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THE RELATIONSHIPS BETWEEN COMPLAINTS, MELATONIN LEVELS AND SOME INDICATORS OF THE STATE OF THE ANTIOXIDANT SYSTEM AND ANGIOGENESIS IN WOMEN WITH UTERINE LEIOMYOMA

The aim of the study - to research the relationships between complaints, melatonin levels and some indicators of the state of the antioxidant system and angiogenesis in women diagnosed with uterine leiomyoma.

Materials and Methods. 60 women of reproductive age with a diagnosis of uterine leiomyoma (experimental group) were examined. The control group consisted of 20 healthy women of the same age group. The concentrations of melatonin and reduced glutathione, as well as the levels of vascular endothelial growth factor VEGF in the blood plasma of the examined women were studied. Comparisons between the groups were carried out using the t-test for unequal samples. Calculations of logistic regression between qualitative (presence or absence of those described below clinical symptoms) and quantitative (listed biochemical parameters) parameters were performed.

Results and Discussion. The level of melatonin in the blood plasma of patients with uterine leiomyoma was significantly lower compared to the level of this hormone in healthy women, namely, 111.01 ± 18.5 ng/ml compared to 153.5 ± 8.5 ng/ml in the control group, $p<0.01$. The plasma concentration of reduced glutathione in women with leiomyoma was significantly lower compared to healthy women (0.77 ± 0.13 μmol/L versus 1.02 ± 0.14 μmol/L in the control group, respectively, $p<0.01$). The level of VEGF in patients with uterine leiomyoma was significantly higher (90.4 ± 23.6 pg/ml) compared to the control group (35.1 ± 8.3 pg/ml), $p<0.01$. With a decrease in plasma melatonin levels below 100.0 ng/ml in women of reproductive age, patients with uterine leiomyoma have an 82% chance of painful menstruation, and with an increase in the concentration of reduced glutathione above 0.78 μmol/L, the chance of profuse bleeding during menstruation is approximately 67%. The level of VEGF in the blood plasma of women with leiomyoma above 96.13 pg/ml, according to our data, is almost always associated with the presence of spontaneous miscarriages in the anamnesis of such patients.

Conclusions. 1. In patients with uterine leiomyoma, there is a reduced activity of the antioxidant system compared to healthy women: in the experimental group, the average level of melatonin in the blood plasma was significantly ($p<0.01$) lower compared to the control group (the difference was 27.6 percent), as well as the average plasma level of reduced glutathione (the difference was 24.5 percent at $p<0.01$, compared to the control). 2. In the group of women diagnosed with uterine leiomyoma, there was a significant (2.58-fold) increase in the average concentration of VEGF in blood plasma compared to healthy women ($p<0.01$), which indicates the activation of angiogenesis in this disease. 3. The relationships between the clinical signs of uterine leiomyoma and the studied biochemical parameters are described using logical regression tools.

Key words: uterine leiomyoma; melatonin; reduced glutathione; VEGF; lipid peroxidation; antioxidant system.

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ВЗАЄМОЗВ'ЯЗОК МІЖ СКАРГАМИ, РІВНЕМ МЕЛАТОНІНУ ТА ДЕЯКИМИ ПОКАЗНИКАМИ СТАНУ АНТИОКСИДАНТНОЇ СИСТЕМИ Й АНГІОГЕНЕЗУ В ЖІНОК ІЗ ЛЕЙОМІОМОЮ МАТКИ

Мета дослідження – встановити взаємозв'язок між скаргами, рівнем мелатоніну та деякими показниками стану антиоксидантної системи і ангіогенезу в жінок із лейоміомою матки.

Матеріали та методи. Обстежено 60 жінок репродуктивного віку з діагнозом «лейоміома матки» (дослідна група). Контрольну групу склали 20 практично здорових жінок тієї ж вікової групи. Вивчали концентрації мелатоніну та відновленого глутатіону, а також рівні фактора росту ендотелію судин VEGF у плазмі крові обстежених жінок. Порівняння між групами проводили з використанням t-тесту для неоднакових вибірок. Здійснювали розрахунки логістичної регресії між якісними (наявність чи відсутність описаних нижче клінічних симптомів) та кількісними (перераховані біохімічні показники) параметрами.

Результати дослідження та їх обговорення. Рівень мелатоніну в плазмі крові пацієнток, хворих на лейоміому матки, був вірогідно нижчий порівняно з рівнем даного гормону у практично здорових жінок: (111,01±18,5) ng/ml (в контролі (153,5±8,5) ng/ml, $p<0,01$). Концентрація відновленого глутатіону в плазмі крові жінок із лейоміомою була достовірно нижчою порівняно зі здоровими жінками ((0,77±0,13) мкмоль/л проти (1,02±0,14) мкмоль/л у контрольній групі, $p<0,01$). Рівень VEGF у пацієнток із лейоміомою матки був вірогідно вищий ((90,4±23,6) пг/мл) порівняно з контрольною групою ((35,1±8,3) пг/мл, $p<0,01$). При зниженні рівня мелатоніну в плазмі крові нижче 100,0 ng/ml у жінок репродуктивного віку, хворих на лейоміому матки, імовірність появи болісних менструацій складає 82 %, а при підвищенні концентрації відновленого глутатіону вище за 0,78 мкмоль/л шанс на появу рясних кров'янистих виділень під час менструації складає близько 67 %. Рівень VEGF у плазмі крові жінок із лейоміомою, вищий за 96,13 pg/ml, майже завжди, відповідно до наших даних, асоціюється з наявністю в анамнезі таких пацієнток самовільних викиднів.

Висновки. У пацієнток із лейоміомою матки спостережено знижену порівняно зі здоровими жінками активність антиоксидантної системи: в дослідній групі середній рівень мелатоніну в плазмі крові був вірогідно ($p<0,01$) нижчий, порівняно з контрольною групою (різниця склала 27,6 %), як і середній рівень у плазмі крові відновленого глутатіону (різниця склала 24,5 % при $p<0,01$ порівняно з контролем). У групі жінок із діагнозом «лейоміома матки» відмічали значне (в 2,58 раза)

збільшення середньої концентрації VEGF у плазмі крові порівняно зі здоровими жінками (p<0,01), що свідчить про активізацію ангіогенезу при даному захворюванні. Взаємозв'язки між клінічними ознаками лейоміоми матки та дослідженими біохімічними показниками описано за допомогою інструментів логічної регресії.

Ключові слова: лейоміома матки; мелатонін; відновлений глутатіон; VEGF; перекисне окиснення ліпідів; антиоксидантна система.

INTRODUCTION. Uterine leiomyoma is a benign monoclonal tumor consisting mainly of myometrial smooth muscle cells. According to some authors, the incidence of this disease in the population of Caucasian women reaches up to 50%, including asymptomatic forms [1]. This disease affects the quality of life of patients and their reproductive function, and can also cause secondary complications, in particular, iron deficiency anemia and impaired trophism of the myomatous node [2]. To date, many scientists study this disease in various aspects, one of which is the search for ways for inhibition of the pathological proliferative process in myomatous nodes and reduction of the secondary damage to uterine tissues associated with this process.

In uterine leiomyoma, secondary tissue damage occurs mainly due to oxidative stress and hypoxia [3 – 7]. On the other hand, hormonal signaling mechanisms are also involved in the formation of myomatous nodes (primarily due to the activation of proliferative processes by estradiol) [8, 9]. However, proliferation is impossible without adequate blood supply to the corresponding area, so another prerequisite for the formation of leiomyoma is a local increase in angiogenesis [10], which, in turn, is activated in response to tissue hypoxia by increasing the expression of Vascular Endothelial Growth Factor (VEGF) [11]. Reperfusion damage to tissues, in this case, to the myometrium, causes oxidative stress with the appearance of a large number of reactive oxygen species that damage cell membranes in the focus of leiomyomatous node formation, thus closing the pathophysiological "vicious circle" [12]. In addition, free radicals damage DNA helices and cause increased proliferation by expressing specific genes [13].

Promising areas for the treatment of uterine leiomyoma will be aimed at breaking the described pathophysiological mechanisms, which are the basis for the growth of the myomatous node. To date, the leading role belongs to the surgical treatment of this disease, which has a number of disadvantages: first of all, surgical intervention is mostly performed on patients with a pronounced clinical picture of uterine leiomyoma, and in such cases it is not always possible to preserve a woman's important reproductive organ [14]; in addition, surgery and anaesthesia always carry certain risks. Thus, conservative methods of treating uterine fibroids are becoming more important, especially in the early stages of the development of the disease. In this aspect, the effect on the process of lipid peroxidation and the antioxidant system in the body of a woman with uterine leiomyoma is especially important [15, 16].

Among the molecules that have antioxidant properties, melatonin and glutathione attract the most attention from researchers. Melatonin is known to be one of the body's strongest free radical cleansers; in addition, melatonin inhibits angiogenesis induced by VEGF, as it has the ability to bind to receptors for this factor [17–20]. There is evidence suggesting the ability of melatonin to trigger cell apoptosis in leiomyoma foci [19].

Glutathione (L-γ-glutamylcysteinylglycine) is a key metabolite in eukaryotes and plays an important role in protecting cells from oxidative damage [21]. Glutathione directly absorbs a variety of oxidizing agents, such as superoxide anion, hydroxyl radical, nitric oxide, and carbon radicals, and it is a cofactor for various antioxidant enzymes, including glutathione peroxidase and glutathione-S-transferase [22, 23]. There are two states of glutathione in cells: reduced glutathione and oxidized glutathione disulfide. Importantly, reduced glutathione is the main tissue antioxidant, while glutathione disulfide accumulates when cells are subjected to oxidative stress. Thus, an increase in the ratio between these two forms of glutathione in favor of glutathione disulfide is indicative of oxidative stress, and reduced glutathione levels are crucial for the body's antioxidant homeostasis [24]. In a recent study, we found that the concentrations of specific glutathione cofactor enzymes, namely glutathione-S-transferase and glutathione peroxidase, are significantly lower, and the levels of plasma malonaldehyde and erythrocytes, which are products of lipid peroxidation of cell membranes, are significantly higher in patients with uterine leiomyoma compared to healthy women [25].

Thus, today studies have particular importance that will allow combining information about the state of angiogenesis and the antioxidant system of the body of women with uterine leiomyoma and clinical complaints. Therefore**, the aim of the study** was to establish the relationship between complaints, melatonin levels and some indicators of the state of the antioxidant system and angiogenesis in women with uterine leiomyoma.

MATERIAL AND METHODS. We selected 60 women of reproductive age (study group). Diagnosis, examination, treatment was carried out in accordance with the Order of the Ministry of Health of Ukraine dated 25.01.2023. No. 147, standard of medical care "Uterine leiomyoma". The control group consisted of 20 healthy women of reproductive age. The study was approved by the Commission on Biological and Medical Ethics of the Higher State Educational Institution of Ukraine "Bukovinian State Medical University" (Minutes No. 4 of December 22, 2020) and was conducted in strict accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects. All patients signed an informed consent.

The groups were parity in age and social status. The age of women in the experimental group ranged from 27 to 46 years and averaged 36.3 ± 1.8 years, the age of patients in the control group ranged from 24 to 45 years and averaged 35.6 ± 2.9 years (p = 0.20 according to the t-test), which indicated statistical homogeneity of the groups by age. Women with severe extragenital pathology were not included in the study.

Determination of melatonin levels in the blood serum of the examined patients was carried out by enzyme-linked

immunosorbent method using the Human MT (Melatonin) ELISA Kit reagent kit (manufactured by Wuhan Fine Biotech Co. Ltd, China) and the automatic analyzer Maritizer EiaQuant (manufactured by Merik Diagnistic LTD, India). The level of melatonin in the blood of the examined women was established once at the beginning of the study, on an empty stomach, venipuncture was performed at 8 o'clock in the morning.

The concentration of vascular endothelial growth factor was studied using the enzyme-linked immunosorbent kit Human VEGF (Vascular Endothelial Cell Growth Factor) ELISA Kit (manufactured by Wuhan Fine Biotech Co. Ltd, China). Determination of the content of reduced glutathione in the blood plasma of the examined women was carried out according to the method of Travina O.V. [26].

Statistical processing was performed using the MedCalc software package (Ostende, Belgium). The data are presented as an arithmetic mean with the standard deviation for each sample. Comparisons between groups were carried out using the t-test for unequal samples. Calculations of logistic regression between qualitative (presence or absence of clinical symptoms described below) and quantitative (listed biochemical parameters) parameters were carried out. The results were considered to be statistically significant at $p<0.05$.

RESULTS AND DISCUSSION. The distribution of complaints in patients of the examined groups is shown in Table 1. As expected, women diagnosed with uterine leiomyoma were significantly more likely to have complaints such as pain during menstruation, profuse spotting during menstruation (more than three pads with maximum absorption capacity per day for at least five days), menstruation lasting longer than 7 days, the presence of intermenstrual spotting, the presence of discomfort in the hypogastrium, constipation, frequent urination, and irregular menstruation. It is clear that in women diagnosed with symptomatic uterine leiomyoma, the frequency of such complaints is higher than in healthy individuals. The presence or absence of the complaints listed in Table 1 was used by us as a qualitative parameter for calculating logistic regression, taking into account the studied biochemical parameters.

As for the biochemical parameters we studied, we found that the level of melatonin in the blood plasma of patients with uterine leiomyoma was significantly lower compared to the level of this hormone in healthy women: 111.01 ± 18.5 ng/ml (in the control group 153.5 ± 8.5 ng/ml, $p<0.01$). The difference was 27.6 percent.

Figure 1 shows a graphical representation (in the form of a comparative diagram) of the difference in melatonin concentrations between the experimental and control groups of the examined patients. Thus, in patients of reproductive age with uterine leiomyoma, melatonin had a much lower antioxidant and membrane-protective effect compared to healthy women.

The results of research of reduced glutathione levels in the blood plasma of women included in the study groups are given below. We found that the plasma concentrations of reduced glutathione in women with leiomyoma were significantly lower compared to healthy women (0.77 ± 0.13 μmol/L versus 1.02 ± 0.14 μmol/L in the control group, $p<0.01$, Figure 2). The difference was 24.5 percent.

As a result of the study of the concentrations of vascular endothelial growth factor VEGF in the blood plasma of women included in the examination groups, the following has been found: the level of VEGF in patients with uterine leiomyoma was significantly (2.58 times) higher (90.4 ± 23.6 pg/ml) compared to the control group (35.1 ± 8.3 pg/ml, $p<0.01$). This may indicate the activation of angiogenesis in patients with leiomyoma, which results in proliferation in the tissue of the myomatous node. Figure 3 shows a comparative diagram of VEGF concentrations in the experimental and control groups of the women we examined.

To investigate the relationships between the studied biochemical parameters and clinical signs, we used the methodology of constructing an "error curve", also known as the ROC-curve. This technique is widely used in clinical practice to make a diagnosis on a yes/no basis ("1/0", diagnosis or clinical sign is present/absent). This methodology can be used to predict the occurrence of a disease or the appearance of a certain symptom, describing the influence of the studied quantitative parameter on the occurrence of a discrete indicator. In the case of the established impact, the threshold value of the quantitative indicator is determined [27]. We were able to establish relationships between the studied biochemical parameters in the blood plasma of patients with uterine leiomyoma and the presence of the described clinical signs in them.

Table 1. **The occurrence of complaints in women with uterine leiomyoma**

	Study group (n=60)	Control group (n=20)
Pain during menstruation, % (n)	83.3 (50) ***	15 (3)
Profuse spotting during menstruation, % (n)	51.7 (31) ***	0
Menstruation lasting longer than 7 days, % (n)	25.0 (15) **	0
Presence of discomfort in the hypogastrium % (n)	18.3 (11) *	0
Presence of intermenstrual spotting, % (n)	63.3 (38) ***	0
Frequent urination, % (n)	28.3 (17) **	0
Constipation, % (n)	30.0 (18) **	0
Irregular menstruations, % (n)	20.0 (12) *	5 (1)

Notes: 1. *– $p<0.05$ compared to the control group, ** – $p<0.01$ compared to the control group, *** – $p<0.001$ compared to the control group.

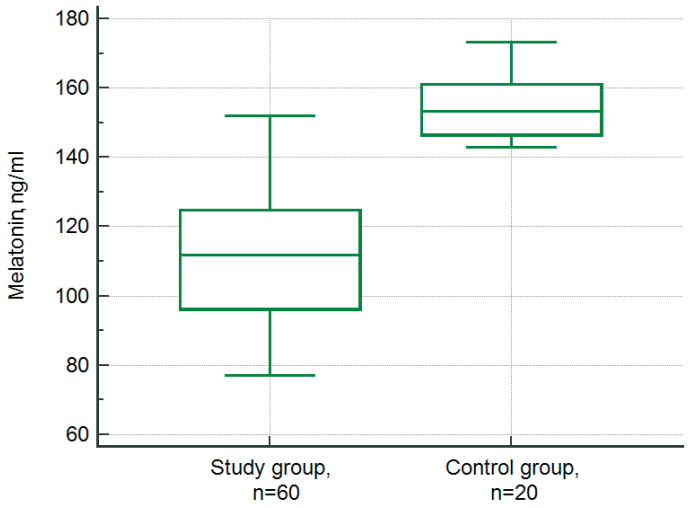

Figure 1. Comparative diagram of melatonin concentrations in the blood plasma of women with uterine leiomyoma and practically healthy women.

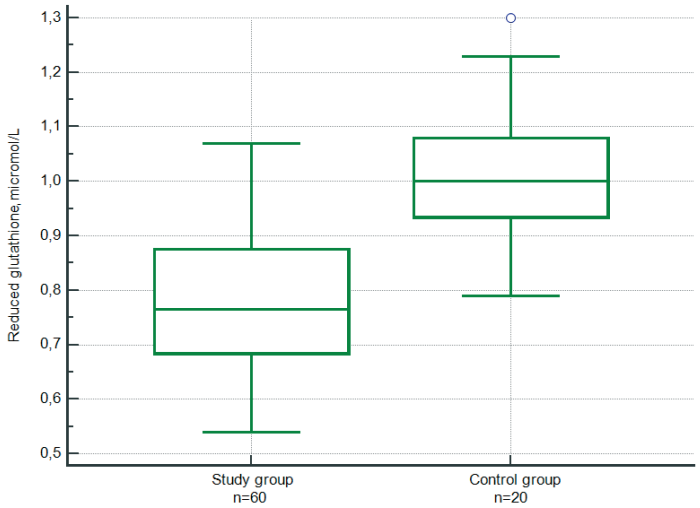

Figure 2. Comparative diagram of reduced glutathione concentrations in the blood plasma of women with uterine leiomyoma and practically healthy women.

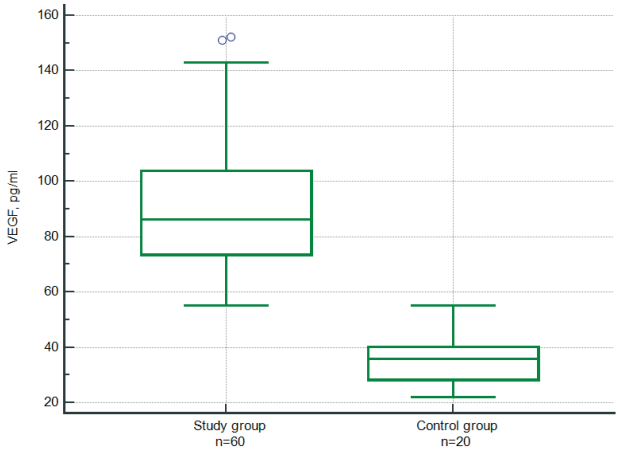

Figure 3. Comparative diagram of the concentrations of vascular endothelial growth factor VEGF in the blood plasma of women with uterine leiomyoma and practically healthy women.

Parameters of the model "Melatonin in blood plasma – pain during menstruation" in patients of the experimental group (Figure 4):

- area under the AUC curve: 0.770,
- standard error: 0.079,
- reliability criterion: p = 0.0007,

associated threshold level for melatonin: ≤ 100.0 ng/ml,
sensitivity: 82.00%,
specificity: 80.17%.

Parameters of the model "Reduced glutathione in blood plasma – profuse spotting" in women with uterine leiomyoma (Figure 5):

- area under the AUC curve: 0.721,
- standard error: 0.0722,
- reliability criterion: p = 0.068,
- associated threshold level for reduced glutathione: >0.78 μmol/L,
- sensitivity: 66.67%,
- specificity: 80.17%.

Parameters of the model "VEGF in blood plasma – miscarriages in anamnesis" in women with uterine leiomyoma (Figure 6):

- area under the AUC curve: 0.896,
- standard error: 0.0685,

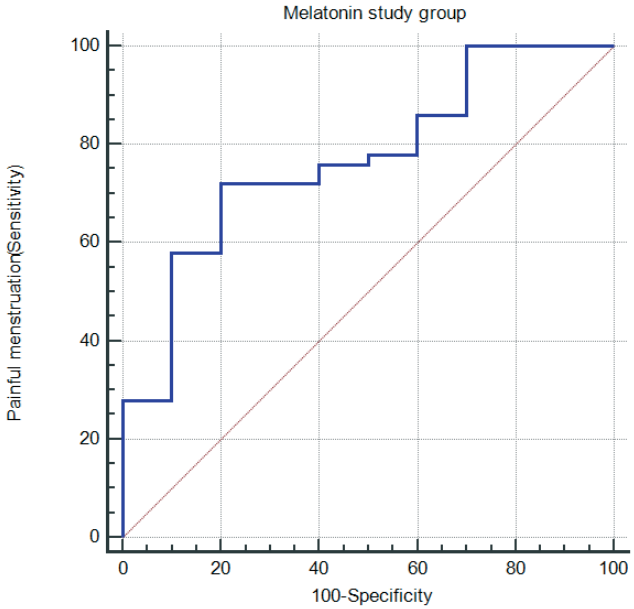

Figure 4. ROC curve of a model describing the relationship between the concentration of melatonin in the blood plasma of women of reproductive age with uterine leiomyoma and the presence of complaints of pain during menstruation.

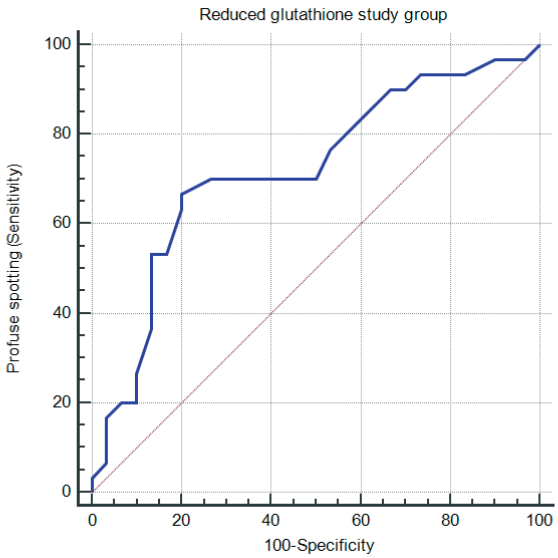

Figure 5. ROC curve of a model describing the relationship between the level of reduced glutathione in the blood plasma of women of reproductive age with uterine leiomyoma and the presence of complaints of profuse spotting during menstruation.

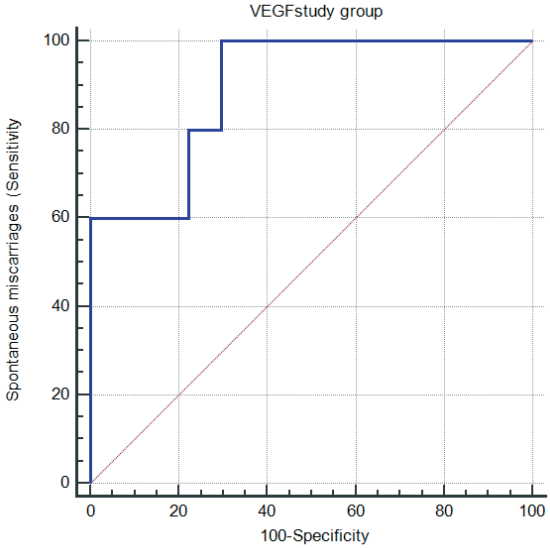

Figure 6. ROC curve of a model describing the relationship between the level of VEGF in the blood plasma of women of reproductive age with uterine leiomyoma and their history of spontaneous miscarriages.

- reliability criterion: $p < 0.0001$,
- associated threshold level for VEGF: > 96.13 pg/ml,
- sensitivity: 100.00%,
- specificity: 70.37%.

Our research allowed us to establish the following. Firstly, in patients with uterine leiomyoma, the concentration of melatonin in the blood plasma significantly decreases. We took the sampling at 8 a.m., when the daily activity of the pineal gland is minimal, to reduce the impact of daily melatonin fluctuations on the interpretation of our results. The consequence of such a decrease is the insufficient performance of melatonin's physiological functions: antioxidant function (cleansing from free radicals) [28, 29] and antiangiogenic function, when melatonin competitively binds to VEGF receptors, preventing excessive angiogenesis provoked by hypoxia inside the leiomyomatous node [13]. Secondly, another important parameter of the body's antioxidant defence system – the concentration of reduced glutathione in blood plasma – is also significantly lower in women with uterine leiomyoma compared to healthy women. Thirdly, in patients with leiomyoma, there is a pathological activation of angiogenesis, which was manifested in a significant increase in the average concentration of VEGF in the blood plasma of patients included in the study group compared to the control. These changes in biochemical parameters provoke oxidative stress in the body of patients with fibroids and the growth of leiomyomatous nodes, which affects the clinical picture [30]. Our research, namely, the construction of ROC curves based on logistic regression, allowed us to reveal more deeply the relationships between the quantitative (biochemical) and qualitative (clinical) parameters of a disease such as uterine leiomyoma. As a result, we were able to establish that when the level of melatonin in the blood plasma decreases below the threshold value of 100.0 ng/ml in women of reproductive age with uterine leiomyoma, the probability of painful menstruation is 82%, and with an increase in the concentration of reduced glutathione to 0.78 μmol/L or more, the chance of profuse spotting during menstruation is approximately 67%. In our opinion, in this case, the cut-off indicator of reduced glutathione is the evidence of the tension of the compensatory mechanisms of the body's antioxidant system. Finally, we would like to note that the level of VEGF in the blood plasma of women with leiomyoma below 96.13 pg/ml, according to our data, is almost always associated with the presence of spontaneous miscarriages (abortions) in the anamnesis of such patients. We can say that this is not a prognostic, but a retrospective sign, but this fact needs to be studied as thoroughly as possible.

Conclusions. 1. In patients with uterine leiomyoma, there was a reduced activity of the antioxidant system compared to healthy women: in the experimental group, the average plasma melatonin level was significantly lower ($p<0.01$) compared to the control group (the difference was 27.6 percent), as well as the average plasma level of reduced glutathione (the difference was 24.5 percent at $p<0.01$, compared to the control).

2. In the group of women diagnosed with uterine leiomyoma, there was a significant (2.58-fold) increase in the average concentration of VEGF in the blood plasma compared to healthy women ($p<0.01$), which indicates the activation of angiogenesis in this disease.

3. With a decrease in the level of melatonin in the blood plasma below the threshold value of 100.0 ng/ml in women of reproductive age with uterine leiomyoma, the probability of painful menstruation is 82%, and with an increase in the concentration of reduced glutathione above 0.78 μmol/L, the chance of profuse bleeding during menstruation is approximately 67%. The level of VEGF in the blood plasma of women with leiomyoma above 96.13 pg/ml, according to our data, is almost always associated with the presence of spontaneous miscarriages in the anamnesis of such patients.

Prospects for further research are to study the pathophysiological impact of melatonin on the processes of angiogenesis and lipid peroxidation in uterine leiomyoma, as well as to study the effectiveness of oral melatonin administration to women with leiomyoma.

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