterus during the

second phase of menstruation compared to the first phase. Although this is statistically insignificant, it is still a noticeable difference.

In clinical groups 2 and 3, there was a statistically significant increase in uterine size during the first and second phases of the menstrual cycle, compared to group 4 (the control group) and clinical group 1.

Table 1. The size of the uterus and its structural elements in the dynamics of the menstrual cycle in patients with adenomyosis

Groups	Group 4 n = 25 (control - 1)		Group 1 n = 24		Group 2 n = 23		Group 3 n = 24	
	Early proliferative phase	The phase of medium secretion	Early proliferative phase	The phase of medium secretion	Early proliferative phase	The phase of medium secretion	Early proliferative phase	The phase of medium secretion
Length of the uterine body, mm	52 ± 1,2	53 ± 1,4	53 ± 1,3	54 ± 2,1	62 ± 2,4	65 ± 2,1	76 ± 2,5	78 ± 2,5
Front and rear size, mm	38 ± 1,2	40 ± 1,3	38 ± 1,4	44 ± 2,1	58 ± 2,4	64 ± 2,3	62 ± 2,3	68 ± 2,5
Width, mm	51 ± 1,2	52 ± 1,2	51 ± 1,1	56 ± 1,5	63 ± 2,1	65 ± 1,4	72 ± 2,3	76 ± 3,2
Uterine size, sm ³	52,7 ± 1,2	57,6 ± 1,4	53,7 ± 1,3	76,1 ± 1,3	118,5 ± 3,2	141,42 ± 2,5	177,4 ± 2,6	210,8 ± 3,4
Thickness "transition zone"	3, 2 ± 2,3	3,2 ± 1,6	5,2 ± 1,9	7,2 ± 2,6	12,4 ± 2,6	13,6 ± 3,4	-	-

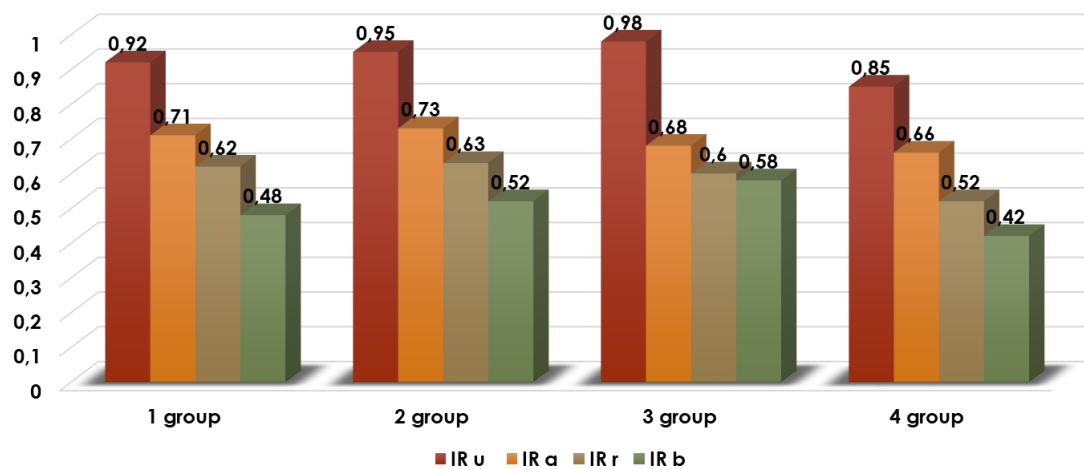


Figure 1. Resistance Index (RI) in Healthy Women and Patients with Adenomyosis

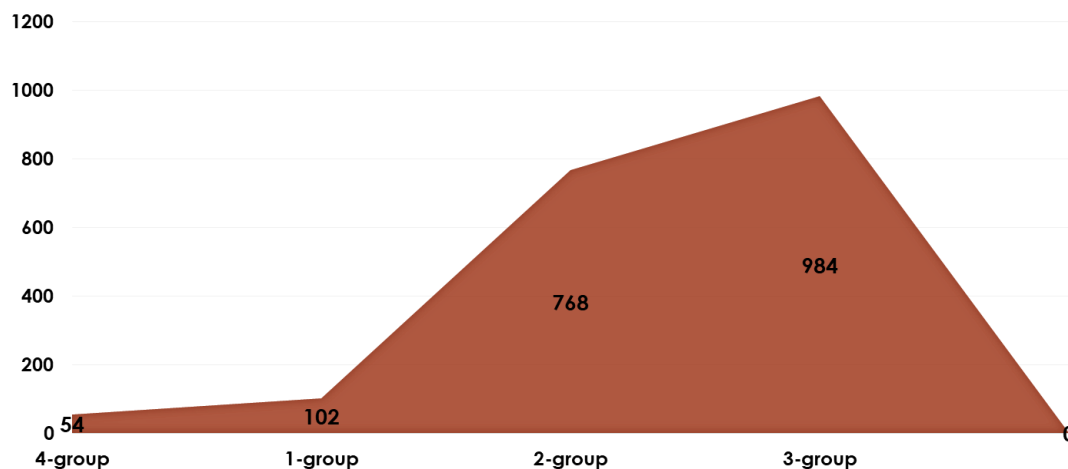


Figure 2. VEGF-A Levels in Cervical Mucus of Patients with Adenomyosis Compared to Group 4

In our research, special attention was given to the “transition zone” (the layer of the myometrium immediately adjacent to the endometrium) during ultrasound examination, considering the development of adenomyosis with the penetration of the endometrium from the basal layer into the myometrium. In all patients of Group 1, ultrasound examination revealed that the echogenic zone adjacent to the endometrium (“transition zone”) measured up to 5.2 ± 1.9 mm during the proliferative phase of the menstrual cycle and up to 7.2 ± 2.6 mm during the secretory phase. In patients with Grade II adenomyosis (Group 2), the “transition zone” had a thickness of up to 12.4 ± 2.6 mm. In contrast, in Group 3, comprising patients with a more extensive form of adenomyosis, the “transition zone” was poorly delineated, possibly due to the deep and widespread infiltration of endometrial elements into the myometrium.

In our research, one of the most characteristic echographic features of adenomyosis observed in patients with stages II and III adenomyosis (Groups 2 and 3) was an increase in the thickness of the anterior and posterior uterine walls, noted in 17 patients (73.9%) and 24 patients (100%), respectively.

Furthermore, the greatest increase in uterine wall thickness in Groups 2 and 3 was observed during the conditional second phase of the menstrual cycle (Table 1). A characteristic echographic feature of adenomyosis — the rounded (spherical) shape of the uterus — was observed in 77.6% of patients with stage II adenomyosis and 89.4% of patients with stage III adenomyosis during the second phase of the cycle. In contrast, the characteristic sphericity was noted in only 65.4% of patients with stage III adenomyosis and 32.2% of patients with stage II adenomyosis during the first phase of the cycle. Thus, early-stage adenomyosis is typically not characterized by uterine enlargement or the development of a rounded (spherical) uterine shape.

In Group 1, the “transition zone” ($M_0 = 5.2$ mm) was observed, whereas in Group 2, it was detected in 79.6% of patients, with its thickness increasing to 12.4 ± 2.6 mm ($M_0 = 10$ mm). In Group 3, the “transition zone” was not defined (appearing as indistinct or blurred). Thus, according to ultrasound findings, the exogenic zone (“transition zone”) adjacent to the endometrium was identified in all patients of Group 1, with a thickness of 5.2 ± 1.9 mm.

According to the results of our research, Dopplerometric examination in women of Group 4 (Control-1) revealed a statistically significant decrease ($p < 0.05$) in the systolic-diastolic ratio in the uterine and arcuate arteries during the mid-secretory phase of the menstrual cycle compared to the early proliferative phase (7.9 ± 0.21 vs. 5.7 ± 0.12 and 6.0 ± 0.23 vs. 4.4 ± 0.21 , respectively). In the radial and basal arteries, the dynamics of R values were minimal, showing no statistically significant differences (2.9 ± 0.11 vs. $2.8 \pm 0.21/6.9\%$ and 1.7 ± 0.23 vs. $1.6 \pm 0.01/5.9\%$, respectively) ($p > 0.05$). In patients of Group 1 with stage I adenomyosis, as in the previous observations, a statistically significant increase in the systolic-diastolic ratio was noted across the entire uterine artery basin compared to Group 4 (Control 1).

In all research groups and the control group (Group 4), the

resistance index (RI) did not show statistically significant variations throughout the menstrual cycle dynamics. At the same time, a high level of resistant blood flow was observed in all clinical groups of patients with adenomyosis, with the highest statistical significance, detected in the entire uterine artery basin in cases of stage III adenomyosis (IRm 0.98 ± 0.01 , IRa 0.68 ± 0.05 , IRr 0.60 ± 0.03 , IRb 0.58 ± 0.03), compared to the control group (IRm 0.85 ± 0.12 , IRa 0.66 ± 0.03 , IRr 0.52 ± 0.01 , IRb 0.52 ± 0.01), where $p < 0.42 \pm 0.40$).

In patients of Group 1, significant differences in IR were observed only in the uterine arteries (IRm 0.92 ± 0.12 versus IRm 0.85 ± 0.12 in Group 4 (control-1), $p < 0.05$). In patients with second-degree adenomyosis, significant differences were found in both the uterine and ovarian arteries (IRm 0.95 ± 0.15 , IRa 0.73 ± 0.01 versus IRm 0.85 ± 0.12 , IRa 0.66 ± 0.03 in the control group, $p < 0.05$).

Thus, based on the research of hemodynamic parameters in the uterine artery and its branches during the phases of the menstrual cycle at different stages of adenomyosis, it was found that even at stage I of adenomyotic disease, there is an increase in resistance. The blood flow in the basin of the uterine artery basin was lower compared to healthy women, which indicates a decrease in arterial circulation intensity in the uterus in patients with adenomyoma.

To investigate non-invasive markers reflecting the state of local angiogenesis in patients with adenomyosis, we analyzed cervical secretions by determining VEGF-A expression levels. It is known that the functional composition of uterine artery tests includes both inhibitors and activators of processes associated with local tissue homeostasis and soluble forms of neoangiogenesis in uterine tissues.

The VEGF-A concentration was 101.5 ± 19.3 pg/mL in Group 1, 767.6 ± 84.3 pg/mL in Group 2, and 973.3 ± 57.6 pg/mL in Group 3, showing a statistically significant difference compared to the control group (Group 4) at 53.3 ± 12.5 pg/mL ($p < 0.05$). Additionally, statistically significant differences were also observed between the groups ($p < 0.05$).

The increased activity of the anti-angiogenic factor in the functional test coefficient (FTC) during the early stages of adenomyosis indicates altered autocrine regulation of angiogenesis in the endometrium, resulting in enhanced vascular proliferative activity. Overall, these findings suggest that this parameter can be used as an additional early non-invasive diagnostic marker.

In general, our research demonstrated that during the initial stages of adenomyosis formation, despite the increased proliferative processes in the endometrium and myometrium within the “transition zone”, there is an accumulation of excess amorphous substances and active pathological neoangiogenesis in the “transition zone”. This is accompanied by reduced microvascular resistance in response to high hemodynamic loads and elevated reserve resistive capacity. These changes lead to the normalization blood flow in the uterine arteries, confirmed by a high uterine artery functional test coefficient. Conversely, with the progression of the disease and the deepening of the adenomyosis stage, there is an increased production of collagen both in the myometrium and in the

abnormal vessels, as well as hypertrophy of the smooth muscle tissues in the myometrium within the “transition zone”, leading to a restriction in the elasticity of the vascular walls. At the same time, against the background of the increasing initial disturbances in blood flow parameters, the functional test becomes less indicative.

The research that was conducted enabled the development of a non-invasive early diagnostic method for adenomyosis, incorporating a set of ultrasound and biochemical indicators. When the thickness of the “transition zone” is visualized at 5 mm or more, vascular blood flow in the uterine arteries is measured using a functional stress test, and a uterine artery functional test coefficient is calculated. In addition, VEGF-A levels in the central nervous system are determined for these patients. If the “transition zone” thickness exceeds 5 mm, the uterine artery functional test coefficient is greater than 1.2, and the VEGF-A level is above 70 pg/mL, the presence of stage I adenomyosis is collectively identified and confirmed.

5. Conclusions

1. As the degree of adenomyosis increases, the severity of its clinical symptoms, as well as the findings from ultrasound and hysteroscopy, also increase. Grade I adenomyosis is characterized by minimal, non-patognomonic signs, and standard ultrasound examination lacks specificity.
2. The imbalance between the increase of anti-angiogenic and pro-angiogenic growth factors in the “transition zone” of the endometrium and the functional test coefficient (direct positive correlation), even in Grade I adenomyosis, indicates a change in the autocrine regulation of angiogenesis. This alteration in the early stages of adenomyosis leads to an increase in the pathological proliferative activity of blood vessels.
3. The ultrasound visualization of the “transition zone” with a thickness greater than 5 mm and the significant increase in vascular resistance in the uterine artery basin are echographic signs of Grade I adenomyosis. The use of a functional stress test during Dopplerometry of the uterine vascular basin increases the specificity and sensitivity of early ultrasound diagnosis.
4. The ultrasound visualization of the “transition zone”, combined with the determination of VEGF-A expression levels in the cervical mucosa, and the use of a functional stress test, serves as a specific and sensitive non-invasive method for diagnosing Grade I adenomyosis.

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