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# Levels of melatonin and some indicators of angiogenesis, antioxidant system and lipid peroxidation in blood plasma in women with uterine leiomyoma



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## ABSTRACT

Uterine leiomyoma is one of the most common gynaecological diseases, which often leads to loss of fertility. It is known that in this pathology, damage to the tissues of the uterus is accompanied by oxidative stress, and the mechanisms of its compensation play a decisive role in the process of myometrium regeneration, especially when performing organ-preserving operations.

**THE AIM OF THE STUDY** was to determine the levels of melatonin, reproductive hormones and state of angiogenesis, antioxidant system and lipid peroxidation in women of reproductive age who were diagnosed with uterine leiomyoma.

**MATERIAL AND METHODS.** 60 women of reproductive age with uterine leiomyoma (study group) were examined. The control group consisted of 20 healthy women of the same age group. Melatonin levels in women's blood were determined once, on an empty stomach, at 8 a.m.; the concentrations of estradiol and progesterone in the follicular phase of the menstrual cycle were also measured. The state of the angiogenesis system was studied by examining the levels of vascular endothelial growth factor (VEGF) and the content of the final metabolites of nitric oxide NO in blood plasma. The activity of the antioxidant system was assessed by blood plasma concentrations of reduced glutathione, glutathione peroxidase, and glutathione transferase. Indicators of lipid peroxidation were investigated by the content of malondialdehyde (MDA) in blood plasma and erythrocytes.

**RESULTS.** It was found that in the patients of the study group with leiomyoma, there was a significantly lower level of melatonin in blood plasma ( $111.1 \pm 18.5$  ng/mL) compared to the control group ( $153.5 \pm 8.5$  ng/mL), while the concentration of estradiol was almost three times higher ( $107.4 \pm 25.3$  pg/mL) compared to the control group ( $36.2 \pm 3.2$  pg/mL), and the concentration of progesterone was 1.9 times higher ( $2.1 \pm 0.4$  ng/mL compared to  $1.1 \pm 0.5$  ng/mL in the control group). The level of VEGF in blood plasma in women with uterine leiomyoma was also higher ( $90.4 \pm 23.6$  pg/mL) compared to the control group ( $35.1 \pm 8.3$  pg/mL), as well as the concentration of final metabolites of nitric oxide, which reached  $25.3 \pm 5.9$  pg/mL compared to  $9.9 \pm 3.9$  pg/mL in the control group. The reduced glutathione level in the blood plasma of women with uterine leiomyoma was significantly lower ( $0.77 \pm 0.13$   $\mu$ mol/L) compared to healthy women ( $1.02 \pm 0.14$   $\mu$ mol/L) in the control group, while the concentrations of glutathione-S-transferase and glutathione peroxidase enzymes were higher ( $161.3 \pm 22.3$  ng/mL and  $235.7 \pm 35.9$  ng/mL, respectively), whereas in the control group these indicators were  $118.9 \pm 18.0$  ng/mL and  $105.3 \pm 41.2$  ng/mL, respectively. The MDA content in women of the study group was higher, measuring  $5.2 \pm 0.8$  nmol/L in plasma and  $10.8 \pm 1.1$  nmol/L in erythrocytes compared to  $2.3 \pm 0.8$  nmol/L and  $5.3 \pm 0.8$  nmol/L in the control group, respectively.

**CONCLUSIONS.** The levels of melatonin and reduced glutathione in the blood plasma of women with uterine leiomyoma were significantly lower, while the concentrations of estradiol, progesterone, glutathione-S-transferase, glutathione peroxidase, VEGF, final metabolites of nitric oxide, as well as the average MDA content in plasma and erythrocytes, were significantly higher compared to healthy women. In the study group, the relationship between the level of progesterone in blood plasma and the content of malondialdehyde in erythrocytes was described using a linear regression equation, which, as we suggest, indicates an activating effect of progesterone on oxidative stress mechanisms in the myometrium. In our opinion, oxidative stress in uterine leiomyoma occurs due to inadequate activity of the antioxidant system, an integral part of which is melatonin, leading to enhanced angiogenesis against the background of estrogen-induced myometrial proliferation.

**KEY WORDS:** uterine leiomyoma; melatonin; estradiol; progesterone; vascular endothelial growth factor (VEGF); malondialdehyde (MDA); oxidative stress

Uterine leiomyoma is one of the most common neoplasms of the female reproductive system. This is the most common benign proliferative pathology, which accounts for up to 30 % of gynaecological diseases, ranking second after pelvic inflammatory processes. There is evidence that the incidence of uterine leiomyoma (symptomatic and asymptomatic forms) reaches 50-70 % in the population of white Caucasian women and up to 80 % in the population of black women [1].

Uterine leiomyoma arises in the myometrium, from smooth muscle tissue and consists of randomly located myofibroblasts that are buried in the extracellular matrix, which makes up a significant part of the tumour volume, regardless of the presence or absence of a fibroblastic component in the tumour nodes and the level of hormone dependence [2]. One of the main factors in pathological cell growth in uterine leiomyoma is the level of estrogens [3]. By localization, uterine leiomyoma is most often classified into subserous (outside the uterus), intramural (inside the myometrium) and submucosal (in the uterine cavity) [3]. Despite the fact that uterine leiomyoma is a benign tumour, often asymptomatic, which impairs timely diagnosis and treatment, patients may experience a number of unpleasant clinical symptoms, including menstrual disorders – irregular, prolonged and heavy menstrual bleeding; iron deficiency anaemia; dysmenorrhoea; pelvic pressure and pain; urinary incontinence, frequent urination; infertility (primary infertility occurs in 18-24 % of patients, and secondary – in more than 25 % of cases); early and recurrent miscarriages [3].

Leiomyoma of the uterus is undoubtedly a hormone-dependent tumour. It is believed that in the early stages, its development occurs with the involvement of physiological fluctuations in the concentration of hormones during the menstrual cycle [4]. The modern model of pathogenesis of uterine leiomyoma includes the genetic transformation of myometrial stem cells into tumour cells that initiate and maintain the growth of clonal cells in foci of local growth; each of these foci is an increase in the size and number of smooth muscle cells and excessive production of the extracellular matrix under the influence of endocrine, autocrine and paracrine growth factors and hormonal receptor signalling [5]. The tissues formed in this way trigger local mechanisms to support tumour growth, primarily through local production of estrogens from androgens and excessive formation of connective tissue [4-6].

However, even now the exact pathophysiological mechanisms of the formation and subsequent growth of uterine leiomyoma remain not fully understood. There is evidence that under the influence of certain factors (possibly infectious inflammation) in the myometrial tissue, there is an overexpression of receptors for steroid female hormones: estrogens and progesterone, which causes the activation of several signalling pathways that trigger growth, proliferation and angiogenesis with the participation of mitogen-activated protein kinase (MEK) and extracellular strongly-regulated kinase (ERK) [3]. Both estradiol and progesterone promote the proliferation of uterine leiomyoma. Recent studies have demonstrated that there is a relationship between the signalling pathways of progesterone and estrogen, suggesting that both hormones are involved in the development of uterine leiomyoma [4, 7, 8]. However, more research is needed to fully understand the mechanism of steroid hormone signalling to the cells and extracellular structures that form uterine leiomyoma and to elucidate the interaction between different pathways and their role in the etiopathogenesis of this disease.

In recent years, a lot of studies have been published in which the classical view of the development of uterine leiomyoma as an exclusively hormone-dependent (steroid-dependent) process is refuted. Those links in the pathogenesis of this disease, which were previously omitted by researchers, are being actively studied. In particular, in the development of pathological changes in the myometrium, the role of oxidative stress and antioxidant mechanisms is specified, which is generally a classic component of the pathogenesis for destructive and hyperplastic disorders in tissues [9-12]. Under normal conditions, the processes of tissue kinetics include free radical reactions, which under pathological conditions lead to their disorders. By oxidation, reactive oxygen species (ROS)

with a short life cycle are formed; they are neutralized by the antioxidant defense system [11]. When the defense mechanisms are violated, free radicals damage DNA strands and lead to cellular changes and pathological proliferation, as well as to the processes of disruption of the activity of the enzymatic link of the antioxidant system. These enzymes include superoxide dismutase, glutathione peroxidase, glutathione transferase, catalase, etc. There is an accumulation of lipid peroxidation products of cell membranes, in particular, malondialdehyde (MDA) in plasma and erythrocytes; an increase in the level of this metabolite in the blood indicates oxidative stress of tissues [13].

Redox signalling plays an important role in regulating cell death and survival. At major levels ROS function as signals that promote cell proliferation and survival, while the accumulation of ROS induces apoptosis by damaging cellular components and simultaneously enhancing antioxidant pathways to maintain redox homeostasis. In recent years, there has been growing evidence that stimulation of ROS or inhibition of antioxidants can be a promising therapeutic strategy for the treatment of neoplasms, which include uterine leiomyoma [14].

Another important growth factor that stimulates endothelial cell proliferation is Vascular Endothelial Growth Factor (VEGF), whose function promotes cell migration and proliferation; this factor binds to specific receptors, enhancing angiogenesis, which is crucial for the occurrence of fibroids [15]. A systematic meta-analysis was conducted with the inclusion of 15 relevant studies and the following observations were obtained: the expression of VEGF in the endometrium was increased in patients with fibroids. From these data, it can be concluded that abnormal angiogenesis is present in such patients, which potentially includes impaired maturation of blood vessels, which leads to the formation of immature and fragile blood vessels [16].

VEGF is a key activator of endothelial cell function and is involved in all stages of the angiogenesis cascade, including migration and proliferation; in addition, VEGF also increases the expression of matrix metalloproteinases and plasminogen activators, which degrade the extracellular matrix [17]. As a result, in patients with uterine leiomyoma a greater number of “venous lakes” are formed in the pathological focus compared to patients without this tumour. This is also a sign of the pathological state of blood vessels, in particular, their abnormal dilatation [18]. It is important to note that melatonin has the property of inhibiting VEGF-induced angiogenesis by preventing the binding of vascular endothelial growth factor to its own receptors [19-21]; in addition, melatonin triggers programmed cell death in leiomyoma foci [22].

Reactive oxygen species can be divided into two groups: free radicals and non-radicals. Free radicals are molecules that contain one or more unpaired electrons, which give them high reactivity. ROS, which share unpaired electrons, are non-radical forms [23]. It is known that reactive oxygen species formed because of ischemic-reperfusion tissue damage have a negative effect on the condition of the organ after transplantation, in particular, in case of uterine transplantation [24]. There is also evidence that in patients who have undergone operative treatment for uterine leiomyoma (conservative myomectomy), there is an increase in blood indicators characteristic of oxidative stress, in particular, the end products of oxidation of proteins and protein carbonyls [25], which, certainly, affects tissue regeneration after surgical intervention. Regarding the relationship between angiogenesis and oxidative stress in uterine tissues, the following is known: in a study on rats it was found that the addition of the antioxidant acetyl-L-carnitine to the solution in which the donor's uterus is stored during its transplantation reduces the expression of VEGF-2 in all layers of the organ and prevents the formation of free radicals [26]. However, the current approach is mainly concerned with signalling pathways and internal regulation of cells; therefore, further research is needed to achieve a complete understanding of the role of oxidative stress in transplantation practice and ways to reduce it to protect transplanted organs.

The presented latest data on the pathogenesis of uterine leiomyoma development led to the choice of the investigated indicators for our study.

Since data on oxidative stress and its compensation are important in the context of myometrial regeneration in organ-preserving surgeries, **THE AIM OF THE STUDY** was to determine the levels of melatonin, reproductive hormones, the state of the antioxidant system and lipid peroxidation in women of reproductive age who were diagnosed with uterine leiomyoma.

## MATERIALS 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 Bukovinian State Medical University (Minutes № 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 appropriate informed consent.

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

Serum melatonin levels of the examined patients were determined by enzyme-linked immunoassay method using the Human MT (Melatonin) ELISA Kit (Wuhan Fine Biotech Co. Ltd, China) and the automatic analyzer Maritizer EiaQuant (Merik Diagnostik LTD, India), which was also used for all other enzyme-linked immunosorbent assays performed. The levels of melatonin in the women's blood were established once, on an empty stomach, venepuncture was performed at 8 o'clock in the morning.

Vascular endothelial growth factor concentrations were studied using the Human VEGF (Vascular Endothelial Cell Growth Factor) ELISA Kit (Wuhan Fine Biotech Co. Ltd, China). The method for determining the content of final stable metabolites of nitric oxide in the blood was based on the reduction of nitrates to nitrites with the determination of the nitrites by reaction with the Griss reagent. Optical density was measured on the SF-46 spectrophotometer at a wavelength of 540 nm. The calculation of the level of nitrites was carried out according to a calibration graph based on nitrogen nitrite [27].

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. [28]. To measure the concentration of glutathione peroxidase in the blood of women included in the study groups, the Human GPX1 (Glutathione peroxidase 1) ELISA Kit (Wuhan Fine Biotech Co. Ltd, China) was used, and for the study of glutathione transferase levels – the Human GSTθ1/GSTt1 (Glutathione S Transferase Theta 1) ELISA Kit (Wuhan Fine Biotech Co. Ltd, China) was applied. Both diagnostic kits are based on sandwich-type enzyme-linked assay technology. To determine the concentrations of malondialdehyde in the blood plasma of the examined patients, the Human MDA ELISA Kit (Wuhan Fine Biotech Co. Ltd, China) based on the Competitive-ELISA enzyme-linked immunosorbent technique was used.

To determine the levels of steroid reproductive hormones in the blood of patients included in the examination groups, venous blood sampling was carried out in the follicular phase of the menstrual cycle. Diagnostic kits "MAGLUMI Estradiol", "MAGLUMI Progesterone" (Snibe, China) were applied. Measurements of the concentrations of these steroid hormones were carried out by chemiluminescence immunoassay *in vitro* using the full automated chemiluminescence immunochemiluminescence analyzer of the MAGLUMI 1000 series (Snibe, China). Blood sampling by venepuncture was carried out at 8 a.m. on the 5<sup>th</sup> day of the menstrual cycle.

Statistical processing was performed using the MedCalc software package (Ostende, Belgium). The data are presented as an arithmetic mean with an indication of the standard deviation for each sample. Comparisons between groups were made using the t-test for unequal samples. Linear regression equations were also derived to establish the relationships between the indicators studied. The results were considered to be significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

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:  $111.01 \pm 18.5$  ng/mL (in the control  $153.5 \pm 8.5$  ng/mL,  $p < 0.01$ ). The difference was 27.6 percent (Fig. 1).

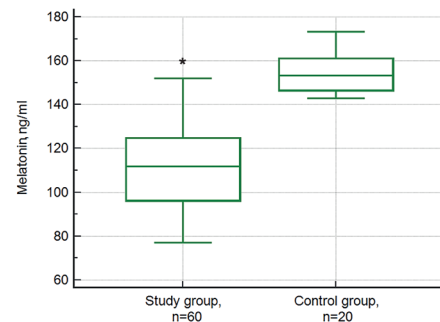


Fig. 1. Comparative diagram of melatonin concentrations in the blood plasma of women with uterine leiomyoma and healthy women. Note: \* –  $p < 0.01$  compared to the control group.

We suggest that in patients of reproductive age with uterine leiomyoma included in the study group, melatonin had a significantly lower antioxidant and membrane-protective effect compared to women included in the control group.

The results of the study of angiogenesis indicators are as follows: the plasma content of vascular endothelial growth factor (VEGF) in the group of patients with uterine leiomyoma was significantly (2.7 times) higher compared to the control:  $90.4 \pm 23.6$  pg/mL (in the control group  $35.1 \pm 8.3$  pg/mL,  $p < 0.01$ , Fig. 2), and the plasma concentration of the final metabolites of nitric oxide NO was higher in the study group by 2.6 times compared to the control group ( $25.3 \pm 5.9$  pmol/L, in the control group  $9.9 \pm 3.9$  pmol/L,  $p < 0.05$ , Fig. 3). Quantitative changes in the studied angiogenesis indicators, in our opinion, testify to an increase in the formation of new blood vessels in patients with uterine leiomyoma against the background of increased secretion of nitric oxide NO by the endothelium, which leads to local vasodilation in the focus of myometrial hyperproliferation.

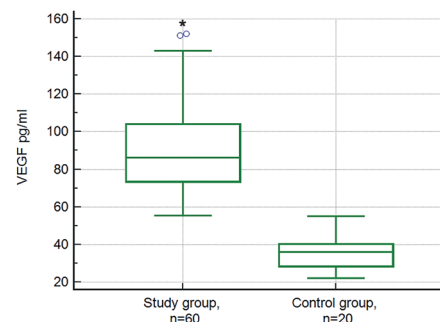
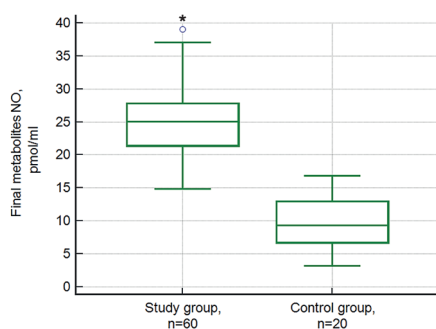


Fig. 2. Comparative diagram of the content of vascular endothelial growth factor in the blood plasma of women with uterine leiomyoma and healthy women. Note: \* –  $p < 0.01$  compared to the control group.



**Fig. 3.** Comparative diagram of the content of final metabolites of nitric oxide NO in the blood plasma of women with uterine leiomyoma and healthy women. Note: \* –  $p < 0.05$  compared to the control group.

Among the indicators of the antioxidant protection system and lipid peroxidation that we studied, which are represented in **Table 1**, the changes in the content of MDA in the blood plasma and erythrocytes deserve the closest attention. We found that both in the blood plasma and in erythrocytes of women of reproductive age who were diagnosed with uterine leiomyoma, the average content of MDA was significantly higher (more than 2 times) compared to the control group at  $p < 0.01$ .

**Table 1.** Indicators of lipid peroxidation in the blood plasma of women with uterine leiomyoma (Mean  $\pm$  SD).

	Study group (n = 60)	Control group (n = 20)
Glutathione reduced, $\mu\text{mol/L}$	0.77 $\pm$ 0.13*	1.02 $\pm$ 0.14
Glutathione-S-transferase, ng/mL	161.3 $\pm$ 22.3*	118.9 $\pm$ 18.0
Glutathione peroxidase, ng/mL	235.7 $\pm$ 35.9*	105.3 $\pm$ 41.2
Plasma MDA, nmol/L	5.2 $\pm$ 0.8*	2.3 $\pm$ 0.8
MDA in erythrocytes, nmol/L	10.8 $\pm$ 1.1*	5.3 $\pm$ 0.8

Note: \* –  $p < 0.01$  compared to control group. MDA – malondialdehyde.

As already mentioned, MDA is a product of the process of lipid peroxidation of cell membranes. An increase in the content of this metabolite in the blood plasma and in erythrocytes, in our opinion, indicates the activation of this process in patients diagnosed with uterine leiomyoma, which is undoubtedly a sign of oxidative stress.

We also found that the concentration of reduced glutathione, the main component of the body's antioxidant system, was significantly lower in the study group compared to the control group against the background of higher levels of glutathione-S-transferase and glutathione peroxidase (at  $p < 0.01$ ).

In order to comprehensively study the links in the pathogenesis of uterine leiomyoma, we also measured the levels of steroid hormones (estradiol and progesterone) in patients with uterine leiomyoma and in healthy women included in the control group. The results are presented in **Table 2**. It was found that in the blood of women with uterine leiomyoma, the level of estradiol was almost three times higher, and the level of progesterone was 1.9 times higher, compared to healthy patients included in the control group.

**Table 2.** Estradiol and progesterone concentrations measured in blood plasma of women with uterine leiomyoma (Mean  $\pm$  SD).

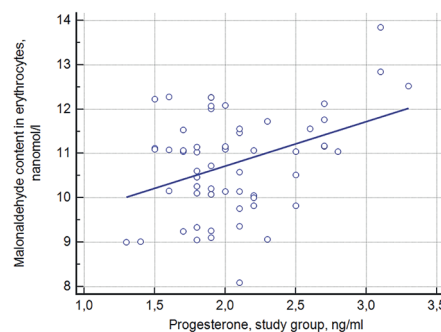
	Study group (n = 60)	Control group (n = 20)
Estradiol, pg/mL	107.4 $\pm$ 25.3*	36.2 $\pm$ 3.2
Progesterone, ng/mL	2.1 $\pm$ 0.4*	1.1 $\pm$ 0.5

Note: \* –  $p < 0.001$  compared to control group.

We were also able to derive a regression equation describing the relationship between the plasma concentration of progesterone in patients with uterine leiomyoma and the content of MDA in their red blood cells. The equation is as follows:

$$y = 8.7268 + 0.9970 x$$

where  $x$  is the concentration of progesterone in blood plasma (ng/mL),  $y$  is the content of MDA in erythrocytes ( $p = 0.003$ ). **Fig. 4** shows a scatter plot with a regression line for the derived equation.



**Fig. 4.** Scatter plot with a regression line describing the relationship between the blood plasma concentrations of progesterone in women with uterine leiomyoma and the content of malondialdehyde in red blood cells.

Considering our results in the context of oxidative stress against the background of a benign hyperproliferative process, which is uterine leiomyoma, we can note the following. First, it is known that oxidative stress is an imbalance between pro-oxidants and antioxidants [29], and this imbalance triggers the processes of pathophysiological cascades involving angiogenesis and hypoxia [30, 31], which results in the realization of genetic and epigenetic factors that cause the proliferation of smooth muscle cells and formation of fibrous fibers [32–34]. The most important compensatory factors on the part of the woman's body under such conditions should be antioxidant agents such as melatonin and glutathione.

In our study, we found a significantly lower level of the concentration of glutathione in the blood plasma of women of reproductive age with uterine leiomyoma, compared to healthy women. In our opinion, the revealed level of activation of enzyme systems that should have maintained the proper level of glutathione and the implementation of its physiological functions is insufficient to fully compensate for oxidative stress, despite the fact that the indicators of glutathione-S-transferase and glutathione-peroxidase between the groups in our research differed considerably, and concentrations of these enzymes were significantly higher in patients with leiomyoma compared to controls.

Moreover, it should be noted that the level of melatonin in the blood of women of reproductive age diagnosed with uterine leiomyoma was significantly lower (by 27.6 %) compared to this indicator in women of the control group, which, in our opinion, did not allow this hormone to fully exert its antioxidant effect. An even more important physiological function of melatonin in this case is to protect VEGF receptors and prevent excessive angiogenesis [19–21]. Thus, insufficient secretion of melatonin in the body of women with uterine leiomyoma does not allow the compensatory mechanisms associated with the action of this hormone to be fully realized. Under such conditions, according to some authors [35–36], angiogenesis is activated against the background of oxidative stress. Our results, namely, higher plasma concentrations of vascular endothelial growth factor and final metabolites of nitric oxide NO in women with leiomyoma, compared to healthy women, coincide with the above data.

Most researchers agree that angiogenesis against the background of oxidative stress is imperfect, which leads to the formation of a hypoxic environment in the core of the myomatous node, and tissue hypoxia itself is a trigger for angiogenesis by increasing the production of VEGF and activating of other pro-angiogenic factors, such as eukaryotic translation initiation factor 2-alpha (eIF2-alpha) and hypoxia-inducible factor 1-alpha (HIF1-alpha) [36]. In addition, it is known that fibroid cells proliferate better in a hypoxic environment, in contrast to normal smooth muscle cells [37]. The same is true about myometrial stem cells, which proliferate in a hypoxic environment (2 % oxygen), but practically do not proliferate in a normoxic environment (20 % oxygen) [38]. At the same time, leiomyoma is believed to contain a much smaller population of stem cells compared to normal myometrium [39], and leiomyoma stem cells themselves differ from normal myometrial stem cells: in particular, they contain mutations in the MED12 gene, which is present in 70 % of leiomyomas. MED12 is a gene that inhibits tumour growth; in the case of its mutation, the growth of leiomyoma is observed [40]. Importantly, leiomyoma stem cells have a reduced number of estrogen and progesterone receptors, and these cells need mature myometrial cells to proliferate, perceiving activating effects through paracrine mechanisms [41]. The hypoxic environment within the leiomyomatous node increases oxidative stress and proliferation, thus closing the pathophysiological circuit.

In this aspect, the role of steroid reproductive hormones, such as estradiol E2 and progesterone, in the progression of pathophysiological processes remains unclear. This is especially true for progesterone. Estradiol, by activating the proliferation of smooth muscle fibers and the extracellular matrix [3], also contributes to the formation of a hypoxic environment within the leiomyomatous node; and progesterone is known to decrease its level at the end of the luteal phase of the cycle what leads to increasing of the production of active oxygen forms in the endometrium [38]. We found that, despite the lack of a significant difference in progesterone concentrations between the groups in our research, there is a fairly clear relationship between the concentration of progesterone in the blood plasma of patients with uterine leiomyoma and the content of MDA in their erythrocytes, which was confirmed by the linear regression equation we derived. Thus, it can be considered as a fact established by us that the accumulation of membrane lipid peroxidation products is directly associated with changes in progesterone concentrations in patients of reproductive age with uterine leiomyoma.

Thus, the revealed pathophysiological changes, in our opinion, indicate the development of oxidative stress in patients with uterine leiomyoma against the background of a decrease in compensatory mechanisms to counteract this condition.

## CONCLUSION

1. *The levels of melatonin and glutathione in the blood plasma of women of reproductive age with uterine leiomyoma are significantly lower, and the concentrations of estradiol and progesterone are significantly higher compared to healthy women.*
2. *Plasma concentrations of VEGF and final metabolites of nitric oxide NO, as well as the mean plasma content of glutathione-S-transferase and glutathione peroxidase, and also plasma and erythrocyte levels of malondialdehyde are significantly higher in women with uterine leiomyoma compared to control group.*
3. *The relationship between the plasma progesterone level of women with uterine leiomyoma and the malondialdehyde content in their erythrocytes can be described using a linear regression equation, which, as we suggest, indicates the activating effect of progesterone on the mechanisms of oxidative stress in the myometrium.*
4. *In our opinion, oxidative stress in women with uterine leiomyoma occurs with insufficient activity of the antioxidant system, an integral part of which is melatonin, which leads to increased angiogenesis against the background of estrogen-induced myometrial proliferation.*

## PROSPECTIVE

Prospective for further research are to reveal the signalling pathways and molecular mechanisms of the pathophysiological relationship between oxidative stress, changes in melatonin concentrations and angiogenesis processes in women with uterine leiomyoma. These data will be important in the studies of the mechanisms of uterine regeneration after organ-preserving operations in this pathology, as well as in transplantation of this important reproductive organ.

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# Рівні мелатоніну та деяких показників ангиогенезу, антиоксидантної системи та перекисного окиснення ліпідів в плазмі крові у жінок з лейоміомою матки

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## РЕЗЮМЕ

Лейоміома матки – одне з найбільш поширених гінекологічних захворювань, що часто призводить до втрати фертильності. Відомо, що при цій патології ураження тканин матки супроводжується оксидативним стресом, а механізми його компенсації відіграють вирішальну роль в процесі регенерації міометрію, особливо при виконанні органозберігаючих операцій. Тому метою дослідження було визначити рівні мелатоніну, репродуктивних гормонів, стан показників ангиогенезу, антиоксидантної системи та перекисного окиснення ліпідів у жінок репродуктивного віку, у яких діагностовано лейоміому матки.

**МАТЕРІАЛ І МЕТОДИ.** Обстежено 60 жінок репродуктивного віку, хворих на лейоміому матки. Контрольну групу склали 20 практично здорових жінок тієї ж вікової групи. Рівні мелатоніну в крові жінок встановлювалися однократно, натще, о 8-й годині ранку; також вимірювали концентрації естрадіолу та прогестерону у фолікулярній фазі менструального циклу. Стан системи ангиогенезу визначали, досліджуючи рівні фактору росту ендотелію судин VEGF та вміст кінцевих метаболітів оксиду азоту NO в плазмі крові. Активність антиоксидантної системи оцінювали за концентраціями відновленого глутатіону, глутатіон-пероксидази та глутатіон-трансферази в плазмі крові. Показники перекисного окиснення ліпідів досліджували за вмістом малонового альдегіду в плазмі крові та еритроцитах.

**РЕЗУЛЬТАТИ.** Встановлено, що у пацієток дослідної групи вірогідно менші рівні мелатоніну в плазмі крові ( $111,1 \pm 18,5$  нг/мл), порівняно з контрольною групою ( $153,5 \pm 8,5$  нг/мл), тоді як концентрація естрадіолу була більша майже втричі ( $107,4 \pm 25,3$  пг/мл) порівняно з контролем ( $36,2 \pm 3,2$  пг/мл), а концентрація прогестерону – більшою в 1,9 рази ( $2,1 \pm 0,4$  нг/мл проти  $1,1 \pm 0,5$  нг/мл в контрольній групі). Рівень VEGF в плазмі крові при лейоміомі матки також був вищий ( $90,4 \pm 23,6$  пг/мл), порівняно з контрольною групою ( $35,1 \pm 8,3$  пг/мл), як і концентрація кінцевих метаболітів оксиду азоту, що сягала  $25,3 \pm 5,9$  пг/мл при  $9,9 \pm 3,9$  пг/мл в контрольній групі.

Рівень відновленого глутатіону в плазмі крові жінок з лейоміомою був достовірно нижчим, порівняно зі здоровими жінками ( $0,77 \pm 0,13$  мкмоль/л проти  $1,02 \pm 0,14$  мкмоль/л в контрольній групі), а концентрації ферментів глутатіон-S-трансферази та глутатіон-пероксидази – підвищеними до  $161,3 \pm 22,3$  нг/мл та  $235,7 \pm 35,9$  нг/мл відповідно, у той час як в контрольній групі ці показники склали  $118,9 \pm 18,0$  нг/мл та  $105,3 \pm 41,2$  нг/мл, відповідно. Вміст малонового альдегіду у жінок дослідної групи був більшим і складав  $5,2 \pm 0,8$  нмоль/л в плазмі та  $10,8 \pm 1,1$  нмоль/л в еритроцитах проти  $2,3 \pm 0,8$  нмоль/л та  $5,3 \pm 0,8$  нмоль/л в контрольній групі, відповідно.

**ВИСНОВКИ.** У пацієток з лейоміомою матки рівні мелатоніну та відновленого глутатіону в плазмі крові достовірно менші, тоді як концентрації в плазмі крові естрадіолу, прогестерону, глутатіон-S-трансферази та глутатіон-пероксидази, VEGF та кінцевих метаболітів оксиду азоту NO, а також середній вміст малонового альдегіду в плазмі та еритроцитах вірогідно більші, порівняно з практично здоровими жінками. В дослідній групі взаємозв'язок між рівнем прогестерону в плазмі крові та вмістом малонового альдегіду в еритроцитах було описано за допомогою рівняння лінійної регресії, що, як ми вважаємо, свідчить про активуючий вплив прогестерону на механізми оксидативного стресу в міометрії. На нашу думку, оксидативний стрес при лейоміомі матки перебігає за недостатньої активності антиоксидантної системи, складовою частиною якої є мелатонін, що призводить до підсилення ангиогенезу на тлі естроген-індукованої проліферації міометрію.

**КЛЮЧОВІ СЛОВА:** лейоміома матки; мелатонін; естрадіол; прогестерон; VEGF; малоновий діальдегід; оксидативний стрес