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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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CORRELATIONS BETWEEN HOMOCYSTEINE AND VITAMIN B12 IN TYPE 2 DIABETES TREATED WITH METFORMIN

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Abstract.

Study Objective: The study aimed to assess the prevalence of vitamin B12 deficiency in patients with type 2 diabetes mellitus treated with metformin, determine the relationship between serum vitamin B12 and homocysteine concentrations, and evaluate the diagnostic value of vitamin B12 for identifying elevated homocysteine levels.

Material and Methods: The study included 126 patients with type 2 diabetes mellitus, whose age ranged from 31 to 71 years (mean age 55.57±7.14 years). All patients were treated with metformin. Serum vitamin B12 and homocysteine concentrations were determined by direct chemiluminescent enzyme immunoassay (CLEIA) using a MAGLUMI X3 analyzer. HbA1c and other clinical parameters were also assessed. Correlation and linear regression analyses were used to assess the relationship between metformin dose, vitamin B12, and homocysteine levels. ROC analysis was used to determine the diagnostic efficiency, based on which optimal cutoff values, sensitivity, specificity, and area under the curve (AUC) were determined. Statistical analysis was performed using SPSS 23 software, and values of $p<0.05$ were considered statistically significant.

Results: Vitamin B12 deficiency or insufficient levels were detected in 50.79% of patients. A weak, but statistically significant positive correlation was found between the daily dose of metformin and homocysteine concentration. At the same time, a strong and statistically significant negative correlation was observed between vitamin B12 and homocysteine concentrations ($\beta = -0.760$, $p<0.001$). ROC analysis showed that vitamin B12 concentration has a high diagnostic ability for detecting elevated homocysteine levels (AUC = 0.904; 95% CI: 0.845–0.964). It was determined that a vitamin B12 value of <443.8 pg/mL represents the optimal cutoff for identifying elevated homocysteine levels, with a sensitivity of 84.97% and a specificity of 85.0%.

Conclusion: Vitamin B12 deficiency is widespread in patients with type 2 diabetes mellitus treated with metformin, and it is closely associated with increased homocysteine levels. The results indicate that reduced vitamin B12 concentrations play an important role in the increase in homocysteine. Assessment of vitamin B12 levels can be used for the early detection of metabolic disorders associated with increased homocysteine. Therefore, joint monitoring of vitamin B12 and homocysteine in patients treated with metformin will contribute to a more effective assessment of metabolic and cardiovascular risks.

Key words. Type 2 diabetes, metformin, vitamin B12 deficiency, homocysteine, ROC analysis.

Introduction.

Type 2 diabetes mellitus is one of the most important global problems of modern medicine, characterized by chronic hyperglycemia, insulin resistance, and multiorgan complications. Long-term pharmacotherapy plays a central role in the management of the disease, with metformin remaining the first-line drug due to its high efficacy, metabolic safety, and cardioprotective potential [1,2]. However, in recent years, increasing scientific evidence indicates that long-term use of metformin is associated with a decrease in serum vitamin B12 levels, which may progress to subclinical or clinically manifest cobalamin deficiency [3,4].

Vitamin B12 is a key cofactor in one-carbon metabolism and is involved in the remethylation of homocysteine to methionine. Cobalamin deficiency leads to the accumulation of homocysteine in plasma, which is associated with endothelial dysfunction, neurodegenerative processes and increased cardiovascular risk [5,6]. Hyperhomocysteinemia is considered an independent risk factor for the development of atherosclerosis, microvascular damage and neuropathy, which is of particular importance in patients with type 2 diabetes, who are already at increased risk of vascular complications [7].

Vitamin B12 deficiency during metformin therapy may not be readily recognized clinically, as its symptoms often overlap with those of diabetic neuropathy. In this setting, elevated homocysteine levels may serve as an early biochemical marker of one-carbon cycle dysfunction and potential metabolic imbalance [5,8]. Therefore, it is of particular interest to assess the correlation between homocysteine and vitamin B12 in patients receiving metformin for long-term treatment.

Although individual studies confirm the effect of metformin on cobalamin levels and also indicate an increase in homocysteine, the question of assessing their interrelationships in depth in the context of type 2 diabetes still requires systematic analysis [9-11]. Of particular importance is the determination of the dose-dependent effect, the duration of therapy, and the relationship to complications.

Research objective.

The aim of the study is to determine the relationship between homocysteine and vitamin B12 levels in patients treated with metformin.

Materials and Methods.

We studied 126 patients with type 2 diabetes aged 31-71 (55.57+7.14) years, including 67(61%) women, 59 men (49%).

All patients were treated with metformin, we studied: values of vitamin B12, homocysteine, HbA1c% and patient

characteristics on the background of treatment with metformin and other antiglycemic drugs.

Serum vitamin B12 and homocysteine levels were measured by direct chemiluminescent enzyme immunoassays (CLEIAs) using a Snibe diagnostic maglum X3 analyzer (reagents - MAGLUMI Vitamin B12 (CLIA) - cyanocobalamin and MAGLUMI Homocysteine (HCY) assay, respectively).

For the interpretation of biochemical parameters, standard clinical laboratory reference ranges were applied. Serum vitamin B12 status was categorized according to widely accepted clinical thresholds. Vitamin B12 deficiency was defined as serum vitamin B12 concentration <200 pg/mL, while vitamin B12 insufficiency was defined as concentrations between 200–300 pg/mL. Values >300 pg/mL were considered within the normal physiological range.

Hyperhomocysteinemia was defined as a plasma homocysteine concentration ≥15 μmol/L, which corresponds to the commonly accepted clinical threshold used in metabolic and cardiovascular risk assessment. For the purposes of ROC analysis, elevated homocysteine levels were used as the outcome variable, and serum vitamin B12 concentration was evaluated as the diagnostic test variable.

These thresholds were applied to classify the study population and to evaluate the diagnostic performance of vitamin B12 in identifying patients with elevated homocysteine concentrations.

We determined the relationship between homocysteine and B12 concentrations

For quantitative indicators, we determined the mean and standard deviation, for qualitative indicators, we determined the frequency and % value, the difference between groups for quantitative indicators was determined by the Student's t-test, the equality of variances was assessed by Levene's Test, for qualitative indicators, the difference was determined by Fisher's exact test (F); Correlation analysis was performed using Pearson's correlation for quantitative indicators and Spearman's rank correlation for qualitative indicators. In all cases, the results were considered reliable when $p<0.05$. Sensitivity and specificity were determined by Rhoak analysis, and disease risk was assessed by regression analysis. Statistical analysis was performed using the SPSS 23 software package.

Results.

Patient characteristics are given in Table 1.

Insulin-dependent diabetes without complications was present in 84 (66.67%) patients, while unspecified metabolic disorders were observed in 32 (33.33%). The average duration of the disease was 66.22+25.61 months, the duration of metformin intake was 19.32+9.10 months - 850-3000 mg per day. The average values of homocysteine and B12 were 12.89+4.74 and 370.24+211.16, respectively.

Correlations between patient characteristics are presented in Table 2.

A significant negative correlation with patient age was found for: B12 - r=-0.228*, p=0.010; BMI - r=.204*, p=0.022; systolic blood pressure - r=-0.212*p=0.017; HR - r=0.221*, p=0.013.

A significant positive correlation with homocysteine concentration was found for metformin dose - r=0.182*, p=0.0142, and a negative correlation was found for B12 concentration - r=-0.760**, p<0.001.

However, the correlation between metformin dose and homocysteine concentration was weak, and there was a non-significant negative correlation between metformin dose and B12 concentration.

The relationship between homocysteine and metformin dose is presented in Table 3 and figure 1.

The regression equation is represented in the following form:

Y = 10.840 + 0.001X,

Where X is the daily dose of metformin, and Y is the homocysteine concentration.

These data show that the higher the daily dose of metformin, the higher the homocysteine concentration.

In our study population, 64 patients (50.79%) had B12 deficiency.

In the next stage of the study, we divided the patients according to B12 deficiency, with the first group including patients without B12 deficiency, and the second group including patients with B12 deficiency (Table 4).

In B deficiency, the average value of homocysteine is significantly higher.

In turn, there is a significant negative correlation between homocysteine and B12 concentrations. The results of linear regression analysis are presented in Table 5 and Figure 2, which also confirm that homocysteine concentration is negatively correlated with B12 concentration in the presence of metformin

The relationship between homocysteine and vitamin B12 concentration is given by regression analysis.

Linear regression analysis demonstrated a strong and statistically significant negative association between vitamin B12 and homocysteine concentrations in patients with type 2 diabetes treated with metformin. Vitamin B12 was identified as an independent predictor of homocysteine levels ($B = -0.017$, $\beta = -0.760$, $p < 0.001$). The 95% confidence interval for the regression coefficient ranged from −0.019 to −0.014, confirming the stability and statistical significance of the observed association. These findings indicate that decreasing vitamin B12 concentrations are significantly associated with increased homocysteine levels.

Metformin-induced reduction in B12 levels in diabetes is associated with increased homocysteine (Figure 2).

The regression equation has the following form:

Y = 19.204 − 0.017X,

Where:

Y - homocysteine concentration,

X - vitamin B12 concentration.

To determine the diagnostic value of B12 for elevated homocysteine, we determined the sensitivity and specificity of vitamin B12 values for elevated homocysteine using ROC analysis (Figure 3, Table 6).

ROC analysis was performed to evaluate the diagnostic performance of serum vitamin B12 levels in identifying elevated homocysteine concentrations in patients with type 2 diabetes treated with metformin. In this analysis, vitamin B12 served as the test variable, whereas increased homocysteine concentration represented the outcome of interest.

As presented in Figure 3 and Table 6, the area under the ROC

Table 1. *Baseline Characteristics of the Study Population.*

Variable	Mean ± SD or n (%)	Min	Max
Male	59 (49%)	–	–
Female	67 (61%)	–	–
Age (years)	55.57 ± 7.14	31.00	71.00
Type 2 diabetes without complications, n (%)	84 (66.67%)	–	–
Unspecified metabolic disorders, n (%)	32 (33.33%)	–	–
Duration of diabetes (months)	66.22 ± 25.61	25.20	118.80
HbA1c (%)	11.43 ± 2.47	7.20	15.80
Homocysteine (µmol/L)	12.89 ± 4.74	2.50	23.00
Vitamin B12 (pg/mL)	370.24 ± 211.16	53.50	895.00
Metformin dose (mg/day)	1729.37 ± 726.40	850.00	3000.00
Duration of metformin therapy (months)	19.32 ± 9.10	5.00	36.00
BMI (kg/m²)	32.66 ± 6.33	19.50	45.00
Systolic blood pressure (mmHg)	150.98 ± 16.99	118.00	200.00
Diastolic blood pressure (mmHg)	89.20 ± 14.85	68.00	92.00
Heart rate (beats/min)	83.39 ± 12.05	56.00	115.00
Vitamin B12 insufficiency and deficiency, n (%)	64 (50.79%)	–	–
Vitamin B12 deficiency, n (%)	36 (28.57%)	–	–

Table 2. *Correlations Between Clinical and Biochemical Parameters.*

Variable	Age	Duration of disease (months)	HbA1c (%)	Homocysteine	Vitamin B12
Duration of disease (months)	r = -0.109	–			
	p = 0.243				
HbA1c (%)	r = -0.077	r = -0.161	–		
	p = 0.394	p = 0.082			
Homocysteine	r = 0.130	r = 0.059	r = -0.075	–	
	p = 0.148	p = 0.527	p = 0.404		
Vitamin B12	r = -0.228*	r = 0.010	r = 0.053	r = -0.760**	–
	p = 0.010	p = 0.914	p = 0.559	p = 0.000	
Metformin dose	r = 0.080	r = -0.124	r = -0.029	r = 0.182*	r = -0.166
	p = 0.371	p = 0.183	p = 0.748	p = 0.042	p = 0.063
Duration of metformin therapy (months)	r = 0.034	r = 0.066	r = 0.020	r = 0.079	r = -0.010
	p = 0.701	p = 0.478	p = 0.827	p = 0.377	p = 0.911
BMI	r = -0.204*	r = 0.093	r = -0.094	r = 0.039	r = 0.026
	p = 0.022	p = 0.317	p = 0.296	p = 0.662	p = 0.776
Systolic blood pressure	r = -0.212*	r = 0.105	r = -0.098	r = 0.072	r = -0.020
	p = 0.017	p = 0.258	p = 0.277	p = 0.423	p = 0.824
Systolic/Diastolic ratio	r = -0.088	r = 0.146	r = -0.022	r = -0.002	r = 0.095
	p = 0.329	p = 0.117	p = 0.810	p = 0.984	p = 0.288
Diastolic blood pressure	r = -0.119	r = -0.014	r = -0.085	r = 0.077	r = -0.095
	p = 0.183	p = 0.884	p = 0.343	p = 0.389	p = 0.291
Heart rate	r = -0.221*	r = 0.103	r = -0.079	r = 0.077	r = -0.021
	p = 0.013	p = 0.269	p = 0.381	p = 0.394	p = 0.818

** $p < 0.05$*
*** $p < 0.001$*

Table 3. *Association Between Homocysteine Concentration and Daily Metformin Dose Coefficientsa.*

Model	Unstandardized Coefficients (B)	Std. Error	Standardized Coefficients (Beta)	t	Sig.	95% Confidence Interval for B (Lower)	Upper
(Constant)	10.840	1.079	–	10.042	0.000	8.703	12.976
Daily metformin dose (mg)	0.001	0.001	0.182	2.057	0.042	0.000	0.002

a. Dependent Variable: Homocysteine.

Table 4. *Comparison of Clinical and Biochemical Parameters According to Vitamin B12 Status.*

Variable	B12 Normal (N=62) Mean	SD	B12 Deficiency (N=64) Mean	SD	t	p
Duration of disease (months)	67.11	24.75	65.29	26.67	0.38	0.704
HbA1c (%)	11.49	2.53	11.37	2.42	0.29	0.773
Homocysteine (µmol/L)	9.77	3.89	15.91	3.32	-9.50	<0.0001
Metformin dose (mg/day)	1612.10	704.36	1842.97	734.79	-1.80	0.074
Duration of metformin therapy (months)	18.53	9.06	20.08	9.14	-0.95	0.342
BMI (kg/m²)	33.31	5.93	32.03	6.67	1.14	0.258
Systolic blood pressure (mmHg)	152.06	17.36	149.92	16.70	0.71	0.482
Diastolic blood pressure (mmHg)	88.60	8.11	89.79	19.32	-0.45	0.651
Systolic/Diastolic ratio	1.71	0.05	1.69	0.13	1.38	0.172
Heart rate (beats/min)	84.10	12.41	82.70	11.76	0.65	0.519

Table 5. *Linear Regression Analysis with Vitamin B12 as Independent Variable Coefficientsa.*

Model	Unstandardized Coefficients (B)	Std. Error	Standardized Coefficients (Beta)	t	p	95% Confidence Interval (Lower)	Upper
Constant	19.204	0.557	–	34.474	<0.001	18.105	20.306
Vitamin B12	-0.017	0.001	-0.760	-13.039	<0.001	-0.019	-0.014

a. Dependent Variable: Homocysteine

Table 6. *Area Under the Curve.*

Test Result Variable(s): B12				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.904	0.030	<0.000	0.845	0.964
The test result variable(s): B12 has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.				
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Table 7. *Diagnostic Characteristics of Vitamin B12 in Detecting Elevated Homocysteine Levels.*

Sensitivity	Specificity	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)	Diagnostic Accuracy
0.849	0.850	0.924	0.723	0.849
0.773	0.739	0.866	0.596	0.787
0.925	0.961	0.982	0.851	0.912

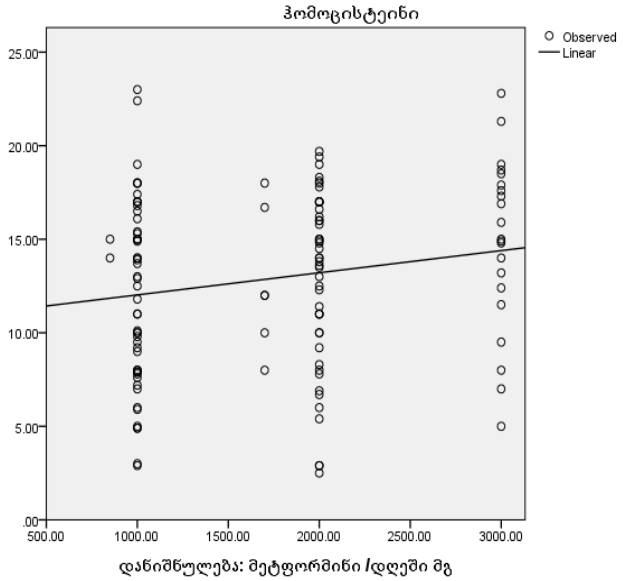

Figure 1. *Association Between Homocysteine Concentration and Daily Metformin Dose.*

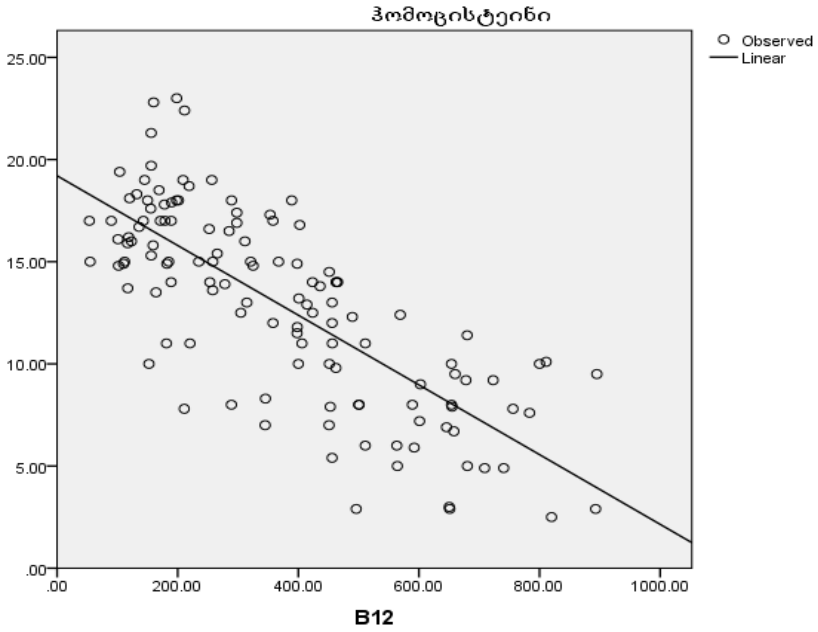

Figure 2. *Association Between Vitamin B12 and Homocysteine Concentrations.*

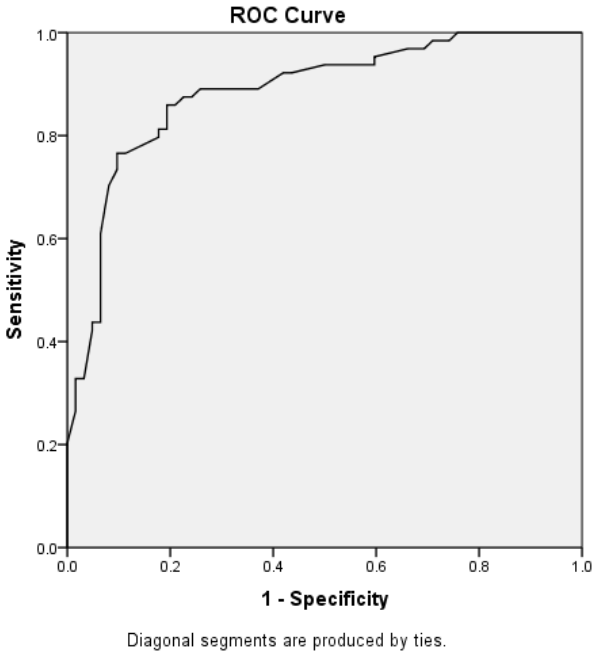

Figure 3. *ROC Curve of Vitamin B12 as a Diagnostic Marker for Hyperhomocysteinemia.*

curve (AUC) was 0.904 (95% CI: 0.845–0.964; $p < 0.001$), indicating excellent diagnostic accuracy of vitamin B12 levels for detecting elevated homocysteine. An AUC value above 0.9 is generally considered to reflect a high level of discriminative ability, suggesting that vitamin B12 concentration reliably distinguishes between patients with normal and increased homocysteine levels.

The ROC analysis identified an optimal vitamin B12 cut-off value of 443.8 pg/mL. At this threshold, the sensitivity of vitamin B12 for detecting elevated homocysteine was 84.9%, while specificity reached 85.0%. These values indicate a strong balance between true positive and true negative detection. The Youden index was calculated as 0.71, confirming that this cut-off provides the best diagnostic trade-off between sensitivity and specificity.

These findings demonstrate that lower vitamin B12 concentrations are strongly associated with increased homocysteine levels and that vitamin B12 measurement can be used as a reliable biochemical indicator for identifying patients at risk of hyperhomocysteinemia during metformin therapy. Given the strong negative correlation observed between vitamin B12 and homocysteine concentrations, ROC analysis further supports the clinical relevance of monitoring these parameters simultaneously in patients with type 2 diabetes.

The value of B12 as a test for elevated homocysteine is characterized by a very good positive predictive value and a good negative predictive value, and good diagnostic accuracy.

Discussion.

Metformin, widely accepted as the first-line drug for the management of type 2 diabetes, has been considered an effective and safe agent for many decades. However, over the past two decades, accumulating scientific evidence has shown that it is associated with reduced vitamin B12 levels [8,9,12]. The high prevalence of vitamin B12 deficiency and insufficiency (50.79%) found in the present study is somewhat higher than some literature data, but is consistent with studies that evaluated long-term (more than one to two years) and relatively high-dose metformin use [8,9,13,14]. A meta-analysis by Chapman et al. confirmed that metformin use significantly reduces serum vitamin B12 concentrations, and the risk of deficiency increases with both dose and duration of therapy [9]. Similarly, Kibirige and Mwebaze indicate that the prevalence of vitamin B12 deficiency is particularly high in patients taking metformin for three years or more [12].

The mechanism of metformin-induced cobalamin absorption impairment is associated with inhibition of calcium-dependent membrane transport in the ileum, which prevents the internalization of vitamin B12 in a complex with intrinsic factor. As a result, a functional deficiency develops, which may not be clinically manifested for a long time. It is at this stage that homocysteine acquires special importance as a functional marker of one-carbon metabolism.

The study revealed a strong and statistically significant negative association between vitamin B12 and homocysteine concentrations ($\beta = -0.760$; $p < 0.001$), indicating that a decrease in vitamin B12 levels significantly contributes to an increase in homocysteine. This relationship is fully consistent with the biochemical logic of one-carbon metabolism, according to which vitamin B12 is an essential cofactor for methionine synthase and its deficiency leads to homocysteine accumulation [3,5]. Petrova et al. note that vitamin B12 deficiency disrupts the methylation cycle and increases systemic homocysteine concentrations, which affects both neurological and cardiovascular risks [3].

Joshi and Jadavji have suggested that homocysteine is a more sensitive indicator of functional vitamin B12 deficiency than serum levels [1]. This is particularly important in patients treated with metformin, where vitamin B12 levels may be at the lower limit of normal but functional deficiency may already be present. McCaddon and Miller have also noted that elevated homocysteine levels often precede the development of clinical anemia and neurological symptoms [15].

The ROC analysis performed in the present study showed that vitamin B12 concentration has a high diagnostic accuracy for identifying elevated homocysteine levels. According to the obtained data, the cut-off value of vitamin B12 of 443.8 pg/mL is the optimal indicator, below which the probability of elevated homocysteine significantly increases. The area under the curve (AUC = 0.904; 95% CI: 0.845–0.964) indicates high diagnostic efficiency, and the obtained sensitivity and specificity indicators confirm that vitamin B12 can be used to reliably identify metabolic disorders associated with elevated homocysteine. These results are consistent with the study of Ramírez-Villalobos et al., where hyperhomocysteinemia is considered as a common metabolic indicator of vitamin B12 deficiency and cardiovascular risk [6].

Furthermore, a meta-analysis by Kataria et al. showed that therapeutic intervention with B vitamins significantly reduces homocysteine levels and may have a positive impact on cardiovascular outcomes [2]. This evidence further reinforces the need for monitoring vitamin B12 in patients treated with metformin to prevent not only hematological but also neurological and vascular complications.

The study also found a weak but statistically significant positive association between daily metformin dose and homocysteine concentrations. Although the strength of the association is limited, it is biologically plausible and consistent with the findings of Hussain et al., who suggest a dose-dependent effect [8]. Abdelgawad's study also confirms that homocysteine levels are significantly higher in patients taking metformin in the presence of low vitamin B12 concentrations [11].

From a clinical perspective, the high prevalence of vitamin B12 deficiency in diabetic patients treated with metformin is important not only because of the risk of anemia, but also in the context of neurological dysfunction and endothelial damage. Homocysteine is considered an independent cardiovascular risk factor and its elevation is associated with the progression of the atherosclerotic process [6,15].

Thus, the results obtained not only confirm the existing literature data, but also strengthen the argument that the joint assessment of vitamin B12 and homocysteine should be considered an important component of metabolic screening in patients with type 2 diabetes mellitus and receiving metformin treatment. Vitamin B12 concentration can be used as a practical biochemical indicator for early detection of risks associated with increased homocysteine and ensuring timely clinical intervention.

Study limitations.

Despite the important findings of the present study, several limitations should be considered when interpreting the results. First, renal function parameters were not systematically assessed in the study population. Impaired kidney function is known to influence homocysteine metabolism and may lead to elevated circulating homocysteine concentrations independently of vitamin B12 status. Therefore, the absence of renal function indicators such as serum creatinine or estimated glomerular filtration rate (eGFR) limits the ability to fully exclude the potential contribution of renal impairment to homocysteine elevation.

Second, information regarding the use of vitamin B-complex supplements, including vitamins B6, B9 (folate), and B12, was not collected systematically. These micronutrients are involved in one-carbon metabolism and may significantly affect homocysteine concentrations. The lack of detailed data on supplement intake represents another potential confounding factor that could influence the observed relationship between vitamin B12 and homocysteine.

Future studies should incorporate renal function assessment and detailed information on micronutrient supplementation in

order to better control for these variables and further clarify the metabolic relationship between vitamin B12 status and homocysteine levels in patients with type 2 diabetes treated with metformin.

Conclusion.

The results of the present study indicate that vitamin B12 deficiency is a widespread phenomenon in patients with type 2 diabetes mellitus and receiving metformin treatment. The data obtained show that in the studied population, vitamin B12 deficiency or insufficient levels were detected in a significant proportion of patients, which indicates that these patients belong to a group at high risk of developing cobalamin deficiency. This circumstance emphasizes the need for regular monitoring of vitamin B12 levels during long-term treatment with metformin.

The study found that the daily dose of metformin is weakly but statistically significantly associated with homocysteine concentrations. Although this association is not strong, it is biologically plausible and suggests that the risk of functional impairment of one-carbon metabolism may increase with increasing metformin dose. Therefore, metformin dose can be considered as an additional factor influencing homocysteine metabolism.

The study also showed a strong and statistically significant negative association between vitamin B12 and homocysteine concentrations in patients with type 2 diabetes treated with metformin. The results support a biochemical mechanism whereby vitamin B12 is involved in the remethylation of homocysteine and its deficiency leads to the accumulation of homocysteine in the blood plasma. The strong standardized coefficient indicates that vitamin B12 is a significant independent determinant of homocysteine levels in this group of patients.

ROC analysis showed that vitamin B12 concentration has a good diagnostic ability for detecting elevated homocysteine levels. According to the results, the vitamin B12 value of 443.8 pg/mL represents a clinically significant threshold value, below which the probability of elevated homocysteine levels increases significantly. This threshold value is characterized by high sensitivity and specificity, which indicates the good diagnostic efficiency of vitamin B12 in the process of identifying metabolic disorders associated with elevated homocysteine.

Thus, the obtained data indicate that in patients with type 2 diabetes mellitus treated with metformin, the assessment of vitamin B12 is important not only for the purpose of controlling the hematological status, but also in the context of assessing the cardiovascular and neurological risks associated with homocysteine metabolism. The combined monitoring of vitamin B12 and homocysteine can be used as a practical clinical tool for early detection of metabolic risk in patients treated with metformin and ensuring timely preventive intervention.

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