НЕОНАТОЛОГІЯ, ХІРУРГІЯ ТА ПЕРИНАТАЛЬНА МЕДИЦИНА Т. XIII, № 2(48), 2023
NEONATOLOGY, SURGERY AND PERINATAL MEDICINE VOL. XIII, № 2(48), 2023
KEY TITLE: NEONATOLOGIÂ, HÌRURGIÂ TA PERINATAL'NA MEDICINA (ONLINE)
ABBREVIATED KEY TITLE: NEONATOL. HÌR. PERINAT. MED. (ONLINE)
ISSN 2226-1230 (PRINT) ISSN 2413-4260 (ONLINE)

UDC: 618.3-06:616.8-009.24-02:612.826.33.015.22
DOI: 10.24061/2413-4260.XIII.2.48.2023.14

EFFECT OF MELATONIN ON THE LEVEL OF CYTOKINES IN PREECLAMPSIA

R.F. Savka, A.M. Berbets, S.H. Pryimak, O.M.Yuzko, D.A. Berbets

Bukovinian State Medical University
(Chernivtsi Ukraine)

Summary

Introduction. *The literature reports a decrease in melatonin levels in preeclampsia, when the degree of decrease correlates with the severity of the process, as well as a decrease in the expression of melatonin receptors in the placental tissue in case of fetal growth restriction.*

Regarding changes in the cytokine profile in PE: there is evidence that the levels of proinflammatory cytokines, namely TNF-α and IL-6, increase in preeclampsia, while the concentrations of anti-inflammatory cytokines, namely IL-4 and IL-10, decrease. Melatonin, on the contrary, reduces the secretion of proinflammatory cytokines, in particular TNF-α, and increases the production of anti-inflammatory cytokines, namely IL-10. Therefore, normalization of melatonin levels may be a promising direction in the treatment of PE in pregnant women.

Objective of the study. *Assess the pathogenetic mechanisms of complications arising from a decrease in melatonin, and, in particular, find out more about the changes in melatonin, IL-6 and IL-10, determine the correlation between them.*

Material and research methods

The study conducted at the Department of Pregnancy Pathology of the Chernivtsi Regional Perinatal Center, involved 32 women whose pregnancy was complicated by preeclampsia. The control group consisted of 33 women with uncomplicated pregnancies observed in the antenatal clinic of the Chernivtsi Regional Perinatal Center.

The patient's informed consent form and the patient's examination card were approved by the Biomedical Ethics Committee of Bukovinian State Medical University (BSMU) of the Ministry of Health of Ukraine (Chernivtsi). The work was guided by the general provisions of the Declaration of Helsinki "Recommendations for Physicians for Biomedical Research Involving Human Subjects" (1964), the World Medical Association on Ethical Principles for Scientific Medical Research Involving Human Subjects (1964-2000), considering the requirements of Directive 2001/20/EC of the European Parliament and of the Council, ICH GCP, the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), Order of the Ministry of Health of Ukraine No. 690 of 23.09.2009.

Statistical data were calculated using MedCalc software developed by MedCalc Software (Ostend, Belgium). The results were evaluated using the Mann-Whitney U-test for small groups. The P value of <0.05 was considered valid. Correlation and regression statistical evaluations were also performed using the aforementioned software.

R&D «Preservation and restoration of reproductive health of women and girls in obstetric and gynecological pathology». State registration number: 0121U110020. Terms of execution: 01.2021–12.2025 yy.

Research results. *The level of melatonin in venous blood taken from women with diagnosed PE was significantly lower (p=0.029), as well as levels of placental growth factor PlGF (p<0.0001), compared to healthy women. In our study we also found that the concentrations of both proinflammatory IL-6 and anti-inflammatory IL-10 were elevated in women with PE compared to women with uncomplicated pregnancies. We performed a prognostic assessment of the impact of the biochemical parameters we studied on the outcome of pregnancies in the examined patients. Melatonin, if present in the blood of a pregnant woman in a concentration higher than the threshold values we established (6.71 pg/ml), reduces the chances of clinical manifestations of severe preeclampsia with a high degree of reliability (p=0.00173). We assume that placental dysfunction in particular leads to impaired synthesis of the antioxidant melatonin, which causes disorders in the tissues of the mother, placenta and fetus and, as a result, provokes clinical manifestations of preeclampsia. The condition of the placenta also worsens the subclinical inflammatory process, as evidenced by an increase in IL-6 levels in patients with diagnosed preeclampsia; an increase in IL-10 in the study group, in our opinion, is a kind of compensatory reaction that prevents preterm labour. In women with pregnancies complicated by preeclampsia, melatonin is a prognostic criterion for delivery before 38 weeks of gestation, and this delivery is mainly associated with the progression of preeclampsia to its severe stage. In the group of patients with uncomplicated pregnancy, proinflammatory interleukin-6 is predictive of spontaneous onset of labour before 38 weeks of gestation. Placental growth factor PlGF was a predictor of the birth of children with a body weight of less than 3000 g in both study groups.*

Conclusions. *Concentrations of melatonin and of placental growth factor in venous blood in women whose pregnancy was complicated by preeclampsia in the third trimester of pregnancy are significantly lower compared to uncomplicated pregnancies. On the other hand, the levels of proinflammatory IL-6 and anti-inflammatory IL-10 were significantly increased in the group of patients with diagnosed preeclampsia compared with normal pregnancy.*

Key words: *Melatonin; PlGF; Cytokines; Preeclampsia.*

Introduction

Preeclampsia (PE) is a systemic disease of the mother-placenta-fetus system typical for pregnancy [1]. PE is characterized by the appearance of arterial hypertension, proteinuria, which is first detected during pregnancy after 20 weeks, as well as damage to other organs, primarily the kidneys, liver dysfunction, changes in blood count, and often fetal growth restriction [2]. A key link in the pathogenesis of preeclampsia is placental dysfunction. Placental damage is associated with excessive secretion of antiangiogenic molecules by the placental tissue [3] and insufficient proangiogenic molecules, in particular, placental vascular growth factor PlGF [4, 15]. Thus, massive endothelial dysfunction occurs in the mother's body, which progressively leads to increased peripheral vascular resistance and activation of procoagulant mechanisms and immune response [2], in particular, to an increase in the production of proinflammatory and a decrease in anti-inflammatory cytokines [5, 6], and progression of systemic multiorgan failure [7].

Oxidative stress is a key factor in placental disorders [8], so many researchers consider melatonin as a potentially useful option for the treatment of PE [1, 9]. Melatonin is a natural antioxidant that acts directly and indirectly. The direct pathway consists in the effective removal of atomic oxygen species from cells directly by the melatonin molecule, while the indirect pathway involves the activation of endogenous antioxidant enzymes, namely glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase [1]. During normal pregnancy, melatonin levels progressively increase [10, 11]; there is evidence of co-expression of melatonin and oxytocin receptors in the myometrium before and during labour [12]. The literature reports a decrease in melatonin levels in preeclampsia, when the degree of decrease correlates with the severity of the process [13], as well as a decrease in the expression of melatonin receptors in placental tissue in case of fetal growth restriction [14].

In pregnant women with PE, there is a decrease in nighttime melatonin concentrations compared to healthy pregnant women [11], which may indicate a dysfunction of the pineal gland. However, a decrease in melatonin levels in this pathology is caused not only by changes in the pineal gland: it is known that the expression of two of the most important enzymes necessary for the synthesis of melatonin, namely aralkylamine N-acetyltransferase and hydroxyindole-O-methyltransferase, which is significantly reduced in the placental tissue of women diagnosed with PE, as well as the expression of melatonin receptors of both types (M1 and M2); therefore, insufficient melatonin synthesis by the placenta in this case is quite accurately confirmed [20].

Regarding changes in the cytokine profile in PE: there is evidence that the levels of proinflammatory cytokines, namely TNF-α and IL-6, increase in preeclampsia, while the concentrations of anti-inflammatory cytokines, namely IL-4 and IL-10, decrease [21]. Melatonin, on the contrary, reduces the secretion of proinflammatory cytokines, in particular TNF-α [22], and increases the production of anti-inflammatory cytokines, namely IL-10 [23]. Therefore, normalization of melatonin levels may be a promising direction in the treatment of PE in pregnant women.

As the key immunosuppressive cytokine, IL-10 is secreted primarily by Th2 cells, macrophages, natural killer cells, granulocytes, dendritic cells, and autoantigen-stimulated B cells; normal pregnancy is characterized by a shift in immunity toward Th2 over Th1; this is commonly referred to as "Th2 polarization," which is reversed in the case of PE [34]. Inhibition of IL-10 during the second half of pregnancy in mice resulted in restricted fetal growth but did not affect gestational age or fetal outcome, illustrating the critical but not essential role of IL-10 in fetal growth [35, 36].

Due to melatonin deficiency, oxidative stress in PE increases, leading to a high amount of circulating reactive oxygen species and reactive nitrogen species; both species play an important role as secondary messengers in many intracellular signaling cascades, but they can also have a critical impact on pathological processes in pregnant women [26]. One of the most important signaling pathways activated by reactive oxygen and nitrogen species is the induction of neutrophil adhesion to the endothelium, accompanied by the release of cytokines and activation of signaling pathways associated with the inflammatory response [26-28]. In addition, poor trophoblast invasion, which has been confirmed as one of the main pathological aspects of PE [29], leads to placental hypoxia, accelerating the apoptotic cascade in the villous trophoblast; the production of several other factors, including leukocyte and platelet membrane particles, cytokines, growth factors, and angiogenic factors, is also altered in the case of abnormal trophoblast invasion, and these factors will then interact with the maternal vascular endothelium, which may already be damaged [29].

The role of placental growth factor in preeclampsia is undoubtedly proven: it is known that the deficiency of the secretion of this pro-angiogenic molecule causes massive endothelial dysfunction in the mother's body, which progressively leads to an increase in the resistance of peripheral vessels and the activation of procoagulant mechanisms [39]. Regarding the relationship between the levels of melatonin and the placental growth factor PlGF, which is key in the pathogenesis of the development of preeclampsia, there is very little data in the modern literature, however, one of the recent studies found that the levels of both melatonin and the placental growth factor decrease in umbilical cord blood collected during childbirth in women whose pregnancy was complicated by fetal growth restriction [32].

Hence, PE is a complex pathological process, a significant role in the development of which is played by a decrease in melatonin levels, which is a consequence of pituitary and placental dysfunction.

The purpose of this study was to assess the pathogenetic mechanisms of complications arising from melatonin deficiency, and in particular - to determine changes in melatonin, IL-6 and IL-10 and the correlation between them.

Material and research methods

R&D «Preservation and restoration of reproductive health of women and girls in obstetric and gynecological pathology». State registration number:

ISSN 2226-1230 (PRINT) ISSN 2413-4260 (ONLINE)
KEY TITLE: NEONATOLOGIÂ, HÌRURGIÂ TA PERINATAL'NA MEDICINA (ONLINE)
ABBREVIATED KEY TITLE: NEONATOL. HÌR. PERINAT. MED. (ONLINE)

0121U110020. Terms of execution: 01.2021–12.2025 yy.

The study, which was conducted at the Department of Pregnancy Pathology of the Chernivtsi Regional Perinatal Center, involved 32 women whose pregnancy was complicated by preeclampsia. The presence of preeclampsia was diagnosed by systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥110 mm Hg, confirmed by a second blood pressure measurement within 10 minutes, as well as proteinuria ≥300 mg per 24-hour urine collection [37]. All women in the study group had a gestational age of 30-32 weeks, confirmed by calculating the labour date based on the first day of the last menstrual period, and the first trimester ultrasound (11-13 weeks).

The control group consisted of 33 women with uncomplicated pregnancies who were observed in the antenatal clinic of the Chernivtsi Regional Perinatal Center.

Patients with extragenital diseases (chronic arterial hypertension, obesity, pregestational and gestational diabetes, endocrine diseases, anemia, etc.) were excluded from both the study and control groups.

Melatonin and PlGF concentrations were determined using an ELISA kit manufactured by IBL (Germany), and concentrations of proinflammatory (IL-6) and anti-inflammatory (IL-10) cytokines were determined using diagnostic kits manufactured by Vector Best (Ukraine). The study was performed in venous blood, all analyzes were collected by vein puncture at the same time: 9:00 am on an empty stomach.

Statistical data were calculated using MedCalc software developed by MedCalc Software (Ostend, Belgium). The results were evaluated using the Mann-Whitney U-test for small groups. The P value of <0.05 was considered valid. Correlation and regression statistical evaluations were also performed using the aforementioned software.

Table 1

Clinical and statistical characteristics of groups of women whose pregnancy was complicated by preeclampsia

	Women with PE (n=32)	Control group (n=33)	p
Age, years	28,6±3,7	31,2±6,6	0,053
Woman's weight, kg	74,0±6,6	65,6±8,7	< 0,0001
Due date of labour, weeks	38,0±0,8	38,7±1,2	0,01

Note: *Data are presented as arithmetic mean ± standard deviation*

We conducted a prognostic assessment of the impact of the biochemical parameters we studied on the pregnancy outcomes of the patients examined. To do this, we used the method of constructing a "curve of errors", also known as a ROC-curve. This technique is widely used in clinical practice to make a diagnosis on a "yes/no" basis ("1/0", diagnosis is/is not present). The methodology also allows calculating threshold values of quantitative indicators used in a diagnostic and/or prognostic model [33]. The result of a diagnostic test is often based on whether the value of the marker selected by the researcher exceeds the threshold value. In this case, the diagnosis for a particular patient is "positive" (present), and if the threshold value is not exceeded, it is "negative" (absent) [34]. The most important aspect in this case is the correct choice of the quantitative variable on which the qualitative indicator "1/0" will depend (or not). We chose the following qualitative indicators that characterized the end of pregnancy in the examined patients: delivery before 38 weeks of gestation, which is usually due to the progression of preeclampsia, as well as the birth of children with a body weight below 3000 g, which is most often due to placental dysfunction. To build ROC curves, we used the "ROC curve analysis" computational block of the MedCalc software package produced by MedCalc, Software Ltd, Ostend, Belgium. The results were taken into account only if p<0.05.

Table 2

Pregnancy outcomes in women, whose pregnancy was complicated by preeclampsia

	Women with PE (n=32)	Control group (n=33)	p
Child's body weight at birth, g	3176,5 ± 274,4	3164,0 ± 405,9	0,88
Height at birth, cm	50,3 ± 1,7	51,5 ± 2,7	0,037
Apgar score (1st min), points	7,7 ± 0,45	7,8 ± 0,69	0,49
Apgar score (5th min), points	8,1 ± 0,46	8,4 ± 0,66	0,038

Note: *Data are presented as arithmetic mean ± standard deviation*

The patient's informed consent form and the patient's examination card were approved by the Biomedical Ethics Committee of Bukovinian State Medical University (BSMU) of the Ministry of Health of Ukraine (Chernivtsi). The work was guided by the general provisions of the Declaration of Helsinki "Recommendations for Physicians for Biomedical Research Involving Human Subjects" (1964), the World Medical Association on Ethical Principles for Scientific Medical Research Involving Human Subjects (1964-2000), taking into account the requirements of Directive 2001/20/EC of the European Parliament and of the Council, ICH GCP, the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), Order of the Ministry of Health of Ukraine No. 690 of 23.09.2009.

Table 3

Melatonin level in the venous blood of the examined patients

	Women with PE (n=32)	Control group women (n=33)	p
Melatonin (pg/ml)	30,98 (19,78 – 42,17)	55,20 (36,23 – 74,17)	0,029

Note: *1. P is calculated by Welch's criterion (assuming unequal variances)*
2. 95% confidence interval for the mean value is given in parentheses

Study results and discussion

Here is a brief clinical and statistical description of the study groups (Table 1).

The mean age in the main group was 28.59 years (95% confidence interval for the average 27.3 - 29.88 years), in the control group - 31.24 (28.97 - 33.51) years, this indicator did not differ significantly between the groups (p=0.053). Instead, as can be seen from Table 1, the average weight of patients whose pregnancy was complicated by preeclampsia was significantly higher compared to healthy pregnant women (p<0.0001). Births in the study group occurred significantly earlier (p=0.01), at an average of 38.09 weeks (in the control group – at 38.67 weeks).

All patients gave birth naturally at a gestational age of more than 37 weeks. Cases of severe fetal distress that required cesarean section, obstetric vice or vacuum fetal extraction were excluded from the study.

The results of pregnancies of women included in the study groups are presented in Table 2.

The data presented in Table 2 show that the average birth weight of children at birth did not differ significantly between the groups (p=0.88), while the height of newborns was significantly higher in the control group (p=0.037). In addition, the Apgar score of newborns at the 5th minute of life was 0.3 points higher in the group of patients with uncomplicated pregnancy (p=0.038).

The analysis of biochemical parameters revealed a significant decrease in melatonin in women with PE compared with patients with uncomplicated pregnancy (Table 3), which is also confirmed by other studies [11, 20].

Table 4

The levels of PlGF in umbilical blood taken at labor from women with diagnosed PE

	Women with preeclampsia (n=32)	Women of control group (n=33)	p
PlGF (pg/ml)	41,90 (33,88 – 49,93)	30,73 (25,97 – 35,49)	0,018

Note: *1. P is calculated by Welch's criterion (assuming unequal variances)*
2. 95% confidence interval for the mean value is given in parentheses

As can be seen in Table 3, the level of melatonin in venous blood taken from women with diagnosed PE was significantly lower (P=0.029) compared to healthy women. Thus, we can conclude that in the case of PE, the level of melatonin in the mother's venous blood decreases during pregnancy.

The levels of PlGF in venous blood taken from the patients of the studied groups are represented in Table 4.

It's been established that the concentration of PlGF in venous blood taken from women with diagnosed preeclampsia was more than 5 times lower than in women who had uncomplicated pregnancies, p<0.0001.

The next step was to determine the consequent pathogenic chain, in particular proinflammatory IL-6 and anti-inflammatory IL-10, as the literature on this issue is different and contradictory in its results. A recent study suggests that endothelial cells in PE produce significantly more IL-6 and its receptor gp130 in soluble form, which is a signal of inflammatory changes in the endothelium [32], but much less IL-10. For example, a study of a preeclampsia model in rats with reduced uterine perfusion showed that placental ischemia was associated with decreased IL-10 levels and endothelial cell dysfunction [33]. Some human studies have shown that individual levels of IL-10 did not differ significantly between patients with preeclampsia and normotensive patients [35, 36].

In our study, we found that the concentrations of both proinflammatory IL-6 and anti-inflammatory IL-10 were elevated in women with PE compared with women with uncomplicated pregnancies (Table 5). In our opinion, this fact can be explained by the inclusion of women with PE, confirmed proteinuria and hypertension in the study group, as well as the exclusion of patients with fetal restriction and fetal distress; thus, IL-10 clearly plays a protective role for the fetus in PE.

The data in Table 5 show a clear difference in cytokine concentrations between the two groups. In the group with PE, we found an increase in the concentration of both IL-6 and IL-10 compared to patients with uncomplicated pregnancies.

To build a prognostic model using the ROC curve, which will be used to predict the selected qualitative indicators in pregnant women with preeclampsia, we analyzed the quantitative variables we monitored.

The results of building ROC curves and the predictive models developed on their basis are shown in Figure 1.

ISSN 2226-1230 (PRINT) ISSN 2413-4260 (ONLINE)
KEY TITLE: NEONATOLOGIÂ, HÌRURGIÂ TA PERINATAL'NA MEDICINA (ONLINE)
ABBREVIATED KEY TITLE: NEONATOL. HÌR. PERINAT. MED. (ONLINE)

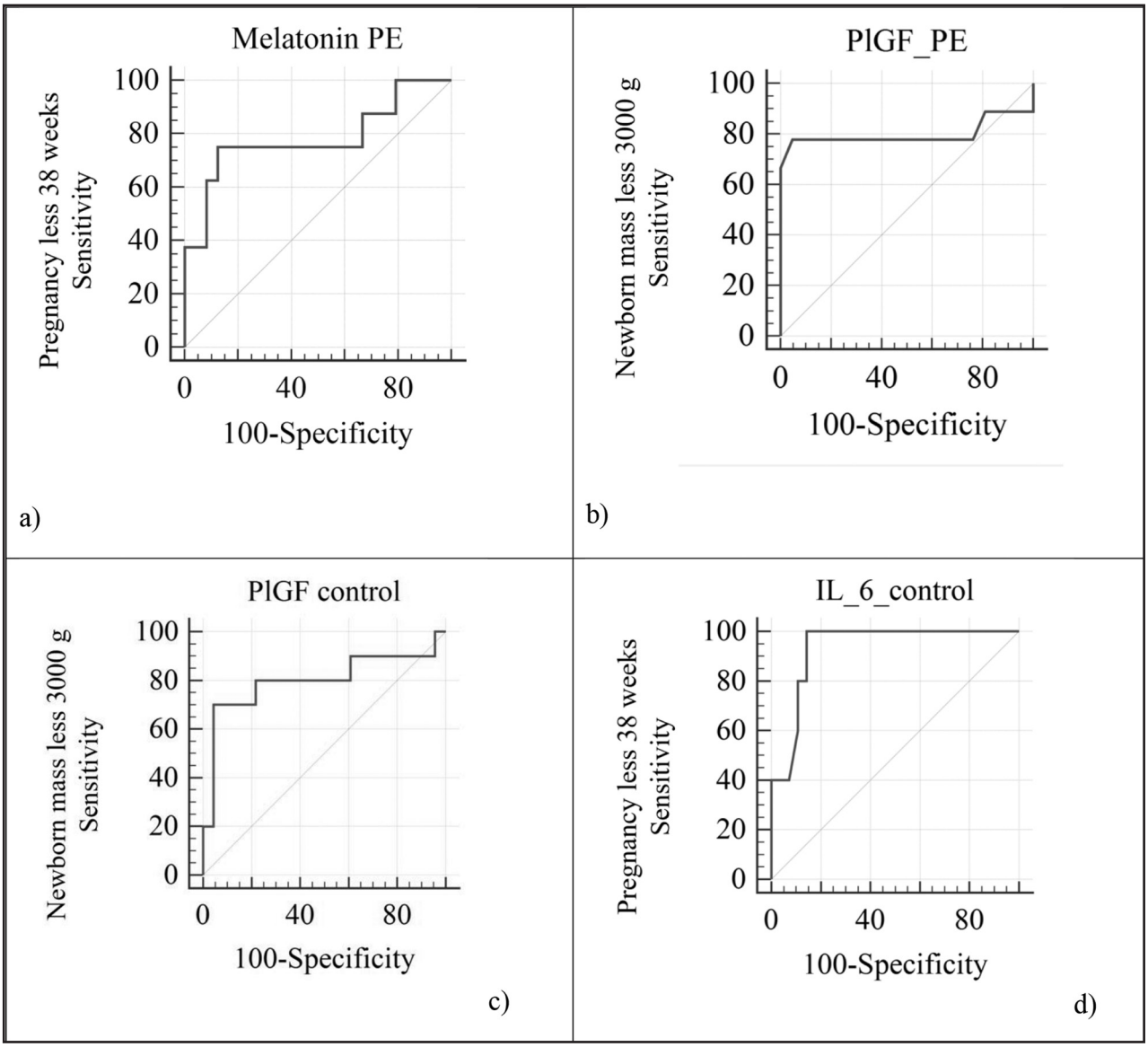

Figure 1: ROC curves for assessing the predictive ability of the monitored indicators:
a) melatonin to predict delivery before 38 weeks (women with preeclampsia),
b) placental growth factor for predicting newborn weight up to 3000 g (women with preeclampsia),
c) placental growth factor for predicting newborn weight up to 3000 g (women in the control group),
d) IL-6 for predicting delivery up to 38 weeks (women in the control group).

Let's take a closer look at the findings.

Thus, we were able to build prognostic ROC curves for predicting the following events during pregnancy complicated by preeclampsia: delivery before 38 weeks by melatonin concentration (Figure 1a), and birth of a child weighing up to 3000 g by placental growth factor concentration (Figure 1b).

First of all, let's pay attention to the prognostic model "Melatonin - delivery before 38 weeks" (Figure 1a). Indications for delivery before 38 weeks of gestation in women with preeclampsia: signs of severe preeclampsia (blood pressure 160/110 mmHg and above and/or proteinuria 3 g per day or more).

Parameters of our prognostic model for women with preeclampsia "Melatonin – delivery before 38 weeks":

- is the area under the AUC curve: 0.781,
- is the standard deviation: 0.118,
- reliability criterion: p = 0.0173,
- associated threshold criterion: ≤ 6.71 pg/ml,
- sensitivity: 75.00%,
- specificity: 87.50%.

The prognostic model was developed for the following tasks and conditions: predicting the onset of severe preeclampsia during pregnancy if the level of melatonin measured in fasting venous blood at 8 am in pregnancy complicated by preeclampsia is ≤ 6.71 pg/ml.

Regarding the prediction of births below 3000 g in women whose pregnancy was complicated by preeclampsia: we were able to reveal the prognostic value of the studied levels of placental growth factor (PlGF) for predicting the birth of a child with such a birth weight (Figure 1b).

The prognostic model for women with preeclampsia "PlGF - birth weight less than 3000 g" has the following parameters:

- is the area under the AUC curve: 0.799,
- is the standard deviation: 0.132,
- reliability criterion: p = 0.0239,

- associated threshold criterion: ≤ 12.88 pg/ml,
- sensitivity: 77.78%,
- specificity: 95.24%.

The prognostic model was developed for the following tasks and conditions: predicting the birth of a child with a birth weight below 3000 g, if the level of placental growth factor PlGF measured in fasting venous blood at 9 am in pregnancy complicated by preeclampsia is ≤ 12.88 pg/ml.

For women with uncomplicated pregnancies (control group), we were able to build prognostic ROC curves to predict the following events: the birth of a child weighing up to 3000 g - by the concentration of placental growth factor PlGF (Figure 1b) and delivery before 38 weeks - by the concentration of IL-6 (Figure 1d).

Parameters of the prognostic model for women with uncomplicated pregnancy "PlGF - birth weight less than 3000 g":

- is the area under the AUC curve: 0.800,
- is the standard deviation: 0.107,
- reliability criterion: p = 0.0051,
- associated threshold criterion: ≤ 66.6 pg/ml,
- sensitivity: 70.00%,
- specificity: 95.65%.

The prognostic model was developed for the following tasks and conditions: predicting the birth of a child with a birth weight below 3000 g if the level of placental growth factor PlGF measured in fasting venous blood at 8 am in an uncomplicated pregnancy is ≤ 66.6 pg/ml.

The parameters of the prognostic model for women with uncomplicated pregnancy "IL-6 - delivery before 38 weeks" are as follows:

- is the area under the AUC curve: 0.932,
- is the standard deviation: 0.448,
- reliability criterion: p < 0.0001,
- associated threshold criterion: > 294.69 pg/ml,
- sensitivity: 100.00%,
- specificity: 85.71%.

The prognostic model was developed for the following tasks and conditions: prediction of labor at up to 38 weeks' gestation if the level of the pro-inflammatory cytokine IL-6 measured in fasting venous blood at 8 am in uncomplicated pregnancy is > 294.69 pg/ml.

We explain the data we obtained in developing our prognostic models as follows. As demonstrated throughout our study, melatonin is a hormone that has a protective effect on the mother and fetus and on the placental tissue. The progression of preeclampsia to it's severe form is a manifestation of maladaptation on the part of the placental tissue. Therefore, melatonin, if present in the blood of a pregnant woman in a concentration higher than the thresholds we have established (6.71 pg/ml), reduces the chances of clinical manifestations of severe preeclampsia with a high degree of reliability (p=0.00173). However, this prognostic model has its drawbacks. Firstly, these are the strict conditions (described above) that must be met when predicting the likelihood of severe preeclampsia based on the level of melatonin in the venous blood of a pregnant woman. Secondly, the model involves invasive medical intervention - venipuncture and blood sampling by a medical professional. In our opinion, the direction of predicting future adverse obstetric events based on changes in salivary melatonin concentrations is promising. Such a study is non-invasive, economically more feasible, can be performed on an outpatient basis, and the patient can donate biological material for it herself. This may be the focus of further research.

To develop prognostic models to calculate the probability of having a child with a birth weight below 3000 g in both groups, the indicator PlGF (placental growth factor) was used.

Placental vascular growth factor is an angiogenic protein whose expression is most pronounced during pregnancy. Its concentration correlates with placental function well. Placental growth factor (PlGF) belongs to the vascular endothelial growth factor (VEGF) family of proteins. It is known that PlGF exerts its angiogenic effects through both direct and indirect mechanisms, causing receptor dimerization and phosphorylation. PlGF directly activates endothelial cells, macrophages, and hematopoietic progenitor cells by binding to a membrane receptor known as fms-like-tyrosin-kinase-1 (Flt-1), and in doing so, not only triggers angiogenesis itself, but also increases the cell's sensitivity to the vascular endothelial growth factor VEGF. PlGF acts indirectly by displacing vascular endothelial growth factor from receptors, while the soluble form of the mentioned receptor, soluble fms-like-tyrosin-kinase-1 (sFlt-1), which is actively expressed by the placenta in preeclampsia has antiangiogenic potential because it binds to PlGF and VEGF in the blood, reduces their bioavailability, and thus interaction with membrane proangiogenic receptors [35]. Thus, PlGF has a proangiogenic effect in the maternal-fetal complex; it is widely known as a predictor and diagnostic marker of preeclampsia [36]. At the same time, this molecule attracts the attention of many researchers in placental insufficiency or fetal distress. For example, Larissa N. Bligh, Ristan M. Greer, Sailesh Kumar (2016) found that reduced PlGF levels were associated with lower Apgar scores, abnormal fetal heart rate changes in labor, and pH changes in the umbilical cord arteries, which are characteristic of fetal hypoxia [37]. In our study, PlGF had a prognostic value for the birth of children with a birth weight below 3000 g at a threshold value of ≤ 12.88 pg/ml (p = 0.0239). We believe that this is due to the delivery of patients with signs of severe preeclampsia, as well as manifestations of placental dysfunction accompanying the development of preeclampsia. However, the prognostic value of PlGF for this qualitative parameter was also observed in the group of women with uncomplicated pregnancy at a threshold value of ≤ 66.6 pg/ml (p = 0.0051). Also in our study, we noted the prognostic value of proinflammatory interleukin IL-6 for predicting the delivery of women with uncomplicated pregnancy at a time earlier than 38 weeks of gestation (associated threshold criterion > 294.69 pg/ml, p < 0.0001), which, in our opinion, is associated with a subclinical inflammatory response in the placental tissue, which, along with other factors, causes spontaneous onset of labor.

Conclusion. Concentrations of melatonin and of placental growth factor in venous blood in women whose pregnancy was complicated by preeclampsia in the third trimester of pregnancy are significantly lower compared to uncomplicated pregnancies. On

KEY TITLE: NEONATOLOGIÂ, HÌRURGIÂ TA PERINATAL'NA MEDICINA (ONLINE)
ABBREVIATED KEY TITLE: NEONATOL. HÌR. PERINAT. MED. (ONLINE)
ISSN 2226-1230 (PRINT) ISSN 2413-4260 (ONLINE)

the contrary, the levels of proinflammatory IL-6 and anti-inflammatory IL-10 are significantly increased in the group of patients with diagnosed preeclampsia compared to normal pregnancies. We assume that placental dysfunction, in particular, leads to impaired synthesis of the antioxidant melatonin, which causes disorders in the tissues of the mother, placenta and fetus and, as a result, provokes clinical manifestations of preeclampsia. The condition of the placenta also worsens the subclinical inflammatory process, as evidenced by an increase in IL-6 levels in patients with diagnosed preeclampsia; an increase in IL-10 in the study group, in our opinion, is a kind of compensatory reaction that prevents preterm birth. In women with pregnancies complicated by preeclampsia, melatonin is a prognostic criterion for delivery before 38 weeks of gestation, and this delivery is mainly associated with the progression of preeclampsia to severe preeclampsia. In the group of patients with uncomplicated pregnancy, proinflammatory interleukin-6 is predictive of spontaneous onset of labor before 38 weeks of gestation. Placental growth factor PlGF was a predictor of the birth of children with a body weight of less than 3000 g in both study groups.

Prospects for further research. In the future, it is planned to continue to elucidate the role of melatonin in preeclampsia combined with extragenital pathology.

Conflict of interest: none

Sources of funding: self-financing

Reference:

1. Sapède D, Cau E. The pineal gland from development to function. Curr Top Dev Biol. 2013;106:171-215. doi: 10.1016/B978-0-12-416021-7.00005-5

2. Patel S, Rahmani B, Gandhi J, Seyam O, Joshi G, Reid I, et al. Revisiting the pineal gland: a review of calcification, masses, precocious puberty, and melatonin functions. Int J Neurosci. 2020;130(5):464-75. doi: 10.1080/00207454.2019.1692838

3. Tan DX, Xu B, Zhou X, Reiter RJ. Pineal Calcification, Melatonin Production, Aging, Associated Health Consequences and Rejuvenation of the Pineal Gland. Molecules [Internet]. 2018[cited 2023 May 12];23(2):301. Available from: https://www.mdpi.com/1420-3049/23/2/301 doi: 10.3390/molecules23020301

4. Tan DX, Manchester LC, Esteban-Zubero E, Zhou Z, Reiter RJ. Melatonin as a Potent and Inducible Endogenous Antioxidant: Synthesis and Metabolism. Molecules. 2015;20(10):18886-906. doi: 10.3390/molecules201018886

5. Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L. Melatonin as an antioxidant: under promises but over delivers. J Pineal Res. 2016;61(3):253-78. doi: 10.1111/jpi.12360

6. Chowdhury I, Sengupta A, Maitra SK. Melatonin: fifty years of scientific journey from the discovery in bovine pineal gland to delineation of functions in human. Indian J Biochem Biophys. 2008;45(5):289-304.

7. Hardeland R, Tan DX, Reiter RJ. Kynuramines, metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. J Pineal Res. 2009;47(2):109-26. doi: 10.1111/j.1600-079X.2009.00701.x

8. Dubocovich ML, Delagrange P, Krause DN, Sugden D, Cardinali DP, Olcese J. International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors. Pharmacol Rev. 2010;62(3):343-80. doi: 10.1124/pr.110.002832

9. Richter HG, Hansell JA, Raut S, Giussani DA. Melatonin improves placental efficiency and birth weight and increases the placental expression of antioxidant enzymes in undernourished pregnancy. J Pineal Res. 2009;46(4):357-64. doi: 10.1111/j.1600-079X.2009.00671.x

10. Berbets AM, Davydenko IS, Barbe AM, Konkov DH, Albota OM, Yuzko OM. Melatonin 1A and 1B Receptors' Expression Decreases in the Placenta of Women with Fetal Growth Restriction. Reprod Sci. 2021;28(1):197-206. doi: 10.1007/s43032-020-00285-5

11. Langston-Cox A, Marshall SA, Lu D, Palmer KR, Wallace EM. Melatonin for the Management of Preeclampsia: A Review. Antioxidants (Basel) [Internet]. 2021[cited 2023 May 12];10(3):376. Available from: https://www.mdpi.com/2076-3921/10/3/376 doi: 10.3390/antiox10030376

12. Lowe SA, Bowyer L, Lust K, McMahon LP, Morton M, North RA, et al. SOMANZ guidelines for the management of hypertensive disorders of pregnancy 2014. Aust N Z J Obstet Gynaecol [Internet]. 2015[cited 2023 May 12];55(5):e1-29. Available from: https://obgyn.onlinelibrary.wiley.com/doi/epdf/10.1111/ajo.12399 doi: 10.1111/ajo.12399

13. Sahu MB, Deepak V, Gonzales SK, Rimawi B, Watkins KK, Smith AK, et al. Decidual cells from women with preeclampsia exhibit inadequate decidualization and reduced sFlt1 suppression. Pregnancy Hypertens. 2019;15:64-71. doi: 10.1016/j.preghy.2018.11.003

14. Maynard SE, Karumanchi SA. Angiogenic factors and preeclampsia. Semin Nephrol. 2011;31(1):33-46. doi: 10.1016/j.semnephrol.2010.10.004

15. Udenze I, Amadi C, Awolola N, Makwe CC. The role of cytokines as inflammatory mediators in preeclampsia. Pan Afr Med J [Internet]. 2015[cited 2023 May 12];20:219. Available from: https://www.panafrican-med-journal.com/content/article/20/219/full/ doi: 10.11604/pamj.2015.20.219.5317

16. Aggarwal R, Jain AK, Mittal P, Kohli M, Jawanjal P, Rath G. Association of pro- and anti-inflammatory cytokines in preeclampsia. J Clin Lab Anal [Internet]. 2019[cited 2023 May 12];33(4):e22834. Available from: https://onlinelibrary.wiley.com/doi/epdf/10.1002/jcla.22834 doi: 10.1002/jcla.22834

17. Turbeville HR, Sasser JM. Preeclampsia beyond pregnancy: long-term consequences for mother and child. Am J Physiol Renal Physiol. 2020;318(6):F1315-26. doi: 10.1152/ajprenal.00071.2020

18. Marseglia L, D'Angelo G, Manti S, Reiter RJ, Gitto E. Potential Utility of Melatonin in Preeclampsia, Intrauterine Fetal Growth Retardation, and Perinatal Asphyxia. Reprod Sci. 2016;23(8):970-7. doi: 10.1177/1933719115612132

19. Olcese J, Beesley S. Clinical significance of melatonin receptors in the human myometrium. Fertil Steril. 2014;102(2):329-35. doi: 10.1016/j.fertnstert.2014.06.020

20. Dou Y, Lin B, Cheng H, Wang C, Zhao M, Zhang J, et al. The reduction of melatonin levels is associated with the development of preeclampsia: a meta-analysis. Hypertens Pregnancy. 2019;38(2):65-72. doi: 10.1080/10641955.2019

21. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med. 2006;12(6):642-9. doi: 10.1038/nm1429

22. Rana S, Karumanchi SA, Levine RJ, Venkatesha S, Rauh-Hain JA, Tamez H, et al. Sequential changes in

antiangiogenic factors in early pregnancy and risk of developing preeclampsia. Hypertension. 2007;50(1):137-42. doi: 10.1161/HYPERTENSIONAHA.107.087700

23. Lu F, Longo M, Tamayo E, Maner W, Al-Hendy A, Anderson GD, et al. The effect of over-expression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice. Am J Obstet Gynecol [Internet]. 2007[cited 2023 May 12];196(4):396.e1-7. Available from: https://www.ajog.org/article/S0002-9378(06)02466-5/fulltext doi: 10.1016/j.ajog.2006

24. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest. 2003;111(5):649-58. doi: 10.1172/JCI17189

25. Hobson SR, Acharya R, Lim R, Chan ST, Mockler J, Wallace EM. Role of activin A in the pathogenesis of endothelial cell dysfunction in preeclampsia. Pregnancy Hypertens. 2016;6(2):130-3. doi: 10.1016/j.preghy.2016.03.001

26. Nakamura Y, Tamura H, Kashida S, Takayama H, Yamagata Y, Karube A, et al. Changes of serum melatonin level and its relationship to feto-placental unit during pregnancy. J Pineal Res. 2001;30(1):29-33. doi: 10.1034/j.1600-079x.2001.300104.x

27. Lanoix D, Guérin P, Vaillancourt C. Placental melatonin production and melatonin receptor expression are altered in preeclampsia: new insights into the role of this hormone in pregnancy. J Pineal Res. 2012;53(4):417-25. doi: 10.1111/j.1600-079X.2012.01012.x

28. Aggarwal R, Jain AK, Mittal P, Kohli M, Jawanjal P, Rath G. Association of pro- and anti-inflammatory cytokines in preeclampsia. J Clin Lab Anal [Internet]. 2019[cited 2023 May 12];33(4):e22834. Available from: https://onlinelibrary.wiley.com/doi/epdf/10.1002/jcla.22834 doi: 10.1002/jcla.22834

29. Liu X, Gong Y, Xiong K, Ye Y, Xiong Y, Zhuang Z, et al. Melatonin mediates protective effects on inflammatory response induced by interleukin-1 beta in human mesenchymal stem cells. J Pineal Res. 2013;55(1):14-25. doi: 10.1111/jpi.12045

30. Chen SJ, Huang SH, Chen JW, Wang KC, Yang YR, Liu PF, et al. Melatonin enhances interleukin-10 expression and suppresses chemotaxis to inhibit inflammation in situ and reduce the severity of experimental autoimmune encephalomyelitis. Int Immunopharmacol. 2016;31:169-77. doi: 10.1016/j.intimp.2015.12.020

31. Berbets AM, Barbe AM, Andriiets OA, Andriiets AV, Yuzko OM. Melatonin Levels Decrease in the Umbilical Cord in Case of Intrauterine Growth Restriction. J Med Life. 2020;13(4):548-53. doi: 10.25122/jml-2020-0128

32. El Farargy MS, Soliman NA. A randomized controlled trial on the use of magnesium sulfate and melatonin in neonatal hypoxic ischemic encephalopathy. J Neonatal Perinatal Med. 2019;12(4):379-84. doi: 10.3233/NPM-181830

33. Kamarudin AN, Cox T, Kolamunnage-Dona R. Time-dependent ROC curve analysis in medical research: current methods and applications. BMC Med Res Methodol [Internet]. 2017[cited 2023 May 12];17(1):53. Available from: https://bmcmedresmethodol.biomedcentral.com/counter/pdf/10.1186/s12874-017-0332-6.pdf doi: 10.1186/s12874-017-0332-6

34. Hayes Ryan D, McCarthy FP, O'Donoghue K, Kenny LC. Placental growth factor: A review of literature and future applications. Pregnancy Hypertens. 2018;14:260-4. doi: 10.1016/j.preghy.2018.03.003

35. Poon LC, Shennan A, Hyett JA, Kapur A, Hadar E, Divakar H, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention. Int J Gynaecol Obstet. 2019;145(1):1-33. doi: 10.1002/ijgo.12802

36. Albonici L, Benvenuto M, Focaccetti C, Cifaldi L, Miele MT, Limana F, et al. PlGF Immunological Impact during Pregnancy. Int J Mol Sci [Internet]. 2020[cited 2023 May 12];21(22):8714. Available from: https://www.mdpi.com/1422-0067/21/22/8714 doi: 10.3390/ijms21228714

37. Bligh LN, Greer RM, Kumar S. The relationship between maternal placental growth factor levels and intrapartum fetal compromise. Placenta. 2016;48:63-7. doi: 10.1016/j.placenta.2016.10.007

ВПЛИВ МЕЛАТОНІНУ НА КІЛЬКІСТЬ ЦИТОКІНІВ ПРИ ПРЕЕКЛАМПСІЇ

Р.Ф. Савка, А.М. Бербець, С.Г. Приймак, О.М. Юзько, Д.А. Бербець

Буковинський державний медичний університет
(Україна, м. Чернівці)

Резюме

Вступ. У літературі є повідомлення про зниження рівня мелатоніну при прееклампсії, коли ступінь зниження корелює з тяжкістю процесу, а також про зниження експресії рецепторів мелатоніну в плацентарній тканині у випадку затримки розвитку плода.

Щодо змін у цитокіновому профілі при прееклампсії: є дані про те, що рівні прозапальних цитокінів, а саме TNF-α та IL-6, підвищуються при прееклампсії, тоді як концентрації протизапальних цитокінів, а саме IL-4 та IL -10, зменшуються. Мелатонін, навпаки, знижує секрецію прозапальних цитокінів, зокрема TNF-α, і збільшує продукцію протизапальних цитокінів, а саме IL-10. Тому нормалізація рівня мелатоніну може бути перспективним напрямком у лікуванні прееклампсії вагітних.

Мета дослідження. Оцінити патогенетичні механізми ускладнень, що виникають при зниженні мелатоніну, зокрема, з'ясувати зміни мелатоніну, IL-6 та IL-10, визначити кореляцію між ними.

Матеріал і методи дослідження.

У дослідженні, яке проводили у відділенні патології вагітності Чернівецького обласного перинатального центру, брали участь 32 жінки, вагітність яких ускладнилася прееклампсією. Контрольну групу склали 33 жінки з неускладненим перебігом вагітності, які спостерігалися у відділенні жіночої консультації Чернівецького обласного перинатального центру.

Формуляр інформованої згоди пацієнта та карта обстеження пацієнта схвалені комісією з питань біомедичної етики Буковинського державного медичного університету (БДМУ) МОЗ України (м. Чернівці). При виконанні роботи керувалися загальними положеннями Гельсінської декларації "Рекомендації для лікарів із проведення біомедичних досліджень із залученням людини" (1964), Всесвітньої медичної асоціації про етичні принципи проведення наукових медичних досліджень за участю людини (1964-2000 рр.) з урахуванням вимог Директиви 2001/20/ЄС Європейського Парламенту та Ради ЄС, ICH GCP, Конвенції Ради Європи про права людини та біомедицину (від 04.04.1997 р.), наказу МОЗ України №690 від 23.09.2009 р.

Статистичні дані розраховували за допомогою програмного забезпечення MedCalc, розробленого компанією

KEY TITLE: NEONATOLOGIÂ, HÌRURGIÂ TA PERINATAL'NA MEDICINA (ONLINE)
ABBREVIATED KEY TITLE: NEONATOL. HÌR. PERINAT. MED. (ONLINE)
ISSN 2226-1230 (PRINT) ISSN 2413-4260 (ONLINE)

"MedCalc Software" (Остенде, Бельгія). Результати оцінювали за допомогою U-тесту Манна-Уітні для малих груп. Значення P <0,05 вважалося достовірним. Кореляційні та регресійні статистичні оцінки також проводили за допомогою згаданого програмного забезпечення.

НДР «Збереження та відновлення репродуктивного здоров'я жінок та дівчат при акушерській і гінекологічній патології». Державний реєстраційний номер: 0121U110020. Термін виконання: 01.2021-12.2025 рр.

Результати дослідження. Рівень мелатоніну у венозній крові, взятій у жінок з діагностованою прееклампсією, був достовірно нижчим (p=0,029), порівняно зі здоровими жінками. У нашому дослідженні ми встановили, що концентрації як прозапального IL-6, так і протизапального IL-10, були підвищені у жінок з прееклампсією порівняно з жінками з неускладненою вагітністю. Нами було проведено прогностичну оцінку впливу вивчених нами біохімічних показників на результати вагітностей обстежених пацієнток. Мелатонін, якщо він присутній в крові вагітної жінки в концентрації, вищій за встановлені нами порогові значення (6,71 pg/ml), з високим ступенем достовірності (p=0,00173) знижує шанси на появу клінічних проявів тяжкої прееклампсії. Ми припускаємо, що плацентарна дисфункція, зокрема, призводить до порушення синтезу антиоксиданту мелатоніну, що спричиняє порушення в тканинах матері, плаценти та плоду та, як наслідок, провокує клінічні прояви прееклампсії. Стан плаценти також погіршує субклінічний запальний процес, про що свідчить підвищення рівня ІЛ-6 у хворих з діагностованою прееклампсією; підвищення ІЛ-10 в досліджуваній групі, на нашу думку, є своєрідною компенсаторною реакцією, яка запобігає передчасним пологам. У жінок з вагітністю, що ускладнилася прееклампсією, мелатонін є прогностичним критерієм розродження до 38 тижнів гестації, причому це розродження переважно пов'язане з прогресуванням прееклампсії до тяжкої. В групі пацієнток з неускладненим перебігом вагітності прогностичне значення щодо спонтанного початку пологової діяльності в терміні до 38 тижнів має прозапальний інтерлейкін-6. Плацентарний фактор росту PlGF виступив предиктором народження дітей з масою тіла менше 3000 г в обох обстежених групах

Висновки. Концентрації мелатоніну у венозній крові у жінок, вагітність яких ускладнилася прееклампсією у III триместрі вагітності, достовірно нижчі порівняно з неускладненою вагітністю. Навпаки, рівні прозапального IL-6 та протизапального IL-10 достовірно підвищені в групі пацієнток з діагностованою прееклампсією порівняно з нормальною вагітністю.

Ключові слова: мелатонін; цитокіни; прееклампсія.

Contact Information:
Ruslan Savka – Postgraduate Student of the Department of Obstetrics and Gynecology of Bukovinian State Medical University (Chernivtsi, Ukraine)
e-mail: savcaruslan@gmail.com
ORCID ID: http://orcid.org/0000-0002-0049-5205

Andriy Berbets – Doctor of Medicine, Full Professor, Professor of the Department of Obstetrics and Gynecology of Bukovinian State Medical University (Chernivtsi, Ukraine)
ORCID ID: http://orcid.org/0000-0002-9027-5256
Researcher ID: http://www.researcherid.com/rid/D-6526-2017
Scopus Author ID: https://www.scopus.com/authid/detail.uri?authorId=57204095952

Svitlana Pryimak – Candidate of Medicine, Assistant Professor of the Department of Obstetrics and Gynecology of Bukovinian State Medical University (Chernivtsi, Ukraine)
e-mail: pryimak.s@bsmu.edu.ua
ORCID ID: http://orcid.org/0000-0001-9086-5128

Oleksandr Yuzko – Doctor of Medical Sciences, Full Professor, Head of the Obstetrics and Gynecology Department of Bukovinian State Medical University (Chernivtsi, Ukraine)
e-mail: yuzko.oleksandr@bsmu.edu.ua
ORCID ID: http://orcid.org/0000-0003-1270-9095
Researcher ID: http://www.researcherid.com/rid/D-8126-2017
Scopus Author ID: https://www.scopus.com/authid/detail.uri?authorId=42962929800

Dmytro Berbets – student of Bukovinian State Medical University (Chernivtsi, Ukraine)
e-mail: berbets.dmytro.mf1@bsmu.edu.ua

Контактна інформація:
Савка Руслан Флорович – аспірант кафедри акушерства та гінекології Буковинського державного медичного університету (м. Чернівці, Україна)
e-mail: savcaruslan@gmail.com
ORCID ID: http://orcid.org/0000-0002-0049-5205

Бербець Андрій Миколайович – доктор медичних наук, професор, професор кафедри акушерства та гінекології Буковинського державного медичного університету (м. Чернівці, Україна)
e-mail: berbec.andrij@bsmu.edu.ua
ORCID ID: http://orcid.org/0000-0002-9027-5256
Researcher ID: http://www.researcherid.com/rid/D-6526-2017
Scopus Author ID: https://www.scopus.com/authid/detail.uri?authorId=57204095952

Приймак Світлана Григорівна – кандидат медичних наук, асистент кафедри акушерства та гінекології Буковинського державного медичного університету (м. Чернівці, Україна)
e-mail: pryimak.s@bsmu.edu.ua
ORCID ID: http://orcid.org/0000-0001-9086-5128

Юзько Олександр Михайлович – доктор медичних наук, професор, завідувач кафедри акушерства та гінекології Буковинського державного медичного університету (м. Чернівці, Україна)
e-mail: yuzko.oleksandr@bsmu.edu.ua
ORCID ID: http://orcid.org/0000-0003-1270-9095
Researcher ID: http://www.researcherid.com/rid/D-8126-2017
Scopus Author ID: https://www.scopus.com/authid/detail.uri?authorId=42962929800

Бербець Дмитро Андрійович – студент Буковинського державного медичного університету (м. Чернівці, Україна)
e-mail: berbets.dmytro.mf1@bsmu.edu.ua

Received for editorial office on 12/02/2023
Signed for printing on 25/05/2023