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How to cite / Як цитувати статтю: Kmyta O, Budko H, Ivakhnyuk T, Shtainberger R. Features of estrogen and progesterone receptor expression in meningiomas depending on gender. *East Ukr Med J.* 2024;12(2):398-405

DOI: [https://doi.org/10.21272/eumj.2024;12\(2\):398-405](https://doi.org/10.21272/eumj.2024;12(2):398-405)

ABSTRACT

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FEATURES OF ESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION IN MENINGIOMAS DEPENDING ON GENDER

Introduction. Meningiomas are non-malignant brain or spinal cord neoplasms originating from meningeal cells. The etiology of meningiomas remains insufficiently studied due to the subclinical course and relatively low incidence. Because meningiomas affect women more than twice as often as men, and there are recorded cases of the disease in pregnant women, it is advisable to investigate the role of female sex hormones in the development of this disease.

Objective. Our work aimed to study the features of the expression of estrogen and progesterone receptors in meningioma tissue depending on the gender of the patients.

Methods and materials. A total of 50 samples were examined, of which 25 samples were from female patients (group W) and 25 from male patients (group M). The average age of patients in group W was 60.92 ± 1.84 years, and group M was 59.88 ± 3.39 years. Regardless of the equivalent indicators of average age, it is worth noting that the age of female patients varied 42 to 79 years and of male patients – 24 to 88 years. We examined the samples macroscopically, histologically and immunohistochemically (antibodies against estrogen and progesterone receptors).

Results. When examined macroscopically, the meningioma had a characteristic spherical or plaque-like shape, grey colour, somewhat bumpy surface, moderate density, and fibrous structure on cross-section. During the histological examination, most samples in both groups were assigned to psammomatous or meningothelial histological subtypes, the characteristic histological patterns of which were concentric structures of flattened tumour cells and psammoma bodies.

Conclusions. Immunohistochemical examination of meningioma tissue with antibodies against estrogen (ER) and progesterone (PR) receptors showed their more significant presence in samples of female

patients compared to samples of male patients ($p < 0.001$), which confirms the critical role of female sex hormones in etiology and pathogenesis meningioma.

Keywords: tumors, meningioma, immunohistochemistry, estrogen, progesterone.

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ОСОБЛИВОСТІ ЕКСПРЕСІЇ РЕЦЕПТОРІВ ЕСТРОГЕНУ ТА ПРОГЕСТЕРОНУ В МЕНІНГІОМАХ ЗАЛЕЖНО ВІД СТАТІ

Менінгіоми – це незлоякісні новоутворення головного або спинного мозку, що походять з клітин мозкових оболонок. Щодо етіології менінгіом, то вона і на сьогоднішній день залишається недостатньо вивченою через субклінічний перебіг та відносно низьку захворюваність. Через те, що менінгіоми вражають жінок більш ніж вдвічі частіше за чоловіків, а також зафіксовані випадки захворювання вагітних, доцільно дослідити роль жіночих статевих гормонів у розвитку даного захворювання.

Метою нашої роботи було вивчення особливостей експресії рецепторів естрогену та прогестерону в тканині менінгіом залежно від статі хворих.

Всього було досліджено 50 зразків, з яких 25 зразків від пацієнтів жіночої статі (група W) та 25 від пацієнтів чоловічої статі (група M). Середній вік пацієнтів групи W склав $60,92 \pm 1,84$ роки, а групи M – $59,88 \pm 3,39$ років. Не зважаючи на рівнозначні показники середнього віку, варто відмітити, що вік пацієнтів жіночої статі варіювався від 42 до 79 років, а чоловічої статі – від 24 до 88 років. Зразки ми досліджували макроскопічно, гістологічно та імуногістохімічно (антитілами проти рецепторів естрогену та прогестерону).

При макроскопічному дослідженні для менінгіом була характерна сфероподібна або бляшкоподібна форма, сірий колір, дещо горбиста поверхня, помірна щільність, волокниста структура на розрізі. При гістологічному дослідженні більшість зразків в обох групах була віднесена до псаммоматозного або менінготеліального гістологічних підтипів, характерними гістологічними патернами яких були концентричні структури зі сплосчених пухлинних клітин та псаммомні тільця.

Імуногістохімічне дослідження тканини менінгіом антитілами проти рецепторів естрогену (ER) та прогестерону (PR) показало їх помітно більшу присутність в зразках пацієнтів жіночої статі у порівнянні зі зразками пацієнтів чоловічої статі ($p < 0,001$), що підтверджує важливу роль жіночих статевих гормонів в етіології та патогенезі менінгіом.

Ключові слова: пухлини, менінгіома, імуногістохімія, естроген, прогестерон.

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INTRODUCTION / ВСТУП

Meningiomas are non-malignant neoplasms of the brain or spinal cord that originate from cells of the meninges and make up 40.8% of all tumours of the central nervous system. In adults (mostly 40-60 years old), these tumours are 20 times more common in the brain than in the spinal cord and 2.2 times more common in women than men [1].

While, in children (aged 0-18 years, less than 1% of all meningiomas), meningiomas of the spinal cord occur three times more often than in adults, and boys and girls are affected with the same frequency [2].

In some patients, intracranial meningiomas may be asymptomatic and be an incidental finding during computed tomography or magnetic resonance imaging ordered for other reasons. In other patients, these brain tumours can lead to various neurological and cognitive disorders (headache, seizures, visual disturbances, hoarseness of voice, behavioural disorders, etc.), which then become the cause of disability and mortality [3, 4].

As for the etiology of meningiomas, it remains insufficiently studied to this day due to the subclinical course and relatively low incidence [5–7]. So far, the main factor whose influence has been proven remains ionizing radiation (increases the risk by 6-10 times). Moreover, relative to this factor, primary meningiomas (environmental factor) and secondary meningiomas (arising after radiation therapy of intracranial tumours) can be distinguished [8, 9]. In addition, these tumours have a certain hereditary predisposition (a meningioma in a close family member increases the risk of the disease by two times) [10].

Because meningiomas affect women more than twice as often as men, as well as recorded cases of the disease in pregnant women, it is advisable to investigate the role of female sex hormones in the development of this disease. This work aims to study the features of the expression of estrogen and progesterone receptors in meningioma tissue depending on the gender of the patients.

Materials and methods

Protocol of the Ethics Commission

This study was approved by the commission on compliance with bioethics in conducting experimental and clinical research at the Educational and Scientific Medical Institute of Sumy State University (protocol No. 5/12 dated December 7, 2023). All studies were performed following the Declaration of Helsinki (6th edition, revised 2008, Seoul) and the Universal Declaration of Bioethics and Human Rights (2006).

Collection of samples

The research was carried out on tissues obtained for histological examination after surgical interventions based in the neurosurgical department of the Sumy

Regional Clinical Hospital and on tissues obtained during autopsies in the pathology department of the Sumy Regional Clinical Hospital (Sumy, Ukraine). A total of 50 meningioma samples were examined, of which 25 samples were from female patients (group W) and 25 from male patients (group M). The samples were examined macroscopically, histologically and immunohistochemically.

Histological examination (hematoxylin-eosin staining)

Meningioma tissue was fixed in a 10% buffered formalin solution for 24 hours. After that, fragments measuring 0.5x0.5x0.3 cm were cut out, placed in histological cassettes and subjected to dehydration and paraffin embedding in the ATM-4M carousel-type apparatus (Ukraine). Paraffin blocks were cut at a thickness of 7 microns using a Shandon Finesse 325 rotary microscope (Thermo Scientific, Waltham, MA, USA). After that, the paraffin sections were stained with hematoxylin-eosin. All photos were taken using a Zeiss Primo Star microscope with a Zeiss Axiocam ERc 5s camera and "Zen 2.0" software (Carl Zeiss, Jena, Germany).

Immunohistochemical study

Serial sections of meningioma tissue with a thickness of 3-4 microns, made from prepared histological paraffin blocks, were applied to SuperFrost adhesive slides (Thermo Scientific) and dried at 37°C for 18 hours. Deparaffinized sections were subjected to unmasking of antigens by the thermal method by heating the sections in a citrate buffer (pH 6.0) at 95–98°C. To visualize the results of an immunohistochemical (IGH) study, the detection system "UltraVision Quanto Detection System HRP Polymer" (Thermo Scientific) was used, which involved blocking endogenous peroxidase activity with 3% hydrogen peroxide, blocking non-specific background staining using "Ultra V Block", enhancing the reaction "Primary Antibody Amplifier Quanto". Diaminobenzidine (DAB) was used as a chromogen. Antibodies against estrogen receptors– ER (clone SP1, Thermo Fisher Scientific, RM-9101-S1) and progesterone receptors– RR (clone 16, VITRO master diagnostic, MAD-000670QD-7) were used.

Statistical analysis

The results of morphometric measurements and immunohistochemical examination were checked for normal distribution using the Shapiro-Wilk test. The Mann-Whitney test was used to assess statistical significance in the non-normal distribution of digital indicators.

If the data samples had a correct distribution, they were compared using the Student's parametric t-test. The results were considered statistically significant with a probability of more than 95% ($p < 0.05$). The statistical

processing of the results of the immunohistochemical study was carried out in the Attestat 11.0 statistical package. Graphic presentation of the statistical analysis results was performed using GraphPad Prism 8.0.

Results

A total of 50 samples were examined, of which 25 samples were from female patients (group W) and 25 from male patients (group M). The average age of patients in group W was 60.92 ± 1.84 years, and group M was 59.88 ± 3.39 years. Regardless of the equivalent indicators of average age, it is worth noting that the age of female patients varied from 42 to 79 years and of male patients - from 24 to 88 years (Fig. 1).

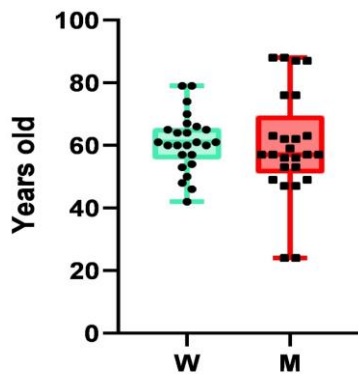


Fig. 1. The age distribution of patients

Histological examination

When examined macroscopically, the meningioma had a characteristic spherical or plaque-like shape, grey colour (with inclusions of dirty grey or brown colours),

a somewhat bumpy surface, moderate density, and fibrous structure on the section (Fig. 2 A, B).

During histological examination, the majority of samples in both groups were assigned to psammomatous (Fig. 3A) or meningotheial (Fig. 3B) histological subtypes, which belong to relatively benign meningiomas (WHO grade 1). A typical histological picture for these subtypes was created by cells of an endothelial phenotype of oval or polygonal shape, closely adjacent to each other with oval nuclei and small fuzzy nucleoli. In addition, single mitoses were observed (up to 4 in 10 fields of view at high magnification) and the absence of brain invasion. Characteristic histological patterns were concentric structures of flattened tumour cells (according to the type of transverse section of the bulb) and specific layered formations, so-called psammoma bodies (in isolated cases, they are the main feature of the meningioma as a whole, in a significant number, the histological subtype is determined as psammomatous).

Immunohistochemical study

Immunohistochemical examination of meningioma tissue with antibodies against estrogen receptors (ER) showed their more significant presence in samples of female patients (Fig. 4A) compared to samples of male patients (Fig. 4B). The number of immunopositive cells (moderate nuclear reaction) in the samples of group W was 81.21 ± 4.97 cells in the field of view with a diameter of 1 mm. It was significantly more than in group M— 54.90 ± 4.18 cells in the field vision with a diameter of 1 mm ($p < 0.001$) (Fig. 5).

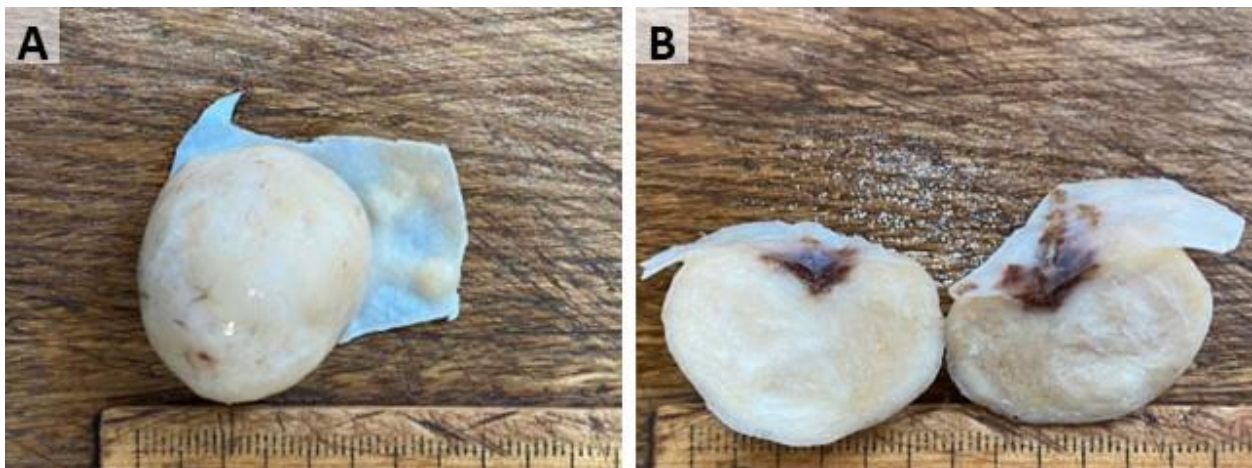


Fig. 2. A. Meningioma tissue with a fragment of the adjacent dura mater. B. Meningioma tissue on section

Immunohistochemical examination of meningioma tissue with antibodies against progesterone receptors (PP) showed their significantly more fabulous presence in samples of female patients (Fig. 6A) compared to samples of male patients (Fig. 6B). The number of immunopositive cells (pronounced nuclear

reaction) in the samples of group W was 174.00 ± 13.30 cells in the field of view with a diameter of 1 mm, and it was significantly more than in group M— 101.37 ± 7.70 cells in the field vision with a diameter of 1 mm ($p < 0.001$) (Fig. 7).

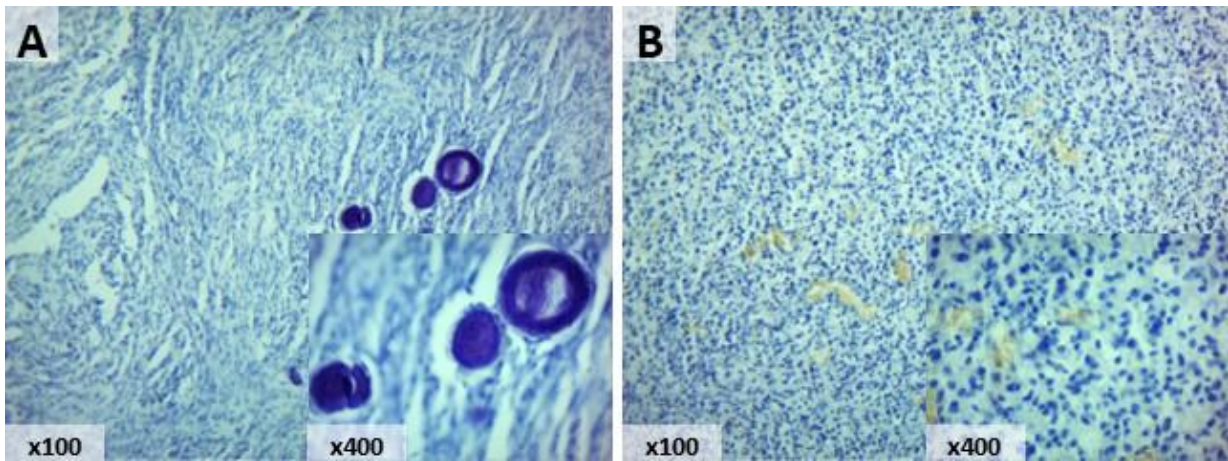


Fig. 3. Histological examination of meningioma tissue (hematoxylin-eosin staining). **A.** Psammomatous meningioma. **B.** Meningothelial meningioma. Drawings in the insets A-B correspond to the enlarged area of this preparation

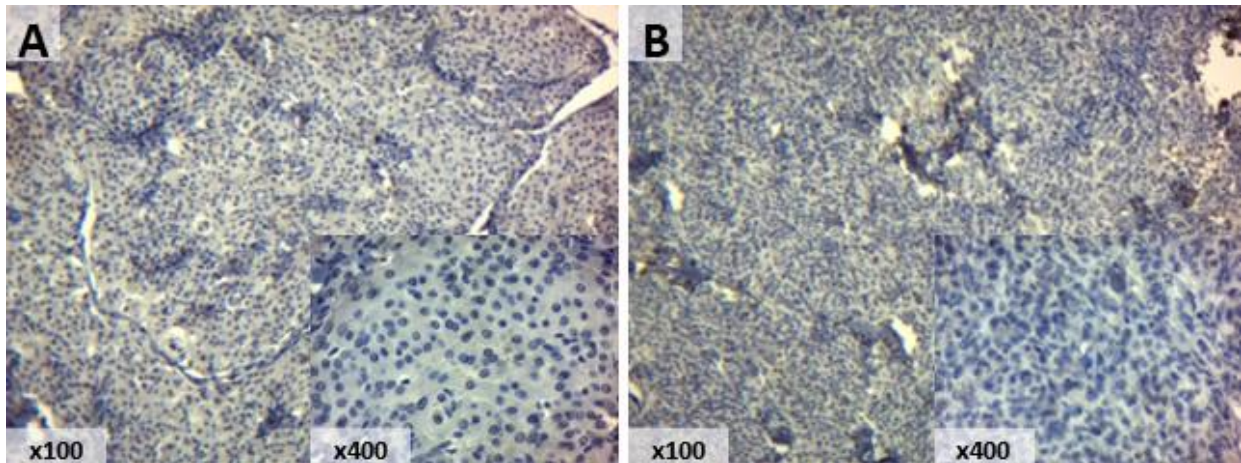


Fig. 4. Immunohistochemical study of meningioma tissue with antibodies against estrogen receptors. **A** is a group of female patients (W). **B** – group of male patients (M). Drawings in the insets A-B correspond to the enlarged area of this preparation

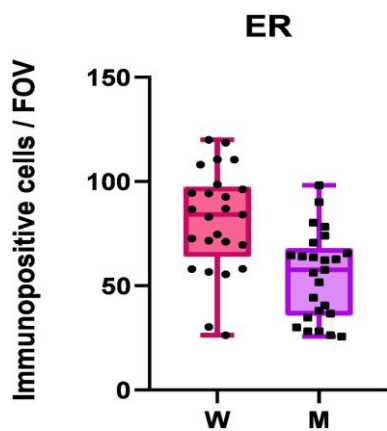


Fig. 5. Immunohistochemical study of meningioma tissue with antibodies against estrogen receptors

Discussion

The role of gonadal hormones in the etiology and pathogenesis of meningiomas has not yet been fully elucidated, even though the first observations about the predominant incidence among women were documented in 1938 by Dr Harvey Cushing in his monograph [11].

Many scientists have been discussing hormones' influence on meningiomas' development for years. First, meningioma is the only intracranial tumour that affects women more often than men (on average, the ratio during life is 2:1, and during the peak reproductive period – 3:1) [12].

Secondly, cases of progression of meningiomas during puberty, during the luteal phase of the menstrual cycle and pregnancy or immediately after childbirth have been recorded (although this phenomenon is more likely to be associated with hypervascularization and oedema rather than proliferation of tumour cells) [13–16].

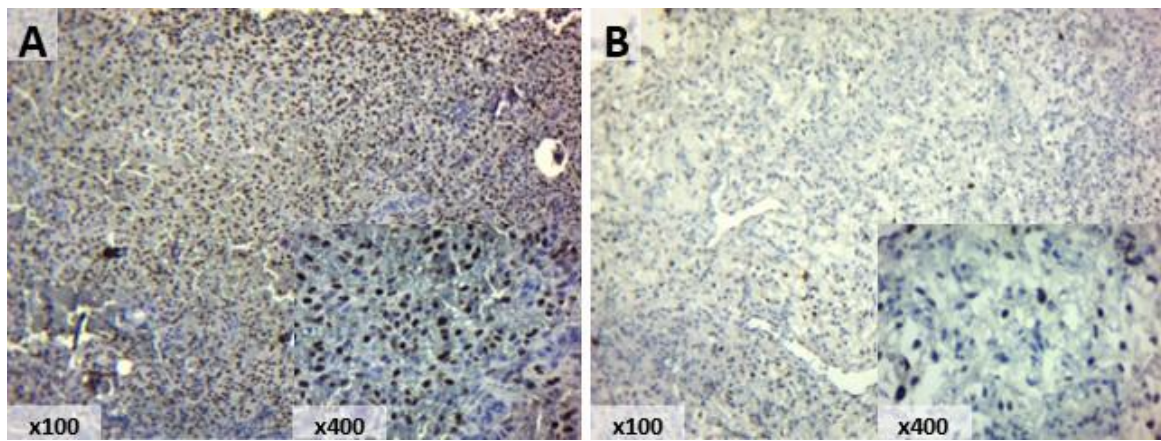


Fig. 6. Immunohistochemical study of meningioma tissue with antibodies against progesterone receptors. A is a group of female patients (W). B – group of male patients (M). Drawings in the insets A-B correspond to the enlarged area of this preparation

Thirdly, cases of the development of meningiomas (including multiple ones) after long-term hormonal therapy, in particular with megestrol acetate, cyproterone acetate, chlormadinone acetate, and their partial regression after discontinuation of therapy have been recorded [17, 18].

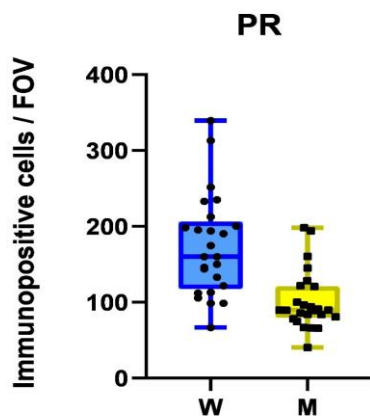


Fig. 7. Immunohistochemical study of meningioma tissue with antibodies against progesterone receptors

On the one hand, some factors indicate a significant role of endogenous or exogenous hormonal changes in the development of meningiomas. On the other hand, no convincing evidence could confirm this.

In this paper, we examined meningiomas macroscopically, histologically, and immunohistochemically (antibodies against estrogen

and progesterone receptors). Macroscopically and histologically, no difference was found between samples from female (group W) and male (group M) patients.

An immunoblot identifies three isoforms of progesterone receptors: PR-A, PR-B and PR-78. They have heterogeneous expression, with PR-A predominating in 2/3 of cases. Some studies indicate that progesterone receptor expression is a favourable prognostic factor, and its decrease may be associated with disease recurrence [19–21].

As for estrogen receptors, there are two isoforms - alpha and beta. There is data on the correlation between the expression of estrogen receptors and Ki-67, but there is no apparent connection with the degree of malignancy of meningioma. Other studies indicate that the expression of these receptors is an adverse prognostic factor. That is, the expression of estrogen receptors has the opposite role relative to progesterone receptors in the further prognosis of meningiomas [22–24].

In our work, immunohistochemical examination of meningioma tissue with antibodies against estrogen receptors (ER) and progesterone receptors (PR) showed their significantly more significant presence in samples of female patients compared to samples of male patients ($p < 0.001$), which confirms the important role of female sex hormones in etiology and pathogenesis of meningiomas.

CONCLUSIONS / ВИСНОВКИ

1. Macroscopically, meningiomas were characterized by a spherical or plaque-like shape, grey colour, somewhat bumpy surface, moderate density, and fibrous structure on cross-section.

2. Histologically, most samples in both groups were assigned to psammomatous or meningothelial histological subtypes, the characteristic histological patterns of which were concentric structures of flattened tumour cells and psammoma bodies.

3. Immunohistochemical examination of meningioma tissue with antibodies against estrogen (ER) and progesterone (PR) receptors showed their significantly more significant presence in samples of

female patients compared to samples of male patients ($p < 0.001$), which confirms the important role of female sex hormones in etiology and pathogenesis of meningiomas.

AUTHOR CONTRIBUTIONS / ВКЛАД АВТОРІВ

Кмита О.П.: концепція та дизайн дослідження, отримання даних, аналіз та інтерпретація даних, оформлення статті, критичний перегляд.

Будко Г.Ю.: отримання даних, аналіз та інтерпретація даних, оформлення статті, критичний перегляд, остаточне затвердження.

Івахнюк Т.В.: отримання даних, аналіз та інтерпретація даних, оформлення статті, критичний перегляд, остаточне затвердження.

Штайнбергер Р.М.: отримання даних, аналіз та інтерпретація даних, оформлення статті, критичний перегляд.

FUNDING / ДЖЕРЕЛА ФІНАНСУВАННЯ

None.

CONFLICT OF INTEREST / КОНФЛІКТ ІНТЕРЕСІВ

The authors declare no conflict of interest.

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Received 09.05.2024**Accepted 30.05.2024****Одержано 09.05.2024****Затверджено до друку 30.05.2024****INFORMATION ABOUT THE AUTHORS / ВІДОМОСТІ ПРО АВТОРІВ**

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