

Influence of maternal cholesterol-enriched diet on chemical composition of teeth enamel in offspring of mice

Inessa I. Yakubova¹, Victor Dosenko², Volodymyr Ostrianko³, Serhii Tsypan¹, Liliia Bielova¹, Ganna Viun¹

¹PRIVATE HIGHER EDUCATIONAL ESTABLISHMENT «KYIV MEDICAL UNIVERSITY», KYIV, UKRAINE

²THE BOGOMOLETS INSTITUTE OF PHYSIOLOGY NAS OF UKRAINE, KYIV, UKRAINE

³SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

ABSTRACT

Aim: To determine the chemical composition of the tooth enamel of two-day-old mice from hypercholesterolemic mothers by energy dispersive X-ray spectroscopy.

Materials and Methods: Forty mature female mice were randomly assigned ($n = 20/\text{group}$) to either a standard chow vivarium diet (control group) or a cholesterol-enriched chow diet (experimental group). After fertilization, pregnancy and birth, on postnatal day 2, the incisor segments of 6 pups from each group were used for energy dispersive X-ray spectroscopy.

Results: Influence of maternal hypercholesterolemic diet on tooth development and mineralization was examined, which revealed changes in enamel chemical composition. First, the results indicate the presence of seven elements (Na, Cl, Ca, P, Mg, S, Fe) in the enamel of both the hypercholesterolemic and normal offspring, but the content of element Ca²⁺ decreased, the content of elements P⁵⁺, Na⁺, Cl⁻ tended to increase in pups from hypercholesterolemic mice. Second, the initial level of mineralization according to the atomic (%) Ca / P in hypercholesterolemic pups ratio was 1.26, comparing with normal pups where level of mineralization was 1.34. Taking into account that irreversible changes in the structure of the enamel were observed when the Ca / P ratio was below 1.33, we can suggest that the eruption of teeth with an imperfect structure could be because of maternal hypercholesterolemic diet.

Conclusions: Results of this study suggest that hypercholesterolemic diet during gestation and lactation leads to altered enamel mineralization in mice because of changes in chemical composition and may link to the early childhood caries.

KEY WORDS: the tooth enamel mice, cholesterol-enriched diet, chemical composition of teeth enamel

Wiad Lek. 2024;77(2):299-304. doi: 10.36740/WLek202402117 DOI

INTRODUCTION

Nutrition during pregnancy and lactation is perhaps the most influential but often overlooked non-genetic factor in fetal development [1, 2]. A maternal diet containing adequate quantities of micro- (vitamins, macro- and microelements) and macronutrients (proteins, fats, carbohydrates) is a key point for maintaining the health of a pregnant woman and plays an important role in the growth and development of the fetal oral cavity and the teeth [3, 4]. A low-protein diet during gestation-lactation results in impaired odontogenesis that may increase susceptibility of dental anomalies [5]. Studies have suggested that enamel hypoplasia, salivary gland hypofunction and saliva compositional changes may be the mechanisms through which the malnutrition is associated with caries, while an altered eruption timing may create a challenge in the analysis of the age specific caries rates [6]. Not only a deficiency, but also an excess of nutrients can negatively affect the course of pregnancy and fetal organism, as well as the

fetal teeth condition in the pre-eruption phase, as it can affect the cellular architecture of the organic matrix and enamel maturation processes [7, 8]. Maternal high-fat diet together with embryonic *Cited2* deficiency significantly reduces the expression of the left-determining gene *Pitx2*, with a dramatic increase in the penetrance of left-right patterning defects, and the appearance of novel defects including cleft palate [9]. Cholesterol intake increases during pregnancy because pregnant women are advised to consume more eggs and other foods rich in cholesterol [10]. However, higher dietary cholesterol from eggs intake during pregnancy was associated with greater risk of gestational diabetes mellitus [11]. The result of a hypercholesterolemic diet, on the one hand, is an increase in the expression of BMP2, which accelerates odontogenesis, on the other hand, a decrease in the expression of the osteocalcin gene leads to insufficient saturation of the tooth hard tissues with hydroxyapatite, which results in the eruption of teeth with low mineralization [12].

AIM

The aim of the study was to determine the chemical composition of the tooth enamel of two-day-old mice from hypercholesterolemic mothers by energy dispersive X-ray spectroscopy. We hypothesized that hypercholesterolemia during gestation and lactation would result in altered mineralization of the enamel.

MATERIALS AND METHODS

ANIMALS AND DIET

Forty mature female mice were purchased and housed in the vivarium of the Bogomolets Institute of Physiology National Academy of Science of Ukraine (Kyiv, Ukraine) in stainless steel cages under controlled conditions (20–24 °C, 50–60% relative humidity, 12-h light–dark cycle) with free access to water. Mice were randomly assigned ($n = 20/\text{group}$) to either a standard chow vivarium diet (24% protein, 11% fat, 48% carbohydrates, 5.5% fiber, 6% vitamin, 5.5% ash) (control group) or a cholesterol-enriched chow diet (a standard chow vivarium diet with the addition of 0.2% Cholesterol (manufactured by Merck, Germany) (experimental group). Female mice were given ad libitum access to their respective diet throughout the study duration. Females in proestrus or estrus phase were mated overnight with male breeders (4 females with 1 male) and separated the following morning. The presence of spermatozoa in the vaginal smear was considered as an indicator of fertilization and first day of pregnancy (gestational *day (GD) 0*). Pregnant females had spontaneous birth, and day of birth was recorded as day 0 of the mouse's life. Throughout the suckling period the dams remained on their respective diets.

TISSUE COLLECTION

At weaning (postnatal day 2) 6 pups from each group (appearance features were nubs in ear area, visible milk spot and manifesting pigment on skin) were by inhalation overdose of carbon dioxide. CO₂ exposure time is recommended 60 minutes for euthanasia of two day old mice (D-2) [13]. The lower jaws were removed and all soft tissue carefully cleaned by dissection. All samples were stored in test tubes (10% streptomycin solution), tightly closed, at a temperature of +2...+4°C. Before the study, the samples were washed with hands wearing rubber gloves in distilled water and passively dried. The incisor segments were used for scanning electron microscopy–Energy dispersive X-ray analysis. The mice used in this experiment were cared for in accordance

with the guidelines established by the «Rules and Regulations for Carrying Out Animal Research Work» [14]. All procedures were reviewed and approved by the Private Higher Educational Institution «Kyiv Medical University».

SCANNING ELECTRON MICROSCOPY–ENERGY DISPERSIVE X-RAY ANALYSIS

When jaws were passively dried, the samples were placed in a vacuum apparatus (Ion Sputter JFC-1600, Jeol, Japan) for 5 min until the residual moisture completely evaporated. The samples were affixed to the scanning electron microscopy (SEM) stubs and sputter-coated with a thin layer of Pt (~25 nm), in order to make the material conductive (Fig. 1). examined with a JSM-6100 JEOL SEM operating at 15 kV and 15–20 mm working distance.

SEM analysis was performed by JSM-6100 JEOL SEM under the standard high vacuum mode, operating at 5–10 kV throughout the analysis and 15–20 mm working distance. Measurements took place on 19 areas of enamel in the control group and on 21 areas in the experimental group. The morphology of each sample was imaged with a variety of magnifications. The size of the areas of enamel spectroscopy ranged from 50x50 µm to 250x250 µm.

The chemical composition of the enamel was obtained using an energy dispersive X-ray spectral analyzer INCA Energy 450 (Oxford Instruments Nanoanalysis, UK). EDX analysis was performed at 10 kV and a 10 mm working distance. The count was conducted on the vestibular surface of incisors (19 areas of enamel in the control group and on 21 areas in the experimental group). The elements quantified were Oxygen (O), Sodium (Na), Chlorine (Cl), Calcium (Ca), Phosphorus (P), Magnesium (Mg), Sulfur (S), Ferrum (Fe). All analyzed elements were normalized to the factory calibration provided by Oxford Instruments Aztec/INCA software and an Oxford Instruments X-Max 50 mm² detector system. C, O, F, Na, Mg, P, and Ca were calibrated based on CaCO₃, SiO₂, MgF₂, albite, MgO, GaP and wollastonite. The element content was calculated as the relative weight percentage of the total element content (100%).

STATISTICAL ANALYSIS

Statistical analysis of the digital data was performed using Excel 2000 and Origin 7.0. Results were expressed mainly as means ± standard error of the mean. Probability distribution of mean ($P < 0.05$) was calculated using Student's t-test.

Table 1. Offspring characteristics of experimental and control groups

Groups	Gestation length, days±SD	Average litter size, ± SD	Male/female (ratio)	Birth weight ± SE, g	Body length at birth ± SE, mm	Survival rate at 2 PNDs
Experimental, n=20	18.9±0.6*	7.21 ± 1.1*, n=144	74/70 (1.06)*	1.22±0.01*	25.00±0.16*	95 %*, n=137
Control, n=20	19.0±0.3	7.14±2.7, n=142	75/67 (1.12)	1.28±0.01	24.93±0.13	97 %, n=139

1. SD standard deviation
2. PND – postnatal days
3. *p ≥ 0.05.

Table 2. Chemical (elemental) composition of D-2 mouse teeth enamel by the EDX method of the control group

	Chemical element	The number of examined samples	Number of samples containing a chemical element		Qualitative composition of samples, %	
			abs	%		
Atomic	O	19	19	100	61.65	
	Na	19	19	100	1.15	
	Cl	19	19	100	0.31	
	Ca	19	19	100	21.82	
	P	19	19	100	14.35	
	Ca/P					1.34
	Mg	19	1	5,26	0.89	
	S	19	1	5,26	0.36	
	Fe	19	9	47,37	0.45	
	Weight	O	19	19	100	41.82
Na		19	19	100	1.13	
Cl		19	19	100	0.47	
Ca		19	19	100	36.55	
P		19	19	100	19.28	
Mg		19	1	5,26	0.92	
S		19	1	5,26	0.5	
Fe		19	9	47,37	1.06	

RESULTS

OFFSPRING PHENOTYPE

Each dam was between 6–9 pups with an average size of 7.2±1.2 in experimental group and 7.1 ± 2.7 in control group (means ± standard deviations). There were no other significant differences in the following parameters between the pups of experimental and control groups: gestation length, survival rate, average litter size, male/female ratio, birth weight, and birth body length (Table 1).

SEM-EDX ANALYSIS

Fig. 1. shows the photo of enamel surface on the vestibular area of incisor of a two-day-old mouse, that was made by scanning electron microscopy.

Characteristic X-ray spectra were obtained on the vestibular area of incisor of a two-day-old mouse with the help of an energy dispersive X-ray spectrum analyzer (Fig. 2).

Different distribution of the trace elements concentration in tooth enamel was revealed using energy-dispersive spectral X-ray diffraction analysis. In the enamel of the experimental and the control groups were found seven elements – Na, Cl, Ca, P, Mg, S, Fe. It was found that the chemical elements Ca²⁺, P⁵⁺, Na⁺, Cl⁻ were found in 100% of the samples in the control group when studying the chemical composition of the D-2 mouse teeth enamel using the EDX method (Table 2).

Elements Mg²⁺, S were found in 5.26% of the samples, Fe³⁺ – in 47.37%. The Ca²⁺ content was 36.55%, the P⁵⁺ content was 19.28%. The initial level of mineralization according to the atomic (%) Ca / P ratio was 1.34. It was found that the chemical elements Ca²⁺, P⁵⁺, Na⁺, Cl⁻ were found in 100% of the samples in experimental group, which received a diet with an increased content of Cholesterol (Table 3).

DISCUSSION

In this study, influence of maternal hypercholesterolemia diet on tooth development and mineralization was

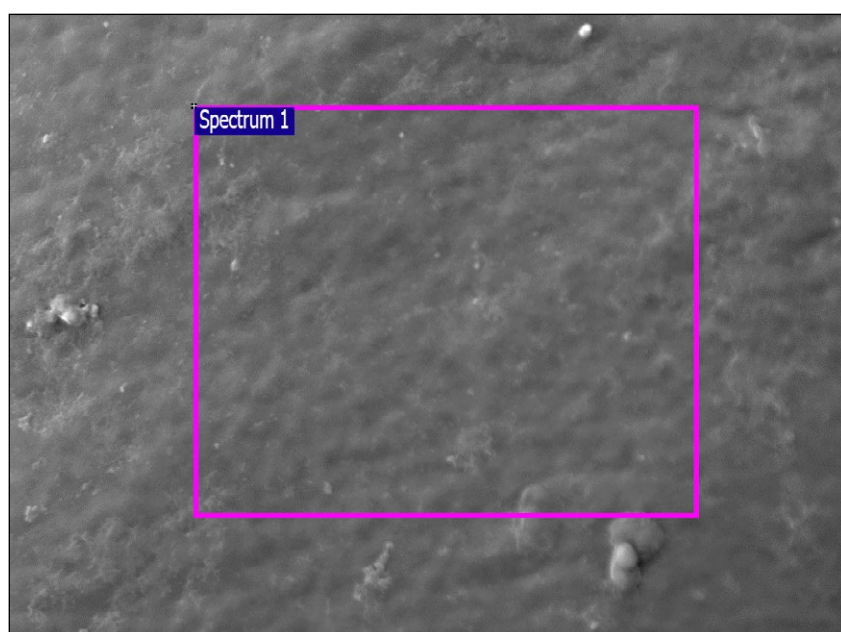


Fig. 1. A section of the tooth enamel (vestibular surface of incisor) of a two-day-old mouse (D-2), obtained by SEM where the chemical (elemental) composition was measured.

Table 3. Chemical (elemental) composition of D-2 mouse teeth enamel by the EDX method of the experimental group

	Chemical element	The number of examined samples	Number of samples containing a chemical element		Qualitative composition of samples, %
			abs.	%	
Atomic	O	21	21	100	61.64
	Na	21	21	100	1.23
	Cl	21	21	100	0.33
	Ca	21	21	100	20.29
	P	21	21	100	16.08
	Ca/P				1.26
	Mg	21	3	14,29	0.92
	S	21	3	14,29	0.36
	Fe	21	11	52,38	0.47
	Weight	O	21	21	100
Na		21	21	100	1.20
Cl		21	21	100	0.50
Ca		21	21	100	34.51
P		21	21	100	21.14
Mg		21	3	14,29	0.92
S		21	3	14,29	0.50
Fe		21	11	52,38	1.12

examined, which revealed changes in enamel chemical composition. We found out few findings that warrant further discussion. First, the results of this study indicate the presence of seven elements (Na, Cl, Ca, P, Mg, S, Fe) in the enamel of both the hypercholesterolemic and normal offspring, but the content of element Ca²⁺ decreased, the content of elements P⁵⁺, Na⁺, Cl⁻ tended to increase in pups from hypercholesterolemic mice. Second, the initial level of mineralization according to the atomic (%) Ca / P in hypercholesterolemic pups ratio was 1.26, comparing with normal pups where level of mineralization was 1.34 (at the lower limit (1.33), that

suggests that the enamel of the teeth of mice that have just erupted is immature). Taking into account that irreversible changes in the structure of the enamel were observed when the Ca / P ratio was below 1.33 [5], we can suggest that the eruption of teeth with an imperfect structure could be because of maternal hypercholesterolemic diet during gestation and lactation.

Although previous work suggests that maternal high-fat diet during pregnancy and lactation can alter fetal cholesterol metabolism and predispose adult offspring to metabolic abnormalities [15, 16], no investigation has assessed the relationship between maternal hyper-

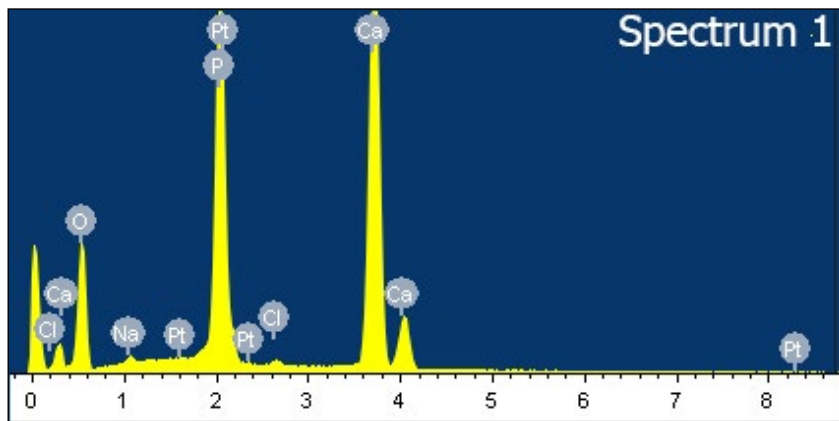


Fig. 2. X-ray characteristic spectrum of the tooth enamel surface (vestibular surface of incisor) of a two-day-old mouse (D-2 mouse) obtained using an energy dispersive X-ray spectrum analyzer.

cholesterolemia and chemical composition of tooth enamel. In our study we have found that a hypercholesterolemic diet during pregnancy and lactation is associated with enamel maturation violation.

The study of the chemical composition of the surface layers of enamel is relevant due to the fact that the variability of the elemental composition of teeth allows to detect metabolic disorders in the process of tooth development and after eruption [17]. The large amounts of free fatty acids, diglycerides, cholesterol and phospholipids as intrinsic components can influence on enamel maturation [18]. A limited number of studies reported significant associations between molar incisor hypomineralization and pre- and perinatal factors such as maternal illness and medication use in pregnancy [19]. There are data in the literature that the intensity of caries of temporary teeth in children is affected by the amount

of sweet food consumed by a pregnant woman [20]. Evidence suggests that cholesterol intake increases during pregnancy, so maternal hypercholesterolemia could be one of the factors that leads to hypomineralization of the enamel and in its turn manifested in early caries [21].

CONCLUSIONS

In summary, results of this study suggest that hypercholesterolemic diet during gestation and lactation leads to altered enamel mineralization in mice because of changes in chemical composition. Based on the rise of cholesterol intake during pregnancy and the early childhood caries because of the eruption of teeth with an imperfect structure mechanism of maternal hypercholesterolemia influence on enamel maturation needs further investigation.

REFERENCES

- Schwarzenberg SJ, Georgieff MK, Daniels S et al. Advocacy for Improving Nutrition in the First 1000 Days to Support Childhood Development and Adult Health. *Pediatrics*. 2018;141(2):e20173716. doi: 10.1542/peds.2017-3716. [DOI](#)
- Marangoni F, Cetin I, Verduci E et al. Maternal Diet and Nutrient Requirements in Pregnancy and Breastfeeding. An Italian Consensus Document. *Nutrients*. 2016;8(10):629. doi: 10.3390/nu8100629. [DOI](#)
- Mousa A, Naqash A, Lim S. Macronutrient and Micronutrient Intake during Pregnancy: An Overview of Recent Evidence. *Nutrients*. 2019;11(2):443. doi: 10.3390/nu11020443. [DOI](#)
- Yenen Z, Ataçağ T. Oral Care in Pregnancy. *J. Turkish-German Gynecol. Assoc.* 2019;20(4):264-268. doi: 10.4274/jtgga.galenos.2018.2018.0139. [DOI](#)
- Calsa B, Bortolança TJ, Masiero BC et al. Maxillary and Dental Development in the Offspring of Protein-restricted Female Rats. *Eur. J. Oral Sci.* 2022. doi:10.1111/eos.12895. [DOI](#)
- Sheetal A. Malnutrition and Its Oral Outcome – A Review. *J. Clin. DIAGNOSTIC Res.* 2013. doi:10.7860/JCDR/2012/5104.2702. [DOI](#)
- Waksmańska W, Bobiński R, Ulman-Włodarz I, Pieliesz A. The Differences in the Consumption of Proteins, Fats and Carbohydrates in the Diet of Pregnant Women Diagnosed with Arterial Hypertension or Arterial Hypertension and Hypothyroidism. *BMC Pregnancy Childbirth*. 2020;20(1):29. doi: 10.1186/s12884-019-2711-y. [DOI](#)
- Dumolt JH, Ma M, Mathew J et al. Gestational Hypercholesterolemia Alters Fetal Hepatic Lipid Metabolism and MicroRNA Expression in Apo-E-Deficient Mice. *Am. J. Physiol. Metab.* 2019;317(5):E831-E838. doi: 10.1152/ajpendo.00138.2019. [DOI](#)
- Bentham J, Michell AC, Lockstone H et al. Maternal High-Fat Diet Interacts with Embryonic Cited2 Genotype to Reduce Pitx2c Expression and Enhance Penetrance of Left-Right Patterning Defects. *Hum. Mol. Genet.* 2010;19(17):3394-401. doi: 10.1093/hmg/ddq251. [DOI](#)
- Ruxton C. Value of Eggs during Pregnancy and Early Childhood. *Nurs. Stand.* 2013;27(24):41-50; quiz 51. doi: 10.7748/ns2013.02.27.24.41.e7343. [DOI](#)

11. Wu Y, Sun G, Zhou X et al. Pregnancy Dietary Cholesterol Intake, Major Dietary Cholesterol Sources, and the Risk of Gestational Diabetes Mellitus: A Prospective Cohort Study. *Clin. Nutr.* 2020;39(5):1525-1534. doi: 10.1016/j.clnu.2019.06.016. [DOI](#)
12. Yakubova I. Vplyv diyety z pidvyshchenym vmistom Cholesterol na morfolohichni zminy zachatkov zubiv ta eksperesiyu heniv, yaki koduyut' kistkovyy morfo-henetychnyy proteyin ta osteokal'tsyn u tkanyakh nyzhn'oyi shchelepy embrioniv myshey [Effect Of Diet with Increasing Cholesterol of Morphological Changes Rudiments of Teeth and Gene Expression That Encode Bone Morphogenetic Protein and Osteocalcin in Tissues Mandible Mouse Embryos]. *Visnyk problem biolohiyi i medytsyny.* 2012;3(2):224–229. (Ukrainian).
13. Pritchett K, Corrow D, Stockwell J, Smith A. Euthanasia of Neonatal Mice with Carbon Dioxide. *Comp. Med.* 2005;55(3):275-81.
14. Rules and Regulations for Carrying Out Animal Research Work. 2008, pp.251–286. doi:10.1142/9789812779595_0003. [DOI](#)
15. Saengnipanthkul S, Noh HL, Friedline RH et al. Maternal Exposure to High-fat Diet during Pregnancy and Lactation Predisposes Normal Weight Offspring Mice to Develop Hepatic Inflammation and Insulin Resistance. *Physiol. Rep.* 2021. doi:10.14814/phy2.14811. [DOI](#)
16. Dumolt JH, Radhakrishnan SK, Moghadasian MH et al. Maternal Hypercholesterolemia Enhances Oxysterol Concentration in Mothers and Newly Weaned Offspring but Is Attenuated by Maternal Phytosterol Supplementation. *J. Nutr. Biochem.* 2018;52:10-17. doi: 10.1016/j.jnutbio.2017.09.013. [DOI](#)
17. Sarna-Boś K, Boguta P, Skic K et al. Physicochemical Properties and Surface Characteristics of Ground Human Teeth. *Molecules.* 2022;27(18):5852. doi: 10.3390/molecules27185852. [DOI](#)
18. Goldberg M, Septier D. Phospholipids in Amelogenesis and Dentinogenesis. *Crit. Rev. Oral Biol. Med.* 2002;13(3):276-90. doi: 10.1177/154411130201300305. [DOI](#)
19. Silva MJ, Scurrah KJ, Craig JM et al. Etiology of Molar Incisor Hypomineralization – A Systematic Review. *Community Dent. Oral Epidemiol.* 2016;44(4):342-53. doi: 10.1111/cdoe.12229. [DOI](#)
20. Riggs E, Kilpatrick N, Slack-Smith L et al. Interventions with Pregnant Women, New Mothers and Other Primary Caregivers for Preventing Early Childhood Caries. *Cochrane Database Syst. Rev.* 2019. doi:10.1002/14651858.CD012155.pub2. [DOI](#)
21. Evans EW, Hayes C, Palmer CA et al. Dietary Intake and Severe Early Childhood Caries in Low-Income, Young Children. *J. Acad. Nutr. Diet.* 2013;113(8):1057-61. doi: 10.1016/j.jand.2013.03.014. [DOI](#)

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Inessa I. Yakubova

Kyiv Medical University

2 Boryspilska Stю, 02099 Kyiv, Ukraine

e-mail: yakubova.inessa@gmail.com

ORCID AND CONTRIBUTIONSHIP

Inessa I. Yakubova: 0000-0003-2780-2460 [A](#) [B](#) [C](#) [D](#) [F](#)

Victor Dosenko: 0000-0002-6919-7724 [A](#) [C](#) [E](#)

Volodymyr Ostriancko: 0000-0001-6525-7526 [B](#) [C](#) [E](#)

Serhii Tsypan: 0000-0003-4451-9990 [C](#) [F](#)

Liliia Bielova: 0000-0001-8546-0222 [F](#)

Ganna Viun: 0000-0002-0473-4031 [E](#)

[A](#) – Work concept and design, [B](#) – Data collection and analysis, [C](#) – Responsibility for statistical analysis, [D](#) – Writing the article, [E](#) – Critical review, [F](#) – Final approval of the article

RECEIVED: 15.09.2023

ACCEPTED: 28.12.2023

