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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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ANALYSIS OF PLASMA MIRNA-497 LEVELS IN THE BLOOD OF PATIENTS WITH BREAST CANCER

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Abstract.

Breast cancer is a heterogeneous disease with a variable clinical course, morphological and clinical features. In clinical observation, we attempted to study the correlation between clinical characteristics of breast cancer patients and plasma miRNA-497 concentration as a possible pathogenic molecular disease factor and a diagnostic indicator. It was established that miRNA-497 levels were significantly higher in the plasma of premenopausal compared to menopausal women, while the opposite is true for healthy women. We did not find a link between miRNA-497 levels and tumor size and clinical stage, though a weak positive correlation between miRNA-497 levels and the N0-N3 stage was noted, with a pronounced increase at the N3 stage, which was reliable in the group of patients after APCT. miRNA-497 levels after the first and second courses of NPCT did not differ statistically significant. There was no correlation between miRNA-497 concentration and the molecular subtype of breast cancer, and the difference between patients with HER2+ type and the triple-negative type was not convincing due to the small patient sample size. Also, no connection was found between the analyzed miRNA-497 levels and follow-up results, and positive initial results require additional research and analysis. In conclusion, analysis of miRNA-497 levels can be useful in the study of the molecular type and stage of breast cancer. Prospects for further research are in analyzing this indicator in a larger sample of breast cancer patients, obtaining remote follow-up results, and comparison with other types of miRNA.

Key words. miRNA-497, breast cancer, blood plasma, chemotherapy, metastasis.

Introduction.

miRNA is a set of heterogeneous non-coding small RNAs, which play a role in the regulation of gene expression of mRNA processing [1]. Disruptions in miRNA regulation can be a factor in the development and progression of oncologic diseases [2]. The number of studies that indicate the role of miRNA-497 in the development of breast cancer (BC) presented in the literature is insignificant [3]. In particular, the role of miRNA-497 in suppressing BC cell proliferation and invasion is already established [3,4], including triple-negative BC [5]. The level of miRNA-497 expression was higher in normal breast tissue compared to BC tissue [4]. Low miRNA-497 expression is closely correlated with a lower degree of tumor differentiation, positive HER2 expression, and a higher frequency of lymph node metastasis, progressing clinical stage, and lower overall 5-year patient survival rates [6,7]. The literature also presents conflicting data on the antitumor role of miRNA-497 in various breast cancer cell lines [8,9]. Though, there is data supporting the role of miRNA-497 as an oncogenesis suppressor, impaired synthesis of which can be a cause of the development of oncological disease. For the possibility of explaining the role of miRNA-497 in the development of breast cancer, and the prognostic value of its concentration in blood plasma we conducted a study of levels of this indicator in the blood of patients with different stages of BC.

Aim of the study.

To study miRNA-497 levels in the blood plasma of BC patients in comparison to relatively healthy donors, and also the correlation of these levels with the clinical course of oncological disease.

Materials and Methods.

88 patients were included in the study, aged 30 to 74 years old: the main group consisted of 70 BC patients; comparison group included 18 people (relatively healthy women). Patients with BC in combined treatment received 4 courses of neoadjuvant polychemotherapy (NPCT) and 6 courses of adjuvant polychemotherapy (APCT). Median age at the time of diagnosis was 45 years old. The distribution of women by age up to 45 years and older was carried out. Tumor volume was calculated using the formula, V=(a×b2)/2 [10], where a — tumor height; b — tumor width; c — tumor width. RNA extraction was carried out according to the manufacturer's instruction (Manufacturer: Zymo Research (USA) Quick-RNA Miniprep Kit (catalog №R1055)). Exogenous internal control – A035 «A set of reagents for determining the quantitative content of RNA», «Ukrgentech» (Ukraine); endogenous internal control – TBP and YWHAZ gene fragments covering the range from the smallest to the longest fragments.

Primer for Real-time PCR synthesized by Metabion (Germany), sequence:

One-step mix with revertase and polymerase – M 08 «One-step RT mix with SYBR Green intercalating dye» «Ukrgentech» (Ukraine). Amplification according to the manufacturer's instructions. A Bio-Rad CFX-96 amplifier (Singapore) was used for the analysis. Bio-Rad CFX Maestro assay and quantification program.

Calibrators for quantification of internal controls:

1 – TCCTTTGCTTGCATCCCACAGACTATTTCCCTCA-TCCTATTTACTGCAGCAAATCTCTCC – 60 bp

2 – TGCACAGGAGCCAAGAGTGAAGAACAGTCCA-GACTGGCAGCAAGAAAATATGCTAGAGTTGTACA-GAAGTTGGGTTTTCCAGCTAAGTTCTTGGACTTCAA-GATTCAGAATATGGTGGGGAGCTGTGATGTG - 132 bp

Quantitative assessment method:

1. An exogenous (added to the sample before extraction at a known concentration) synthetic internal control was used to estimate the level of extraction A035, by comparing the applied concentration and the concentration after the extraction in quantitative indicators calculated using the Bio-Rad CFX Maestro software. As a result, the extracted coefficient ranged from 0.7 to 0.9, samples in which the extraction coefficient was below 0.7 were subject to repeated extraction.
2. For quantification, synthetic calibrators were used as positive controls for each group of reactions separately in duplicates and two dilutions with a two-order difference of 100 and 10,000 copies, respectively, and using Bio-Rad CFX Maestro software, copy per reaction was obtained (duplicate, samples performed in doubles).
3. To determine the number of copies per milliliter: Copies/ml = Amount of miRNA in the reaction (instrument data in copies/reaction)/Extraction coefficient×Number of reactions from one eluate×Number of extractions from one ml. – gene fragment.

Statistical analysis was performed using the SPSS 17.0 software package (SPSS, Chicago, Illinois, USA). Data were presented as mean ± SEM. Univariate analysis of variance (ANOVA, Bonferroni post hoc test) and Mann–Whitney U-test were used to determine the significance of intergroup differences. Spearman's test was used to assess the correlation of miRNA-497 with clinical and pathological features of breast cancer. Differences were considered statistically significant at $p<0.05$.

Results.

miRNA-497 levels differed in pre- and postmenopausal women (<45 and ≥45 years old) (Table 1). If the difference in miRNA-497 levels was not significant in healthy women over 45 years old, it was significant for BC patients. In the general sample of patients under 45 years of age, a significantly higher rate was established (p=0.01), this pattern was confirmed in subgroups of patients who received APCT (p=0.05) or NPCT (p=0.02). At the same time, miRNA-497 levels were significantly higher in premenopausal than in menopausal women. These results can indicate a link between miRNA-497 expression level and the development of BC and the role of age factor in the probability of disease occurrence. The age dependence remains insufficiently understood, because of the small sample size, which is especially true of healthy women, that was investigated, but acquired results indicate a connection between plasma miRNA-497 levels and BC in premenopausal women.

The correlation of the BC clinical stage with miRNA-497 levels was analyzed and no difference was found between the 1st and subsequent stages of the disease (Table 2). At the same time, the probability of the difference in miRNA-497 levels between the groups of patients with the 2nd and 3rd stages in the APCT subgroup was p=0.06, and between the groups with the 1st and 3rd stages in the NPCT subgroup was equal to p=0.07. These results can be viewed as a correlation tendency of the breast cancer stage with an increase in plasma miRNA-497 levels. After clustering patients according to age, this trend was noted in the category of women under 45 years old, namely, the trend of plasma miRNA-497 level increase in the 3rd stage of cancer.

Correlational analysis was conducted (Spearman's criterion) and the intergroup difference was assessed while taking into account the disease stage according to TNM classification. A weak positive correlation between miRNA-497 level and N stage was detected r=0,26; p=0,03). In the general patient sample, the miRNA-497 level increased statistically significantly at the N3 stage (the tendency was also present while comparing N0-N2), and the result of linear regression R2=0.04. There was no discrepancy between the compared treatment groups. Obtained results can be interpreted this way: there is a probability of a positive relationship between plasma miRNA-497 levels and breast cancer stage, though this statement requires further research on larger patient samples.

There was no association between miRNA-497 level and tumor size (r=0,11; p=0,35). In our observations, there is no difference in the general sample of the studied values as well as in the subgroups of the two treatment regimens. Although a trend towards an increase in miRNA-497 level was noted with a tumor volume ranging from 1.0 to 5.0 cm3 (at the same time, the value of variance increases significantly; hypothetically, the difference could be probable if there were more cases in the general sample).

Univariate analysis of variance and group differences according to the non-parametric Mann-Whitney test of miRNA-497 levels in cases of four molecular cancer subtypes were performed: luminal A (n=13), luminal B (n=34), HER2+ (n=4), triple-negative (n=19). No statistically significant difference was found between the comparison groups (high variance in each group). An insufficient sample of analyzed HER2+ cases does not allow for confidence in the obtained statistical assessment results in this molecular subtype patient group. That is, in this study, a reliable link between miRNA-497 and molecular subtype was not established.

We attempted to analyze the dependence of treatment results on the initial miRNA-497 level at the time of diagnosis (Table

Table 1. *miRNA-497 levels in breast cancer (BC) patients (Mean(SEM)×10³).*

Indicator/ patient group		Healthy women			Breast cancer patients								
		miRNA-497 level		p <45 vs ≥45	APCT	p <45 vs ≥45	p healthy vs ill	NPCT	p <45 vs ≥45	p healthy vs ill	All patients	p <45 vs ≥45	p healthy vs ill
Age	<45	4,6(1,4) n=18	3,0(1,2) n=12		11,3(6,4)		0,05	176,8(85,0)		0,02	117,2(56,2)		**0,01**
	≥45		7,8(3,2) n=6	0,09	34,9(31,0)	**0,04**	0,08	49,3(3,2)	**0,04**	0,28	44,8(24,3)	0,01	0,18

Table 2. *The difference in plasma miRNA-497 levels of breast cancer (BC) patients depending on clinical disease course and chemotherapy regimen (Mean(SEM)×10³).*

Indicator/miRNA-497 level		Breast cancer patient group and probability level											
		APCT	p			NPCT	p			All patients	p		
Clinical stage	1	9,3(5,9) n=10				1,0(0,9) n=2				7,9(5,0) n=12			
	2	2,4(7,6) n=10	0,33			94,9(53,4) n=22	NA			66,0(36,2) n=31	0,09		
	3	157,7(139,8) n=3	0,12	0,06		138,0(84,3) n=15	NA	0,43		141,3(72,6) n=18	**0,04**	0,17	
	4	-				19,1(8,5) n=10	NA	0,36	0,41	23,7(10,1) n=8	0,07	0,19	0,31
Tumor size, см³	<1,0	12,6(11,5) n=5				33,0(30,1) n=4				21,6(14,1) n=9			
	1,0-2,0	7,0(5,8) n=4	1,0			224,1(211,3) n=4	1,0			92,8(85,0) n=8	0,31		
	2,0-5,0	2,5(0,5) n=5	1,0	1,0		244,8(146,4) n=9	0,92	1,0		184,3(112,7) n=14	0,18	0,36	
	≥5,0	54,2(47,9) n=9	1,0	1,0	1,0	37,4(22,2) n=30	1,0	0,93	0,18	41,3(14,1) n=39	0,29	0,25	0,21
T	1	11,6(3,5) n=11				254,7(90,0) n=8				114,0(67,0) n=19			
	2	44,9(13,5) n=11	0,41			75,3(13,9) n=29	0,13			66,9(31,3) n=40	0,45		
	3	45 n=1	NA	NA		9,1(7,2) n=5	0,53	1,0		7,6(6,1) n=6	0,28	0,35	
	4	-				17,7(15,8) n=5	0,60	1,0	1,0	17,7(15,8) n=5	0,46	0,49	0,35
N	0	8,7(4,2) n=16				196,9(165,6) n=6				60,1(46,1) n=22			
	1	2,3(0,6) n=4	1,0			39,8(25,6) n=26	1,0			31,8(22,3) n=30	0,15		
	2	-				98,5(76,7) n=11	1,0	1,0		98,5(76,7) n=11	0,11	0,27	
	3	157,7(139,8) n=3	0,02	0,05		263,7(250,3) n=4	0,60	1,0	1,0	218,3(145,4) n=7	**0,03**	0,08	0,21
Metastasis		0								n=77			
		1								n=8			
Molecular subtype		HER2+								0,2(0,1); Me=0,1 n=4			
		Luminal A								18,1(12,9); Me=1,3 n=13	NA		
		Luminal B								55,6(27,7); Me=3,5 n=34	NA	0,11	
		Triple-negative								148,2(78,1); Me=2,7 n=19	**0,04**	0,29	0,36

NA – not analyzed, not evaluated statistically.

3). For that purpose, patients were separated into two categories: stabilization, improvement, no recurrence or metastasis («0»); deterioration, metastasis, relapse, and death («1»). With the research factor set that way, a moderate correlation was found regarding the initial result (r=0.30; p=0.01) and the result of a 2-3-year follow-up (n=70) (r=0.38; p=0,01). Patients with an unfavorable treatment outcome (n=6) had I (n=1), II (n=1), III (n=2), and IV (n=1) stages. It should be taken into count, that in observed patients a weak positive link (r=0,25; p=0,04) between miRNA-497 levels and regional lymph node involvement was present. In the BC group after NPCT a tendency toward increased miRNA-497 levels on stage N3 compared to N1-2, and in the group after APCT the difference was statistically significant (N0 vs N3 p=0,02; N2 vs N3 p=0,05).

Analysis results demonstrated a statistically significant link between miRNA-497 level and initial outcome as well as with 2-3-year treatment follow-up analysis, though the clinical significance of this data requires further research.

Repeated evaluation of plasma miRNA-497 levels after a course of NPCT (n=18) did not show a statistically significant difference between repeated observations (p=0.50).

Hence, there is a link between plasma miRNA-497 levels

Table 3. *Correlation between initial miRNA-497 levels and treatment results.*

Indicator (VAR vs. VAR)	Rho	p
497-RNA vs. Initial results (improvement/=1; no change=2; increase=3)	0,30	0,01
497-RNA vs. Treatment outcome (improvement/ stabilization/no metastasis =0; relapse/prolongation/ metastasis=1)	0,38	0,00
497-miRNA vs. N0-N3	0,25	0,04
497-miRNA vs. Stage (I-IV)	0,23	0,05
Follow-up results (stabilization=1; relapse, death=2 vs. N0-N3	0,04	0,73
Follow-up results (stabilization=1; relapse, death=2 vs. Stage	0,09	0,45
Initial results (improvement/decrease=1; no change=2; increase=3) vs. Treatment outcome (improvement/stabilization/no metastasis =0; relapse/prolongation/metastasis=1)	0,47	0,00
Treatment outcome (improvement/stabilization/no metastasis =0; relapse/prolongation/metastasis=1) vs. N0-N3	0,15	0,21
Treatment outcome (improvement/stabilization/no metastasis =0; relapse/prolongation/metastasis=1) vs. Stage	0,17	0,17

and more advanced disease stage with the involvement of regional lymph nodes in BC patients. miRNA-497 levels were significantly higher in premenopausal women with BC, did not differ between chemotherapy regimens, and did not differ between the first and second NPCT courses.

Discussion.

The assessment of blood plasma parameters is gaining more and more importance in clinical oncology, and new biomarkers are being researched, such as miRNA, for monitoring healthy and ill people [11]. Our study attempted to discover a new way in the area of breast cancer study and diagnosis. Our results show that high plasma miRNA-497 levels are connected to breast cancer and correlate with the progression of the disease. Though there is no correlation with tumor size, the link with tumor cell invasion into the regional lymph nodes is present. Our data also indicates that miRNA-497 level increases significantly in patients younger than 45 years old, while it is lower in older patients, but does not correspond with that of healthy women. These results can be useful in the study of the disease, though they differ from earlier data, which needs to be investigated further. Particularly, published data indicates a decrease in miRNA-497 expression in breast cancer, which then explains the loss or decrease of the role of miRNA-497 as a cell proliferation suppressor [10]. On the contrary, in the analyzed patient sample we found a link between high miRNA-497 levels and a tendency for disease progression.

A low level of miRNA-497 expression was observed in patients with triple-negative breast cancer [12]. Our work did not reveal a dependency of plasma miRNA-497 levels on the molecular type of breast cancer, though a correlation tendency with the triple-negative type was present. Evidently, the sample size of our study was not sufficient for discerning such a correlation, but that does not mean that it cannot be established by further research.

Expression levels of some miRNAs can become a biomarker in predicting the outcome of oncologic disease. Certain miRNA panels can potentially become prognostic biomarkers for breast cancer. In that fashion, some data indicates a potentially better treatment prognosis for cervical and colorectal cancer patients in the case of high miRNA-497 expression levels [8,13]. However, in the case of breast cancer, similar observations were not supported by data sighted in open sources. We attempted to analyze the correlation between miRNA-497 levels in the blood of patients, who received different chemotherapy treatment regimens and we did not find a significant difference before or at the beginning of treatment and after a course of chemotherapy. Analysis of the results, which were obtained based on correlational analysis, indicated an association between higher miRNA-497 levels and a worse condition or prognosis of the disease in breast cancer patients.

Comparison of acquired results with data represented in the literature [6] demonstrated certain differences, which can be explained by multiple variables present in each study, such as differing population patient samples, different chemotherapy regimens, and other factors of patient treatment. In the end, it should be noted, that the potential research possibility of plasma miRNA-497 levels requires further observations and statistical studies.

Conclusion.

1. The correlation between plasma miRNA-497 levels and age in women with breast cancer states that miRNA-497 level increases significantly in premenopausal women, while the opposite tendency is true for healthy women.
2. A weak positive correlation between plasma miRNA-497 levels of breast cancer patients and regional lymph node metastasis was noted. The level of miRNA-497 increased at the N3 stage in patients after APCT.
3. Tumor molecular subtype and received immediate treatment does not affect blood plasma miRNA-497 levels of breast cancer patients.

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Резюме

Рак молочної залози є гетерогенним захворюванням з варіабельним клінічний перебіг, морфологічними та молекулярними особливостями. У клінічному спостереженні зроблено спробу дослідити зв'язок між клінічною характеристикою хворих на РМЗ та рівнем міРНК-497 у плазмі як можливого патогенетичного молекулярного фактору захворювання та діагностичного показника. Встановлено достовірно вищі рівні міРНК-497 у плазмі крові хворих жінок у пременопаузальному віці порівняно з менопаузою, тоді як у здорових жінок відмічено протилежну тенденцію. Ми не виявили залежності між рівнем міРНК-497 і розміром пухлин та стадією раку (Grade), але відмічено слабку позитивну кореляцію між рівнем міРНК-497 та стадією N0-N3, виражену тенденцію зростання показника на стадії N3, яка у групі хворих після АПХТ була достовірною. Рівень міРНК-497 після першого та повторного курсу НПХТ не мав статистично значущої різниці. Не виявлено різниці показника між молекулярними типами РМЗ, а деяка різниця між хворими з HER2+ та тричінегатичний не була переконливою з причини невеликої вибірки таких пацієнтів. Також не виявлено зв'язку між проаналізованим рівнем міРНК-497 та віддаленими результатами, а позитивні безпосередні результати потребують додаткових досліджень та аналізу. Як заключення, аналіз рівня міРНК-497 може бути корисним у дослідженні молекулярного типу та стадії РМЗ. Перспективи наступних досліджень полягають у аналізі цього показника у більшій кількості спостережень хворих з РМЗ, віддалених результатів та порівнянні з іншими типами міРНК.

Ключові слова: міРНК-497, рак молочної залози, плазма крові, хіміотерапія, метастазування.