UDC: 618.3:618.36-007.1:577.15:612.017
DOI: 10.15587/2519-4798.2025.340456

ASSESSMENT OF ENDOTHELIAL NITRIC OXIDE SYNTHASE ACTIVITY AND STABLE NITRIC OXIDE METABOLITE LEVELS IN PREGNANT WOMEN WITH HYPERTENSIVE DISORDERS

Volodymyr Siusiuka, Mykhailo Kyrychenko

The aim. *To evaluate nitric oxide system parameters in pregnant women with hypertensive disorders by assessing the levels of stable NO metabolites and the activity of endothelial NO synthase.*
Materials and methods. *A prospective controlled study included 65 pregnant women in the third trimester of pregnancy. The main group comprised 35 patients with gestational hypertension or preeclampsia, while the control group included 30 women with physiological pregnancies. Endothelial NO synthase (eNOS) activity was assessed spectrophotometrically by the stoichiometric oxidation of NADPH during the conversion of L-arginine to NO. The concentration of stable NO metabolites: nitrates and nitrites (NOx) was determined spectrophotometrically using the Griess reagent with preliminary reduction of nitrates to nitrites. For statistical analysis, the Mann-Whitney U test and Spearman's rank correlation coefficient were applied.*
Results*. In women with hypertensive disorders, eNOS activity was more than 3.5 times lower compared to the control group: 16.70 [16.10-17.60] vs. 60.45 [59.55-62.25] nmol/mg protein/min ($p < 0.05$). The NOx concentration was also nearly halved: 18.60 [17.80-19.25] vs. 34.80 [32.90-36.80] µmol/L ($p < 0.05$). A positive correlation was found between eNOS activity and NOx concentration ($\rho = 0.72$; $p < 0.05$), confirming their physiological interrelation.*
Conclusions. *Pregnant women with hypertensive disorders demonstrated a marked decrease in eNOS activity and stable NO metabolite levels, indicating significantly reduced NO bioavailability. These findings are consistent with current concepts regarding the role of endothelial dysfunction and oxidative stress in the pathogenesis of preeclampsia. Assessment of these biomarkers may have promising value for early diagnosis, prognosis of pregnancy complications, and may provide a rationale for novel preventive strategies*
Keywords: *hypertensive disorders of pregnancy, preeclampsia, nitric oxide, nitric oxide synthase, endothelial nitric oxide synthase (eNOS), stable NO metabolites, endothelial dysfunction, oxidative stress, nitric oxide bioavailability, biomarkers*

How to cite:
Siusiuka, V., Kyrychenko, M. (2025). Assessment of endothelial nitric oxide synthase activity and stable nitric oxide metabolite levels in pregnant women with hypertensive disorders. ScienceRise: Medical Science, 2 (63), 4–9. http://doi.org/10.15587/2519-4798.2025.340456

1. Introduction

Hypertensive disorders of pregnancy (HDP) remain a major challenge in modern obstetrics and are among the leading causes of maternal and perinatal morbidity and mortality [1]. The most common and clinically severe form of HDP is preeclampsia (PE), a condition characterized by complex pathophysiology and a profound impact on pregnancy outcomes [2].

According to several studies, abnormal placentation is considered a key pathogenic mechanism underlying PE. Insufficient trophoblast invasion of the spiral arteries and incomplete remodelling of the uteroplacental circulation result in impaired placental capacity to support adequate maternal-fetal exchange [3]. These alterations lead to hypoxia and ischemia of placental tissues, accompanied by excessive generation of reactive oxygen species (ROS) and the development of oxidative stress (OS). OS further aggravates placental injury and contributes to endothelial dysfunction, which is regarded as one of the central features of PE [4]. One of the main factors contributing to abnormal placentation and endothelial dysfunction in PE is the reduced bioavailability of nitric oxide (NO), a key vasodilator and regulator of arterial pressure in the placenta. Physiological pregnancy is associated with systemic vasodilation, largely mediated by NO, whose role is essential for maintaining effective vascular function throughout gestation [5].

NO is a signalling molecule with a broad range of physiological functions, including regulation of vascular tone and vasodilation, long-term potentiation of synaptic transmission, which underlies memory and learning in the central nervous system, as well as modulation of immune responses [6].

NO is synthesized by enzymes of the nitric oxide synthase (NOS) family, comprising three main isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and

inducible NOS (iNOS) [6, 7]. While iNOS is predominantly induced in immune cells and produces large amounts of NO over prolonged periods, eNOS and nNOS, in contrast, generate moderate amounts of NO, which primarily serve regulatory and signalling functions. An important feature of NOS enzymes is their ability, under certain conditions, to switch enzymatic activity, producing superoxide anion instead of NO, thereby reducing NO bioavailability and enhancing OS [7].

In the endothelium and placenta, NO synthesis is mediated by the endothelial isoform eNOS, which confers vasorelaxant and anti-aggregatory properties to the vascular wall. Moreover, eNOS plays a crucial role in placentation by promoting the synthesis of vascular endothelial growth factor (VEGF), essential for adequate placental vascularization [8].

Adequate NO bioavailability is considered a critical factor in pregnancy biology, as it ensures normal vascular tone and sufficient fetoplacental blood flow [9]. In PE, a marked decrease in NO levels and impaired regulation of its synthesis are observed, contributing to vascular dysfunction and hypertension [5].

One of the main drivers of this reduction is OS, which inhibits eNOS activity and facilitates the formation of peroxynitrite, further damaging the endothelium [4]. The generation of peroxynitrite is regarded as a key mechanism linking OS, NO deficiency, and progression of vascular complications in PE [4].

Thus, NO deficiency and decreased eNOS activity play a pivotal role in the development of endothelial dysfunction in preeclampsia, thereby influencing the clinical course of this complication [5].

Objective. To evaluate the parameters of the nitric oxide system in pregnant women with hypertensive disorders by assessing the levels of stable NO metabolites and the activity of endothelial NO synthase.

2. Materials and Methods

This prospective controlled observational study, conducted from 2020 to 2023, included 65 pregnant women in the third trimester, followed up at the Consultative and Diagnostic Department of the Communal Non-Commercial Enterprise «Regional Perinatal Center» of the Zaporizhzhia Regional Council. The study group comprised 35 women with singleton pregnancies complicated by gestational hypertension (GH) or PE of moderate or severe forms, diagnosed according to the current unified clinical guidelines of the Ministry of Health of Ukraine. The control group consisted of 30 women with uncomplicated singleton pregnancies [10, 11].

The median gestational age at examination was 30 [29–31] weeks in the HDP group and 29 [28–30] weeks in the control group. The mean age of participants was 31 [27–34] years in the study group and 28 [25–32] years in the control group, with no statistically significant differences between groups ($p > 0.05$). No significant differences were observed in social or occupational background ($p > 0.05$).

Blood plasma was analyzed for NO system parameters: eNOS activity and the concentration of stable NO metabolites: nitrates and nitrites (NOx). eNOS activity was determined spectrophotometrically by measuring the stoichiometric oxidation of NADPH during the conversion of L-arginine to NO. The decrease in NADPH concentration was monitored by measuring the reduction in optical density at 340 nm over a 4-minute period. To confirm specificity, the NOS inhibitor N-nitro-L-arginine (1 mM) was added. Results were expressed as nmol/mg protein/min. The concentration of NOx was measured spectrophotometrically using the Griess reagent, based on azo compound formation from the interaction of nitrite ions with naphthyl acetate and sulfanilic acid in an alkaline medium. For quantitative assessment of nitrates, they were first reduced to nitrites using nitrate reductase. Optical density was measured at 540 nm, and results were expressed in μmol/L [12].

Laboratory analyses were performed at the Educational and Scientific Medical Laboratory Center with vivarium, Zaporizhzhia State Medical and Pharmaceutical University. All measurements were conducted on certified equipment in accordance with validated laboratory protocols.

The study was conducted in accordance with ICH/GCP requirements, the Declaration of Helsinki (1964, as amended), the Council of Europe Convention on Human Rights and Biomedicine, and applicable Ukrainian legislation. Written informed consent was obtained from all participants. The protocol was approved by the Bioethics Committee of Zaporizhzhia State Medical University (Protocol No. 1, meeting of January 14, 2020). Zaporizhzhia State Medical University existed until 31 March 2023 and was subsequently reorganized as Zaporizhzhia State Medical and Pharmaceutical University.

Statistical analysis was performed using Microsoft Excel and STATISTICA 13. The Shapiro-Wilk test was used to assess data distribution. Intergroup comparisons were performed with the Mann-Whitney U test, and Spearman's correlation coefficient was applied to evaluate associations between variables.

3. Results

The present study evaluated plasma eNOS activity and NOx concentration in pregnant women with HDP compared with controls with uncomplicated pregnancies. Comparative analysis revealed significant intergroup differences (Table 1).

Table 1

Comparative analysis of eNOS and NOx levels in pregnant women with HDP and in the control group

Marker	Study group (n=35)	Control group (n=30)	p-value
eNOS (nmol/mg protein/min)	16.70 [16.10–17.60]	60.45 [59.55–62.25]	$p < 0.05$
NOx (μmol/L)	18.60 [17.80–19.25]	34.80 [32.90–36.80]	$p < 0.05$

Note: data are expressed as median [Q1–Q3], where Q1 and Q3 represent the 25th and 75th percentiles, respectively

In women with HDP, eNOS activity was more than 3.5-fold lower than in the control group: 16.70 vs. 60.45 nmol/mg protein/min ($p < 0.05$). The concentration of stable NO metabolites was also almost twofold lower: 18.60 vs. 34.80 μmol/L, respectively ($p < 0.05$).

These findings demonstrate a marked reduction in both eNOS activity and NOx concentration, consistent with decreased NO bioavailability in pregnant women with hypertensive disorders (Fig. 1, 2).

Additionally, correlation analysis was conducted to evaluate the relationship between eNOS activity and NOx levels. Spearman's rank correlation showed a strong positive correlation between these parameters ($\rho = 0.72$; $p < 0.05$), indicating that higher eNOS activity was associated with increased levels of stable NO metabolites. This result supports the pathophysiological interconnection of these biomarkers in regulating the nitric oxide system during pregnancy (Fig. 3).

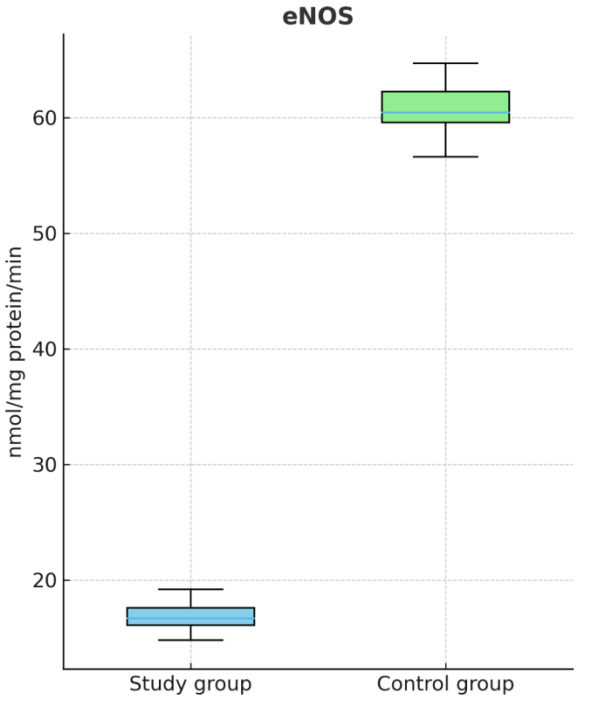

Fig. 1. Levels of eNOS in pregnant women: comparison between the study and control groups (nmol/mg protein/min)

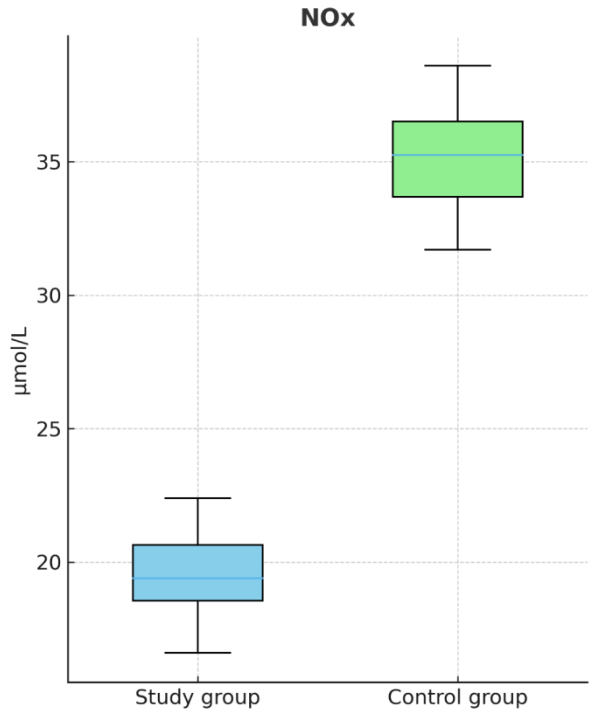

Fig. 2. Concentration of NO metabolites in pregnant women: comparison between the study and control groups (μmol/L)

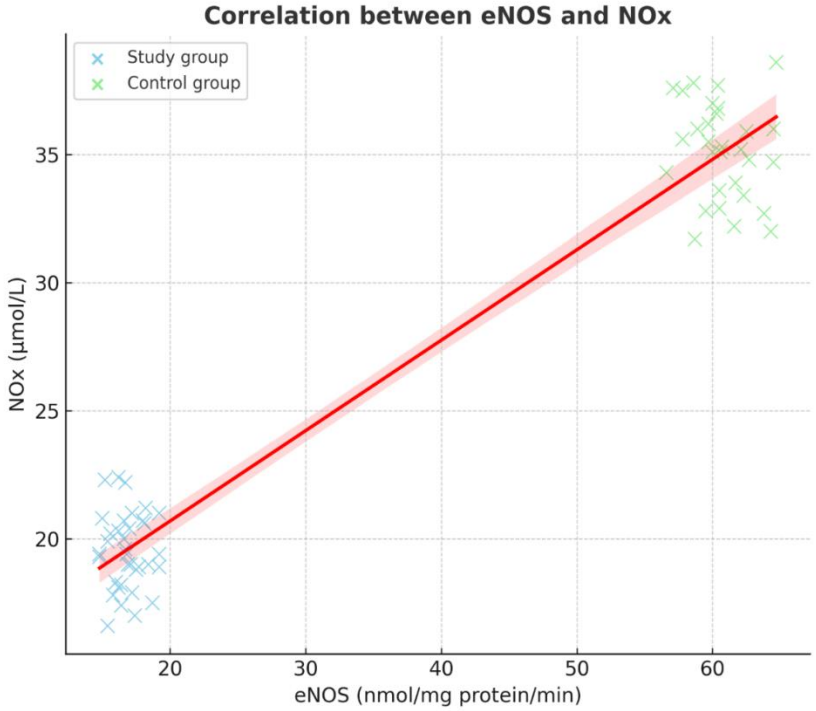

Fig. 3. Correlation between eNOS activity and NO metabolites in pregnant women

4. Discussion

The findings of this study demonstrated significantly reduced eNOS activity and concentrations of stable NO metabolites in pregnant women with HDP compared with the control group ($p < 0.05$). These results are consistent with current research emphasizing the potential contribution of NO deficiency and oxidative stress (OS) to the pathogenesis of PE [3].

Several studies have shown that the excessive generation of reactive oxygen species and an imbalance within the NO system may reduce its bioavailability, thereby contributing to the development of endothelial dysfunction [13]. These mechanisms are incorporated into the updated two-stage model of placentation, which explains the gradual progression of clinical manifestations of PE [14]. Furthermore, review studies have emphasized that endothelial dysfunction is a central element of HDP pathogenesis, as it directly affects vascular tone and placental microcirculation [15]. In this context, the decreased eNOS activity observed in our study may reflect impaired endothelial capacity to synthesize NO, thereby limiting its vasodilatory and anti-aggregatory properties. Similar observations have been reported in the literature, where suppression of eNOS activity has been associated with impaired NO synthesis in women with HDP, particularly PE [13, 16].

In the present study, a significant positive correlation ($p < 0.05$) was observed between eNOS activity and NOx levels, highlighting their pathophysiological interrelationship. This finding is consistent with other reports describing a simultaneous reduction in eNOS activity and stable NO metabolites in patients with PE [14, 17]. These findings suggest that suppression of enzymatic NO synthesis is accompanied by depletion of its metabolites, which may serve as a marker of endothelial alterations in PE.

The reduced NO bioavailability identified in our study is likely to be of clinical significance, as NO is regarded as a critical regulator of vascular tone, placentation, and uteroplacental blood flow [5]. Several publications suggest that NO deficiency may be associated with vasospasm, ischemia, and placental hypoperfusion, which in turn contribute to the clinical manifestations of PE [9]. Thus, our findings are consistent with contemporary concepts regarding the role of disturbances in the NO system in the vascular changes underlying HDP [3, 15].

The demonstrated decrease in eNOS activity and NOx concentration may reflect specific pathogenetic pathways of vascular impairment in pregnant women with HDP. These results provide biochemical evidence of alterations in the NO system; however, they require further confirmation in larger and more representative cohorts. Expanding the panel of NO-related biomarkers in future studies could allow for a more precise evaluation of their diagnostic and prognostic significance in HDP [4, 8].

Study Limitations. This study was performed on a relatively small sample ($n = 65$), and the inclusion of additional biomarkers could have provided further insights into the mechanisms underlying alterations in pregnant women with HDP.

Future Research Perspectives. Future studies should include larger cohorts of pregnant women and assess a broader range of biomarkers related to the nitric oxide system and oxidative stress. Such investigations may enable a more precise evaluation of their clinical relevance and potential prognostic value in hypertensive disorders of pregnancy.

5. Conclusions

1. Pregnant women with hypertensive disorders, including preeclampsia, demonstrated a statistically significant reduction in endothelial nitric oxide synthase activity compared with the control group: 16.70 [16.10–17.60] vs.

60.45 [59.55–62.25] nmol/mg protein/min ($p < 0.05$).

2. The concentration of stable nitric oxide metabolites in women with hypertensive disorders of pregnancy was almost twofold lower than in the control group: 18.60 [17.90–19.80] vs. 34.80 [33.50–36.10] μmol/L ($p < 0.05$), suggesting reduced nitric oxide bioavailability.

3. A significant positive correlation between endothelial nitric oxide synthase activity and the levels of stable nitric oxide metabolites ($\rho = 0.72$; $p < 0.05$) confirms their close interrelationship in pregnant women with hypertensive disorders of pregnancy.

4. The demonstrated association between suppressed endothelial nitric oxide synthase activity and depletion of nitric oxide metabolites supports the role of impaired nitric oxide system regulation in the pathogenesis of preeclampsia. It underscores the potential utility of these biomarkers for clinical monitoring.

Conflict of interest

The authors declare that they have no conflict of interest related to this study, including financial, personal, authorship-related, or any other potential conflicts that could have influenced the research or the results presented in this article**.**

Funding

The study is a fragment of scientific research work of Zaporizhzhia State Medical and Pharmaceutical University on the topic “Prediction and prevention of gestation complications in women with comorbid states”, state registration No. 0121U112325 (2021–2025).

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence Tools

The authors confirm that no artificial intelligence technologies were used in the preparation, analysis, or writing of this manuscript.

References

1. Trends in maternal mortality 2000 to 2023: estimates by WHO, UNICEF, UNFPA, World Bank Group and UNDESA/Population Division (2025). World Health Organization, UNICEF, UNFPA, World Bank Group, UNDESA/Population Division. Geneva: World Health Organization.

2. Brown, M. A., Magee, L. A., Kenny, L. C., Karumanchi, S. A., McCarthy, F. P., Saito, S. et al. (2018). Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. Hypertension, 72 (1), 24–43. https://doi.org/10.1161/hypertensionaha.117.10803

3. Rana, S., Lemoine, E., Granger, J. P., Karumanchi, S. A. (2019). Preeclampsia: Pathophysiology, Challenges, and Perspectives. Circulation Research, 124 (7), 1094–1112. https://doi.org/10.1161/circresaha.118.313276

4. Guerby, P., Tasta, O., Swiader, A., Pont, F., Bujold, E., Parant, O. et al. (2021). Role of oxidative stress in the dysfunction of the placental endothelial nitric oxide synthase in preeclampsia. Redox Biology, 40, 101861. https://doi.org/10.1016/j.redox.2021.101861

5. Sutton, E. F., Gemmel, M., Powers, R. W. (2020). Nitric oxide signaling in pregnancy and preeclampsia. Nitric Oxide, 95, 55–62. https://doi.org/10.1016/j.niox.2019.11.006

6. Lundberg, J. O., Weitzberg, E. (2022). Nitric oxide signaling in health and disease. Cell, 185 (16), 2853–2878. https://doi.org/10.1016/j.cell.2022.06.010

7. Barbouti, A., Varvarousis, D. N., Kanavaros, P. (2025). The Role of Oxidative Stress-Induced Senescence in the Pathogenesis of Preeclampsia. Antioxidants, 14 (5), 529. https://doi.org/10.3390/antiox14050529

8. Osol, G., Ko, N. L., Mandalà, M. (2017). Altered Endothelial Nitric Oxide Signaling as a Paradigm for Maternal Vascular Maladaptation in Preeclampsia. Current Hypertension Reports, 19 (10). https://doi.org/10.1007/s11906-017-0774-6

9. Zullino, S., Buzzella, F., Simoncini, T. (2018). Nitric oxide and the biology of pregnancy. Vascular Pharmacology, 110, 71–74. https://doi.org/10.1016/j.vph.2018.07.004

10. Standarty medychnoi dopomohy «Normalna vahitnist» (2022). Nakaz MOZ Ukrainy No. 1437. 09.08.2025. Available at: https://www.dec.gov.ua/wp-content/uploads/2022/08/2022_1437_smd_nv.pdf

11. Yedynyi klinichnyi protokol pervynnoi, vtorynnoi (spetsializovanoi) ta tretynnoi (vysokospetsializovanoi) medychnoi dopomohy «Hipertenzyvni rozlady pid chas vahitnosti, polohiv ta v pisliapolohovyi period» (UKPMD) (2022). Nakaz MOZ Ukrainy No. 151. 24.01.2025. Available at: https://www.dec.gov.ua/wp-content/uploads/2022/01/2022_151_ykpmd_giprozlvagitn.pdf

12. Chekman, I. S., Bieliienchev, I. F., Nahorna, O. O., Horchakova, N. O., Lukianchuk, V. D., Bukhtiiarova, N. V. et al. (2016). Doklinichne vyvchennia spetsyfichnoi aktyvnosti potentsiinykh likarskykh zasobiv pervynnoi ta vtorynnoi neiroprotektsii. Kyiv, 93.

13. Afrose, D., Alfonso-Sánchez, S., McClements, L. (2024). Targeting oxidative stress in preeclampsia. Hypertension in Pregnancy, 44(1). https://doi.org/10.1080/10641955.2024.2445556

14. Staff, A. C. (2019). The two-stage placental model of preeclampsia: An update. Journal of Reproductive Immunology, 134-135, 1–10. https://doi.org/10.1016/j.jri.2019.07.004

15. Dimitriadis, E., Rolnik, D. L., Zhou, W., Estrada-Gutierrez, G., Koga, K., Francisco, R. P. V. et al. (2023). Pre-eclampsia. Nature Reviews Disease Primers, 9 (1). https://doi.org/10.1038/s41572-023-00417-6

16. Burton, G. J., Redman, C. W., Roberts, J. M., Moffett, A. (2019). Pre-eclampsia: pathophysiology and clinical implications. BMJ, l2381. https://doi.org/10.1136/bmj.l2381

17. Grzeszczak, K., Łanocha-Arendarczyk, N., Malinowski, W., Ziętek, P., Kosik-Bogacka, D. (2023). Oxidative Stress in Pregnancy. Biomolecules, 13 (12), 1768. https://doi.org/10.3390/biom13121768

Received 14.08.2025
Received in revised form 11.09.2025
Accepted 23.09.2025
Published 03.10.2025

Volodymyr Siusiuka, Doctor of Medical Sciences, Professor, Head of Department, Department of Obstetrics and Gynecology, Zaporizhzhia State Medical and Pharmaceutical University, Marii Prymachenko blvd., 26, Zaporizhzhia, Ukraine, 69035
ORCID: https://orcid.org/0000-0002-3183-4556

Mykhailo Kyrychenko*, Assistant, Department of Obstetrics and Gynecology, Zaporizhzhia State Medical and Pharmaceutical University, Marii Prymachenko blvd., 26, Zaporizhzhia, Ukraine, 69035
ORCID: https://orcid.org/0000-0002-8658-9148

***Corresponding author:** Mykhailo Kyrychenko, e-mail: kirichenkomihail93@gmail.com*