The features of the prevention of preeclampsia in pregnant women with gestational endotheliopathy in the first trimester

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The objective: to evaluate the clinical effectiveness of L-arginine in the prevention of preeclampsia and reduction of other perinatal risks in patients with preclinical gestational endotheliopathy (GE).

Materials and methods. A comparative clinical study was performed at the clinical base of the Department of Obstetrics and Gynecology N. 1 of the Vinnytsya National Pirogov Memorial Medical University. 116 pregnant women with preclinical GE (main group), which was diagnosed by laboratory and instrumental research (microalbuminuria and endothelium-dependent vasodilatation), took part in the study. The patients of the main group were divided into clinical subgroups: 31 pregnant women with GE in subgroup A received acetylsalicylic acid (ASA) at a dose of 75 mg per os per day; 33 patients with preclinical GE from subgroup B received L-arginine at a dose of 4-4.2 g per os per day; 52 pregnant women with preclinical GE who refused prophylactic treatment were included in the subgroup C. The control group involved 58 pregnant women with a physiological gestation and without GE.

The clinical effectiveness of the therapy was assessed by comparing the number of cases of perinatal pathology in the I, II and III trimesters.

Results. The use of L-arginine as an alternative preventive therapy for the development of preeclampsia and other perinatal pathology made possible to reduce the rate of preeclampsia significantly (RR 0.19, 95% CI: 0.05-0.77; p=0.02) and placental hyperplasia/hypoplasia (RR 0.17, 95% CI: 0.04-0.68; p=0.01), compared to patients who did not receive any preventive treatment. In pregnant women with early-onset gestational endotheliopathy who received L-arginine, placental dysfunction was not diagnosed in the II and III trimesters of pregnancy and there were no cases of fetal growth retardation.

The use of L-arginine was not associated with side effects and/or adverse reactions in the proposed dose and frequency of administration and can be considered beneficial for the mother and the fetus.

Conclusions. Prescribing ASA and L-arginine drugs for pregnant women with a moderate degree of perinatal risk (preclinical GE) made possible not only to prolong pregnancy, but also to prevent the development of severe perinatal pathology. A more pronounced clinical effectiveness of the course prescription of solution of L-arginine per or (daily dose of L-arginine - 4.0-4.2 g) in pregnant women with preclinical form of GE may be associated with the endotheliotropic protective effect of the drug.

The appropriateness of using L-arginine during pregnancy is still debated, and further researches are needed to determine the optimal dosage, initial period for using and duration for the best prophylactic or therapeutic effect.

Keywords: *pregnancy, gestational endotheliopathy, preeclampsia, fetal growth retardation, placental dysfunction, perinatal pathology, acetylsalicylic acid, L-arginine.*

Особливості профілактики прееклампсії у вагітних з гестаційною ендотеліопатією у I триместрі
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Мета дослідження: оцінювання клінічної ефективності L-аргініну у профілактиці прееклампсії та зниженні інших перинатальних ризиків у пацієнток із попередньо визначеною гестаційною ендотеліопатією (ГЕ).

Матеріали та методи. Порівняльне клінічне дослідження було виконано на клінічній базі кафедри акушерства і гінекології № 1 Вінницького національного медичного університету ім. М. І. Пирогова. У дослідженні брали участь 116 вагітних із попередньо визначеною за допомогою лабораторного та інструментального дослідження (мікроальбумінурія та ендотелійзалежна вазодилатація) ГЕ (осно-

вна група). Пацієнтки основної групи були розподілені на клінічні підгрупи: 31 вагітна із ГЕ у підгрупі А отримувала ацетилсаліцилову кислоту (АСК) у дозі 75 мг на добу перорально; 33 пацієнтки із донозологічною ГЕ з підгрупи В отримували L-аргінін у дозі 4–4,2 г на добу перорально; 52 вагітні із ГЕ, які відмовились від профілактичного лікування, увійшли до підгрупи С. До контрольної групи включено 58 вагітних із фізіологічним перебігом гестаційного процесу без ознак ГЕ.

Оцінювання клінічної ефективності терапії проводили методом порівняння кількості випадків перинатальної патології у I, II та III триместрах.

Результати. Використання L-аргініну як альтернативної профілактичної терапії стосовно розвитку прееклампсії та іншої перинатальної патології дозволило достовірно зменшити кількість випадків прееклампсії (ВР 0,19, 95% ДІ: 0,05–0,77; p=0,02) та гіперплазії/гіпоплазії плаценти (ВР 0,17, 95% ДІ: 0,04–0,68; p=0,01) порівняно із пацієнтками, у яких не використано будь-якої профілактичної стратегії. У вагітних із донозологічною ГЕ, які отримували L-аргінін, не було діагностовано порушення плацентарної функції у II та III триместрах вагітності та уповільнення росту плода.

Використання L-аргініну у наведених дозі та кратності уведення не асоціювалося із побічними ефектами, тож його можна розцінювати як сприятливе для вагітної та плода.

Висновки. Призначення препаратів АСК та L-аргініну вагітним з помірним ступенем перинатального ризику (донозологічна ГЕ) дозволило не тільки пролонгувати вагітність, але й попередити розвиток тяжкої акушерської патології. Більш виражена клінічна ефективність курсового призначення питного розчину L-аргініну (добова доза L-аргініну – 4,0–4,2 г) у вагітних з донозологічною формою ГЕ може бути пов'язана із ендотеліотропним протекторним ефектом препарату.

Доцільність використання L-аргініну під час вагітності все ще обговорюється, потрібні подальші дослідження для визначення оптимальних доз, початку і тривалості використання для найкращого профілактичного або лікувального ефекту.

Ключові слова: вагітність, гестаційна ендотеліопатія, прееклампсія, уповільнення росту плода, плацентарна дисфункція, перинатальна патологія, ацетилсаліцилова кислота, L-аргінін.

Hypertensive disorders of pregnancy (HDP) remain one of the leading causes of maternal and perinatal morbidity and mortality worldwide. Pregnant women with HDP, regardless of the presence of traditional cardiovascular risk factors, have an increased risk of cardiovascular disease in the future after pregnancy [7, 12, 20]. Pre-eclampsia (PE) is the most severe and unfavourable variant of HDP and one of the main causes of maternal morbidity and mortality [3, 20, 37]. Being the main cause of iatrogenic prematurity, PE is also one of the main factors of perinatal losses and fetal growth retardation (FGR) [7, 23, 42].

Placental dysfunction (or uterine and placental vascular insufficiency) is another important problem during pregnancy that threatens adequate fetal nutrition and increases the risk of low birth weight, FGR, preterm birth, and stillbirth [15, 19, 28, 34].

As of today, only one agent, acetylsalicylic acid (ASA), reliably prevents the development of PE. High-quality systematic reviews by Cochrane (2019, 2021), which summarized the pregnancy outcomes of almost 40.000 patients, concluded that ASA reduces the incidence of PE by 18% (relative risk (RR) 0.82; 95% CI: 0.77– 0.86). Although RR of early PE (up to 34 weeks) is more significant, this subtype accounts for less than a third of all cases of PE [9, 38]. Administration of ASA (81 mg/d) at the indicated gestational age led to a significant reduction in the number of cases of preterm labor, PE and FGR in women at high risk of developing PE [38].

Gestational endotheliopathy (GE) involves insufficient generation of vasodilating molecules, such as nitric oxide (NO), which is one of the main vasoactive mediators of the endothelium [3, 13, 21, 46]. During pregnancy, accompanied by hypertensive disorders, there is an increase in the synthesis of asymmetric dimethylarginine (ADMA) [14]. ADMA is an endogenous nitric oxide synthase (NOS) inhibitor that reduces the synthesis of NO from the normal amino acid L-arginine [35]. L-arginine is the main precursor of NO in vivo, which is involved in the pathophysiology of the relevant obstetric conditions [14]. The enzymatic pathway for the conversion of L-arginine includes nitric oxide synthase (NOS_3). In patients with elevated ADMA levels, normal L-arginine competes with ADMA for NOS_3, increasing NO levels [1, 42].

In recent years, various methods of preventing and treating GE have been developed and continue to be improved, but the problem is far from being solved. Preventive correction of endotheliopathy, especially before the clinical manifestation of pathology, can significantly improve perinatal outcomes [2, 4, 22, 40, 47]. Treatment of perinatal complications does not significantly affect the development of the fetus, but, according to some publications, can stabilize its condition, increase its resistance to hypoxia and prepare the fetus for delivery.

In addition, although reliable sources of evidence show encouraging trends in the prevention of PE and related perinatal pathology, it remains unclear whether other drugs prescribed in Ukraine prevent PE [3, 4, 7, 22]. Therefore, there is an urgent need to identify potentially new pharmacological agents for the prevention of PE in women at risk.

NO is a key regulator of maternal and fetal homeostasis during pregnancy, regardless of whether strategies involving NO precursors, NO donors, natural derivatives, or pharmacological modulators of the NO system require more detailed evaluation and randomized trials. NO is essentially the "face" of the endothelium, as it helps maintain vascular homeostasis:

• regulation of vascular tone;
• inhibition of platelet adhesion, aggregation and thrombus formation;
• regulation of proliferation and apoptosis;
• regulation of oxidative processes;
• inhibition of leukocyte adhesion.

Moreover, NO regulates all these processes with a "+" sign, that is, it has a positive and protective effect on all these functions, which means that it is one of the most popular substances in pathology[26, 40, 45, 48].

Most of the entheliotropic drugs are either not recommended during pregnancy due to a lack of reliable data about the absence of teratogenic and embryotoxic effects (resveratrol, meldonium), or are only undergoing clinical trials (statins proton pump inhibitors, metformin), or raise concerns about a possible link between prenatal exposure and neonatal death from pulmonary hypertension (sildenafil) [1-4, 20, 24, 36]. In addition, all of the above drugs have a stimulating effect on the endothelium, which leads to the production of NO, but also to endothelial depletion. Therefore, it is very important to use a NO precursor from which the endothelium can synthesize the necessary substances. Thus, there is a need for a class of endothelioprotective agents that not only stimulate the endothelium to produce NO but also supply the substrate. The only substance that is a substrate for NO synthesis is L-arginine [1, 27, 32, 50].

The objective: to evaluate the clinical effectiveness of L-arginine in the prevention of preeclampsia and reduction of other perinatal risks in patients with preclinical gestational endotheliopathy (GE).

MATERIALS AND METHODS

The study was conducted at Vinnytsya National Pirogov Memorial Medical University as the research work No. 0121U109141 "*Optimization of early diagnosis and preventive treatment of perinatal complications caused by gestational endotheliopathy*".

During the examination of patients, we were guided by international and domestic regulatory documents on biomedical ethics, namely: the Geneva Declaration, the World Medical Association Declaration of Helsinki on Biomedical Research Involving Human Subjects (World Medical Association Declaration of Helsinki, 1994, 2000, 2008), the International Code of Medical Ethics, the International Guidelines for the Ethics of Biomedical Research Involving Human Subjects, COMPS (Geneva, 1993), Declaration on Patients' Rights in Europe, WHO (1994), Guidelines for Good Clinical Practice, prepared by the International Conference on Harmonization, ICH GCP (1996), The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (Council of Europe, 1997) with "Additional Protocols", Order of the Ministry of Health of Ukraine No. 690 of 23.09.2009. "On Approval of the Procedure for Conducting Clinical Trials of Medicinal Products and Examination of Clinical Trial Materials and the Standard Terms of Reference for the Ethics Committee" and No. 110 of February 14, 2012. "Informed voluntary consent of the patient to the processing of personal data", a positive conclusion of the Bioethics Commission of Vinnytsya National Pirogov Memorial Medical University of the Ministry of Health of Ukraine.

This study was also conducted in compliance with the concept of informed consent of patients in accordance with the Order of the Ministry of Health of Ukraine No. 29 of January 21, 2016, insert No. 8 to the form No. 096/o "History of Pregnancy and Childbirth", taking into account the mandatory prevalence of potential benefits over the risk of harm, the principle of confidentiality and respect for pregnant women who participated in the study.

The study involved 116 pregnant women with previously diagnosed prenatally significant GE in the I trimester of gestation (main group). The average age of the examined women in the main clinical group was 25.8±4.4 years.

The control group included 58 pregnant women with a physiological course of the gestational process without signs of GE (mean age of patients - 23.6±3.8 years).

GE was established on the basis of determination of microalbuminuria (MAU) and measurement of endothelium-dependent vasodilation (EDV) after a compression test [1, 3, 5, 13, 45].

MAU was determined by the ratio of albumin to creatinine in the urine using diagnostic test strips. The test is based on the principle of color change of the acid-base indicator under the influence of proteins and on the reaction of creatinine with 3,5-dinitrobenzoic acid in an alkaline medium. Depending on the concentration of albumin and creatinine in the urine, the color

zone on the test strip changes color. GE was diagnosed with an albumin to creatinine ratio of more than 5 mg albumin/mol creatinine [5, 6].

Arterial EDV caused reactive hyperemia in response to the cessation of blood flow by a cuff applied proximal to the measurement site, which was performed using a Toshiba Xario XG ultrasound machine and a 12 MHz linear transducer. The corresponding parameters were determined in a longitudinal section of the brachial artery at the level of the lower third of the upper arm 3 cm proximal to the medial epicondyle in an upright position. The diameter and velocity of blood flow were assessed before and after arterial compression.

The physiological response of the artery to reactive hyperemia was considered to be dilatation by more than 20%. A lower degree of vasodilation and paradoxical vasoconstriction were considered pathological reactions indicative of endothelial dysfunction (GE). The reactive hyperemia test was performed by increasing the cuff pressure by 50 mm Hg from the baseline systolic pressure. The duration of occlusion was 5 minutes, after which the blood flow rate was recorded in the phase of reactive hyperemia (within 15 seconds after decompression). 48 hours before the study, the patient was discontinued from medications that can affect vascular tone (antispasmodics, progesterone and magnesium) [3].

The following measurements were performed using ultrasound Doppler:
1. Resting diameter of the brachial artery, mm (DR1);
2. Brachial artery diameter 60 seconds after decompression, mm (DR2).

We calculated brachial artery end-diastolic velocity (EDV) using the formula:

$$EDV = \frac{DR2-DR1}{DR2} \times 100\%.$$

In addition, in pregnant women in this prospective study, in order to study the synthesis of placental hormones in the comprehensive diagnosis of placental dysfunction, blood serum level of placental lactogen was determined using commercial laboratory kits Micro-Elisa Placental Lactogen (hPL) Diagnostic Kit by Leinco Technologies (USA) and unconjugated (free) estriol - EIA-1612, Free Estriol ELISA by DRG (Germany) according to the manufacturer's instructions.

The criteria for inclusion of patients in the main clinical group were:
1. Single pregnancy
2. Presence of GE (MAU > 5 mg/mol and EDV < 10%)
3. Absence of somatic pathology, auto-immune diseases that could affect the result of the study
4. Willingness and ability to participate in the study.

The criteria for inclusion of patients in the control group were:
1. Absence of any diseases that could affect the outcome of pregnancy
2. Absence of GE (MAU< 5 mg/mol and EDV > 20%)
3. No history of antenatal pathology in previous pregnancy
4. Informed consent of the patient for inclusion in the study.

Pregnant women in the main group were divided into clinical subgroups depending on the pharmacological agent chosen to prevent the development of PE and the clinical manifestation at the beginning of prophylactic treatment. Women with GE in subgroup A received ASA per os at a dose of 75 mg per day (n=31), in subgroup B they received L-arginine per os at a dose of 4-4.2 g per day (n=33), and women with GE who refused prophylactic treatment were included in subgroup C (n=52).

Preventive ASA therapy was performed from 12 to 36 weeks of pregnancy. Prophylactic treatment with L-arginine was carried out in a course regimen. The first course was prescribed from 12 to 14 weeks, the second course - from 16 to 18 weeks, and the third course - from 28 to 30 weeks of pregnancy.

The clinical effectiveness of the therapy was assessed by comparing the number of cases of perinatal pathology in the I, II and III trimesters.

Variational and statistical processing of the study results was performed using the program «STATISTICA 10» Enterprise Portable (2011, ENG) with the definition of the main variation indicators: mean (M), standard error (m) and mean square deviation ( ). The reliability of the results was determined using the Student's t-test. Relative risk (RR), standard error, and 95% confidence interval (CI) were calculated using a calculator MedCalc (version 20.305 – 64-bit) [18].

RESEARCH RESULTS AND THEIR DISCUSSION

Pregnancy outcomes and peculiarities of the course of labor were observed in all patients of the main group and pregnant women from the control group (100.0%). In the I trimester, the main complications of gestation in the examined pregnant women were most often observed as threatened miscarriage, spontaneous miscarriage or missed abortion (MA), as well as nausea and vomiting (Table. 1).

Pregnant women who used ASA, as well as patients who used L-arginine, did not differ in the incidence of early gestational complications either among themselves or in relation to pregnant women who did not receive prophylactic therapy at all. This was due to the fact that drugs for the prevention of PE were prescribed at the end of the I trimester, so they could not affect the incidence of complications in any way.

Таблиця 1

Pregnancy complications due to gestational endotheliopathy in the I trimester, n (%)

Pathology of pregnancy	Main group, n=116			Control group, n=58	p_{1-2} p_{1-3} p_{2-3}
	Subgroup A, n=31	Subgroup B, n=33	Subgroup C, n=52		
Threatening miscarriage	0	0	2 (3.8)	0	-
Spontaneous miscarriage	0	0	0	0	-
MA	0	0	1 (1.9)	0	-
Early gestosis	1 (3.2)	3 (9.7)	6 (11.5)	0	0.36 0.23 0.72
Anemia in pregnant women	3 (9.6)	4 (12.1)	3 (5.8)	0	0.75 0.51 0.31

Note: p_{1-2} – differences between patients in subgroups A and B;; p_{1-3} – differences between patients in subgroups A and C;
p_{2-3} – differences between patients in subgroups B and C; MA – missed abortion.

In the II trimester of pregnancy, we analysed cases of gestational pathology in the form of threatened late miscarriage, perinatal loss, threatened preterm birth, early PE, signs of abnormal development and localization of the placenta (Table 2).

When analysing gestational complications in the II trimester of pregnancy in clinical subgroups in women with preclinical GE in the setting of prophylactic therapy, a certain tendency to increase the number of cases of threatened late miscarriage in pregnant patients who did not receive prophylactic therapy was determined, but there was no statistically significant difference compared with subgroup A (RR 0.60, 95% CI: 0.21-1.72; p=0.34) and pregnant women treated with L-arginine (RR 0.28, 95% CI: 0.067-1.88; p=0.08), were not noted.

The RR for the development of early PE, depending on the type of pathogenetic prophylaxis in the presence of endotheliopathy, calculated using the logistic regression method, was 0.30 for pregnant women receiving ASA and L-arginine (95% CI: 0.07– 1.26; p=0.10) and 0.14 (95% CI: 0.02-1.04; p=0.054), respectively, compared to the same indicator in the subgroup of women who refused preventive therapy.

The RR for cases of pathology of placental development and localization (low placenta, placenta surrounded by a roller or rim, girdle placenta) was 0.55 (95% CI: 0.19– 1.55; p=0.26) and 0.26 (95% CI: 0.06-1.08; p=0.06) for pregnant women in subgroup C.

Placental dysfunction in the II trimester was assessed on the basis of laboratory and sonographic cri-

Table 2

Pregnancy complications due to gestational endotheliopathy in the II trimester, n (%)

Pathology of pregnancy	Main group, n=115			Control group, n=58	p_{1-2} p_{1-3} p_{2-3}
	Subgroup A, n=31	Subgroup B, n=33	Subgroup C, n=51		
Threatening miscarriage	4 (12.9)	2/6.0	11 (21.6)	0	0.36 0.34 0.08
Threatening preterm labor	2 (6.4)	0	5 (9.8)	0	- 0.53 -
Perinatal losses	0	0	1 (2.0)	0	-
PE	2 (6.4)	1 (3.0)	11 (21.6)	0	0.53 0.10 0.054
Anomalies in the development and localization of the placenta	4 (12.9)	2 (6.0)	12 (23.5)	0	0.36 0.26 0.06

Note: p_{1-2} – differences between patients in subgroups A and B; p_{1-3} – differences between patients in subgroups A and C; p_{2-3} – differences between patients in subgroups B and C; PE – preeclampsia.

Table 3

Placental dysfunction in the setting of gestational endotheliopathy in the II trimester, n (%)

Parameter	Main group, n=115			Control group, n=58	p_{1-2} p_{1-3} p_{2-3}
	Subgroup A, n=31	Subgroup B, n=33	Subgroup C, n=51		
Decrease in the level of placental hormones	3 (9.6)	0	6 (11.8)	2 (3.4)	- 0.59 -
Impaired blood flow	0	0	2 (3.9)	0	-
FGR	0	0	2 (3.9)	0	-
Oligohydramnios	2 (6.4)	0	2 (3.9)	0	- 0.62 -

Note: p_{1-2} – differences between patients in subgroups A and B; p_{1-3} – differences between patients in subgroups A and C; p_{2-3} – differences between patients in subgroups B and C; FGR - fetal growth retardation.

teria - FGR, changes in the amount of amniotic fluid, premature placental maturation, uterine-placental and fetal-placental blood flow disorders according to Doppler studies (Table 3).

Also, we did not observe a statistically significant difference in the incidence of placental dysfunction and concomitant FGR between pregnant women with pre-eclampsia and ASA prophylactic treatment and women with preclinical pre-eclampsia diagnosed in the I trimester who refused to have prophylactic therapy (p>0.05). We would like to note that no cases of placental dysfunction and FGR were detected in patients receiving L-arginine during the II trimester (Table. 3)

In the III trimester, complications in pregnant women with GE were observed in the form of threatened preterm labor, PE, placental dysfunction, antenatal fetal death, pathological changes in the placenta and placental abruption (Table 4).

We would like to note a statistically significant decrease in the number of cases of PE in women treated with L-arginine (RR 0.19, 95% CI: 0.05– 0.77; p=0.02). Regarding the reduction of quantitative indicators of placental hyperplasia/hypoplasia, a similar pattern was observed in the subgroup of pregnant women B (RR 0.17, 95% CI: 0.04-0.68; p=0.01) compared with pregnant women who did not receive prophylactic therapy. At the same time, women who received ASA from 12 weeks of gestation showed a significant trend toward a decrease in the number of cases of PE (2.7-fold) and placental hyperplasia/hypoplasia (3-fold) compared with patients in clinical subgroup C, but the difference was not statistically significant (RR 0.61, 95% CI: 0.26– 1.38; p=0.23 and RR 0.54, 95% CI: 0.24-1.21; p=0.13, respectively).

We would also like to note that the difference in the reduction of PE manifestation, in total, in pregnant women from subgroups A and B with pre-eclampsia who received ASA or L-arginine as prophylactic therapy was statistically significant (RR 0.39, 95% CI: 0.18– 0.84; p=0.02).

Table 4

Pregnancy complications in the setting of gestational endotheliopathy in the III trimester, n (%)

Pathology of pregnancy	Main group, n=114			Control group, n=58	p_{1-2} p_{1-3} p_{2-3}
	Subgroup A, n=31	Subgroup B, n=33	Subgroup C, n=50		
Threatening preterm labor	2 (6.4)	0	8 (16.0)	0	- 0.23 -
Perinatal losses	0	0	1 (2.0)	0	-
PE	6 (19.3)	2 (6.0)	16 (32.0)	2 (3.4)	0.135 0.23 0.02
Placental hyperplasia/hypoplasia	6 (19.3)	2 (6.0)	18 (36.0)	0	0.36 0.13 0.01

Note: p_{1-2} – differences between patients in subgroups A and B; p_{1-3} – differences between patients in subgroups A and C; p_{2-3} – differences between patients in subgroups B and C; PE – preeclampsia.

Placental dysfunction in the setting of gestational endotheliopathy in the III trimester, n (%)

Parameter	Main group, n=114			Control group, n=58	p_{1-2} p_{1-3} p_{2-3}
	Subgroup A, n=31	Subgroup B, n=33	Subgroup C, n=50		
Decrease in the level of placental hormones	4 (12.9)	0	9 (18.2)	2 (3.4)	0.55
Impaired blood flow	0	0	6 (12.0)	0	-
FGR	0	0	3 (6.0)	0	-
Low water. high water	1 (3.2)	0	2 (4.0)	0	- 0.86 -

Note: p_{1-2} – differences between patients in subgroups A and B; p_{1-3} – differences between patients in subgroups A and C; p_{2-3} – differences between patients in subgroups B and C; FGR - intrauterine growth retardation of the fetus.

In the analysis of placental dysfunction at the indicated gestational age, there were no cases of placental dysfunction in pregnant women of the clinical group with prenosological form of GE treated with L-arginine (Table. 5).

Thus, in the III trimester of pregnancy, there was a significant decrease in the number of cases of PE, placental and umbilical cord abnormalities in women who were treated with L-arginine for prophylactic purposes compared to patients who refused prophylactic therapy.

In pregnant women in the main first clinical group who received medical correction (ASA or L-arginine) aimed at preventing for PE development, and in the control group, all pregnancies ended in term delivery. In patients from subgroup C, preterm labor occurred in only 2 (4.1%) cases.

Cases of severe PE during pregnancy and in the postpartum period were not diagnosed in women in all subgroups of the study group and the control group.

Cases of acute fetal distress during labor were diagnosed in 1 (3.0%) woman in subgroup B and 1 (2.0%) patient in subgroup C.

In women with preconceptional GE diagnosed in the I trimester of pregnancy (subgroup C), bleeding during labor was observed in 4 (8.2%) of women in labor, while in subgroups A (preconceptional GE, ASA prophylaxis) and B (preconceptional GE, L-arginine prophylaxis) it was recorded in 1 case in each of these subgroups - 3.2% and 3.0%, respectively (RR 0.39, 95% CI): 0.05– 3.37; p= 0.40 and RR 0.37, 95% CI: 0.04-3.17; p=0.36, respectively).

In clinical subgroup C, placental defect was also detected in 4 (8.2%) postpartum patients. At the same time, the above complication of the third period of labor was recorded in one (3.0%) case in subgroup B (RR 0.37, 95% CI: 0.04-3.17; p=0.36) and in two (6.4%) cases in subgroup A (RR 0.79, 95% CI: 0.15– 4.06; p=0.78). In pregnant women with pre-eclampsia who did not receive preventive therapy, the number of cases of premature rupture of membranes was 4 (8.2%), while

the above obstetric pathology was observed in three (9.1%) cases in subgroup B (RR 1.11, 95% CI: 0.27-4.65; p=0.88) and in two (6.4%) – in subgroup A (RR 0.39, 95% CI: 0.05– 3.37; p=0.40).

In clinical subgroup C, six (12.2%) cases of abnormal labor activity were noted, in subgroups A and B, this complication was diagnosed in two persons (RR 0.53, 95% CI: 0.11– 2.45; p= 0.41 and RR 0.49, 95% CI: 0.11– 2.31; p=0.37). The RR in the subgroups where prophylactic therapy was administered (clinical subgroups A and B) and where pregnant women refused prophylactic treatment (clinical subgroup C) was 0.51 (95% CI: 0.15– 1.71; p=0.28).

During the use of the proposed preventive therapy regimens, the absence of any side effects was noted when using ASA and L-arginine.

The current scientific and medical literature describes at least 25 clinical trials in which L-arginine was used for prophylactic or therapeutic purposes. Of these clinical trials, L-arginine was administered orally in sixteen researches, intravenously – in eight, and in one study both routes of administration were combined. Doses for oral administration ranged from 1 g/day to 16 g/day, and the duration of treatment ranged from 8-10 days and throughout pregnancy [27]. Intravenous administration was mainly used in the emergency treatment of hypertension or PE, with doses ranging from 15 g (in 500 ml of 5% glucose solution) to 30 g (in 100 ml of saline) [10].

A study by Camarena Pulido et al. (2016) found that L-arginine supplementation reduced the incidence of PE. The risk reduction in the L-arginine group was estimated at 26% with an effectiveness of 74%. L-arginine significantly reduced the number of cases of severe PE [8]. This finding is consistent with finding F. Vadillo-Ortega et al. [47], but the dose used in the present study was half that used by F. Vadillo-Ortega et al. In the study by F. Vadillo-Ortega et al. (2011) reported side effects such as headache, palpitations, and dizziness, but the Camarena Pulido study did not report such disorders. The main side effect identified

in this study was dyspepsia [8]. Therefore, we believe that doctors should be careful when prescribing such medications and discuss this risk with patients with dyspeptic disorders.

In three studies, L-arginine was administered to women in assisted reproductive technology cycles together with gonadotropin-releasing hormone and follicle-stimulating hormone or in addition to folic acid and vitamin E [44]. As a result of L-arginine treatment, less aborted fertility cycles were observed, the number of oocytes and transferred embryos and the content of NO metabolites in plasma and follicular fluid increased, and in one study, an improvement in Doppler parameters was found [26].

A negative effect of L-arginine on embryo quality and pregnancy rate during controlled ovarian stimulation cycles has also been reported due to an inverse correlation between the concentration of NO metabolites in follicular fluid and embryo quality [26]. On the contrary, S. So et al. (2020) reported that when L-arginine was prescribed, especially in cases of male infertility, the rate of both biochemical and clinical pregnancy significantly increased [44].

In eleven studies, L-arginine was used to treat women with hypertensive disorders of pregnancy, chronic hypertension (CH), or PE. In three studies, L-arginine was administered intravenously, in seven studies - orally, and in one study - orally or intravenously (in cases where oral administration was not possible) [26].

Five studies included women diagnosed with PE, where treatment lasted from 2 days (emergency treatment) to 3 weeks [26]. L-arginine reduced systolic blood pressure, diastolic blood pressure, and mean arterial pressure [26]. In addition, L-arginine significantly promoted fetal growth, improved fetal condition and neonatal outcome [39]. It also contributed to the normalization of blood pressure and kidney function on the 10th day after delivery in the mother [17].

There have been 3 studies involving women with HDP who were administered L-arginine intravenously (20 g/day for a maximum of 5 days). Infusion of L-arginine demonstrated a pronounced hypotensive effect on both systolic and diastolic blood pressure, which contributed to the prolongation of pregnancy [11, 30, 31].

Other studies included women at high risk of PE or patients with chronic hypertension [27, 32]. Prophylactic oral administration of L-arginine was started in the I trimester and continued from 10 weeks to more than 30 weeks of gestation. Although L-arginine had no effect on blood pressure, a smaller percentage of women required antihypertensive medication; it was found that L-arginine improved uterine artery impedance [27].

PE was less pronounced, and the frequency of PE superimposed on hypertension, which is a factor in preterm birth (<34 weeks of gestation), showed a tendency to reduce the frequency of preterm birth. L-arginine

treatment was also associated with higher birth weight and fewer preterm births [8].

There were 2 studies involving women with a threat of preterm labor, in one of which L-arginine was used orally at a dose of 3 g/day from the moment of hospitalization to delivery [39], in the other L-arginine was administered intravenously 20 g/500 ml for 3 hours [10]. Oral administration of L-arginine increased fetoplacental blood flow, and in the case of intravenous administration, there was a decrease in uterine contractions, and increased levels of NO metabolites in the blood serum.

In 7 studies, L-arginine was used in pregnant women with FGR: in three of them, L-arginine was administered intravenously, and in four - orally. In general, studies have demonstrated that L-arginine infusion affected uterine placental blood flow by reducing uterine artery pulsatility index [27], increasing birth weight [43], reducing apoptosis in the placenta [26]. L-arginine also reduced the incidence of abnormal blood flow in the umbilical artery and contributed to the disappearance of early diastolic notch in the uterine artery, but was ineffective in severe FGR, possibly due to severe growth retardation (3rd percentile) and premature pregnancy (28 weeks) [49].

One study evaluated the L-arginine/NO system and its role in insulin signaling and endothelial function in pregnant women with different body mass indexes. Women with overweight/obesity have been found to have impaired endothelial function and insulin regulation. L-arginine decreased insulin levels in the I trimester and only in the case of normal body weight - in the II trimester [33].

Overall, the results demonstrated that the use of L-arginine during pregnancy can be beneficial in several circumstances, especially in maternal chronic hypertension and for the prevention of FGR. It reduces blood pressure levels, preventing PE and improving blood circulation, as well as placental function.

However, it should be emphasized that many of the included RCTs were conducted more than 15 years ago and had poor power and heterogeneous populations. In addition, there are no data on the most severe outcomes (i.e., stillbirth, placental abruption, or severe FGR), in contrast to other studies with NO donors and precursors [16], which, however, failed to demonstrate efficacy in terms of preterm birth and perinatal mortality and morbidity.

Although pharmacologic NO precursors are associated with low efficacy, L-arginine, which is the physiologic substrate of endothelial NO synthase, shows a better risk/benefit profile. Perhaps, in case of administration of this amino acid, peroxynitrites are not formed due to excessive bioavailability of NO [29].

Short-term administration of L-arginine, especially in late pregnancy, did not improve hemodynamics in the mother and did not mitigate the effects of severe FGR. This may indicate that the use of L-arginine

should be started as early as possible and continued throughout pregnancy to have a positive effect on blood pressure or placental vascular insufficiency through the arginine-NO pathway [24]. These results confirm the findings of a recent meta-analysis by E. Goto (2018) suggested that L-arginine should be recommended for women with compromised previous pregnancies, those at high risk of developing PE, and those with pre-existing HDP [15]. However, we agree with the authors who argue that more research is needed to draw more convincing conclusions, as the effects are small.

The use of L-arginine during pregnancy significantly reduced the number of cases of infants with low birth weight for gestational age (LBW) in women with HDP (RR 0.51, 95% CI: 0.31-0.83), and in patients with existing FGR (RR 0.46, 95% CI: 0.25– 0.88). Subgroup analysis demonstrated that administration of L-arginine at a dose of <4 g/day, either for ≥1 month or in the III trimester, had a significant effect on birth weight in women with HDP without proteinuria. However, a higher dose of L-arginine was more beneficial in prolonging gestational age and reducing the risk of LBW in later pregnancy.

In addition, intravenous infusion of L-arginine, but not oral administration, significantly increased birth weight in pregnant women with FGR, although the recommended dose of L-arginine should be limited to <4 g/day [50].

In a systematic review of C.-N. Hsu and Y.-L. Tain (2019) analysed the role of arginine synthesis and metabolism during pregnancy and provided evidence of a link between disruption of the arginine metabolic pathway and the pathogenesis of compromised pregnancy and fetal programming. Interestingly, the authors presented the use of L-arginine as a potential reprogramming strategy during pregnancy to prevent non-communicable diseases in offspring. Much of the evidence supporting this idea focuses on the ability to improve fetal growth and development also in cases where placental function is compromised [17].

Among the studies conducted previously, none reported serious adverse reactions to L-arginine, which confirms its previously reported safety profile during pregnancy [25]. Significant side effects were recorded only in the population of patients with recent coronary heart disease during long-term treatment (6 months) with high doses (9 g/day) [41]. In addition, in a controlled study, L-arginine was found to be safe when used at a dose of 15-30 g/day for 90 days.

However, it should be noted that the use of L-arginine during pregnancy is still under discussion, as not all NO donors and precursors are considered safe during this period. Sildenafil, for example, when used for severe early FGR, not only did not reduce the risk of perinatal death or severe neonatal morbidity, but actually increased the risk of neonatal pulmonary hypertension [36].

Our results indicate that L-arginine can be used as a means to prevent the development of PE. It should be noted that the benefits of L-arginine in preventing PE exceed the cost of its treatment. In addition, in the current study, L-arginine demonstrated good results when administered to patients at 12-14 weeks of pregnancy, and its valuable pharmacological properties (indirect vasodilatory effect, increased microcirculation, antioxidant, cytoprotective, antihypoxic, detoxifying, membrane stabilizing effects) fit perfectly into the concept of adequate prevention of PE in the setting of endotheliopathy.

L-arginine therapy may be important because many patients with PE first consult a doctor when it is too late for other treatments, such as ASA, to be effective. The combined use of ASA and L-arginine should be evaluated to improve efficacy. Thus, oral treatment with L-arginine 4.0-4.2 g per day has a significant effect not only on preventing the development of PE in patients with prenosological forms of GE, but also on the development of placental dysfunction.

CONCLUSIONS

1. In the analysis of the clinical course of pregnancy and childbirth in women with gestational endotheliopathy (GE), on the background of the prescribed pathogenetically based prophylactic therapy, a positive effect of the proposed preventive treatment regimens (ASA and L-arginine) was found.

2. The early administration of ASA and L-arginine to pregnant women with a moderate degree of perinatal risk (preconceptional GE) allowed not only to prolong pregnancy but also to reliably prevent the development of preeclampsia (RR 0.39, 95% CI: 0.18– 0.84; p=0.02).

3. The more pronounced clinical efficacy of a course of L-arginine drinking solution (daily dose of L-arginine - 4.0-4.2 g) in pregnant women with pre-eclampsia may be associated with the endotheliotropic protective effect of the drug - a decrease in the number of preeclampsia cases (RR 0.19, 95% CI: 0.05-0.77; p=0.02) and placental hyperplasia/hypoplasia (RR 0.17, 95% CI: 0.04-0.68; p=0.01) compared with pregnant women who were diagnosed with GE and did not receive prophylactic therapy.

4. The use of L-arginine in the given dose and frequency of administration was not associated with side effects, so the safety profile of the drug can be considered favourable for pregnant women and the fetus.

5. The prophylactic use of L-arginine in clinical practice during pregnancy is still under discussion, and more researches are needed to determine the optimal dose, initiation and duration of use for the best preventive or therapeutic effect.

Conflict of interest. There is no conflict of interest between the authors.

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