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Study of iron deficiency conditions in patients with gluten-related disorders who are on a gluten-free diet

Abstract. Background. Iron deficiency anemia (IDA) is the most common extraintestinal symptom of celiac disease in adults. Studies of the presence or absence of iron deficiency, including cases of latent anemia, in patients with both celiac disease and non-celiac gluten sensitivity (NCGS) has not been previously carried out in our country. The purpose was to study and analyze general clinical and biochemical blood parameters of patients who are on a gluten-free diet with regards to the presence of anemia and/or iron deficiency. **Materials and methods.** The study included 38 adults previously diagnosed with one of the major types of cereal intolerance — celiac disease or NCGS. All of them were on a gluten-free diet. The following hematological parameters were studied and analyzed for each subject: erythrocyte count, hemoglobin, ferritin, total iron-binding capacity, iron, and blood transferrin levels. **Results.** The analysis revealed that 13.16 % of 38 patients had mild IDA as measured by hemoglobin levels, and latent iron deficiency was found in 18.42 % patients. In general, analysis of serum ferritin concentrations revealed the rate of iron deficiency of 31.58 % despite following a gluten-free diet for 6.5 (interquartile range of 4–12) years, suggesting that the rate of all iron deficiency cases (including the latent ones) almost twice exceeds the rate of IDA detected by hemoglobin analysis. Withal, the red blood cell count was normal in 100 % of the examined people, but a decrease in the concentration of serum iron in the blood was observed in 23.08 % of patients. Among individuals with iron deficiency, people with celiac disease are more likely to be found than those with NCGS (83.3 and 16.7 %, respectively). **Conclusions.** Patients with celiac disease and NCGS remain at risk for the development of IDA despite following a gluten-free diet. The isolated indicator of red blood cells is not informative for the diagnosis of not only latent iron deficiency but IDA as well. Isolated serum iron levels are also not indicative for the diagnosis of these conditions. In this regard, ferritin becomes the most significant marker of iron deficiency. Thus, it is necessary to carefully monitor this group of patients to identify latent forms of nutritional deficiency, in particular, iron deficiency, and to carry out the well-grounded individualized correction of their consequences.

Keywords: celiac disease; non-celiac gluten sensitivity; iron deficiency anemia; latent iron deficiency

Introduction

Celiac disease is an autoimmune disease that affects the small intestine in genetically predisposed individuals and is associated with absolute gluten intolerance [1]. The consequence of morphological changes in celiac disease is impaired absorption (malabsorption) with the subsequent formation of various types of macro- and micronutrient insufficiency [2].

Non-coeliac gluten sensitivity (NCGS) is a pathological condition associated with cereals intolerance; it has other mechanisms of development. Patients with NCGS also, as patients with celiac disease, are typically prescribed gluten-free diet (GFD), which significantly improves their quality of life. However, despite the mechanisms of pathogenesis and the intestinal mucosa damage, which differ from celiac disease, NCGS is also often accompanied by iron deficient anemia (IDA) [3]. According to world data, the incidence of IDA in patients with NCGS is 15.9% [4].

GFD is the only treatment for both celiac disease and NCGS. It helps to restore the absorption of iron, and, accordingly, to normalize its content in the body. However, there are some publications showing incomplete recovery of intestinal mucosa against GFD, which may support the persistence of deficient states in adult patients. [5]. Thus, atrophy of intestinal mucosa has been shown to persist in patients with celiac disease on GFD with prevalence from 4% [6] to 55% [7]. Iron is known to be absorbed in the small intestine, so untreated celiac disease is often accompanied by a deficiency of this element, and iron deficiency anemia becomes a common complication and extra-intestinal clinical manifestations in a timely undiagnosed gluten enteropathy [8%]. On the other hand, prolonged recovery of intestinal mucosa can also cause prolonged delay in the recovery of absorption processes, in particular iron - and lead to the development of IDA in patients following GFD. Thus, iron deficiency becomes a reflection of the mismatch between it's the intake and loss of the body. Common criteria for the diagnosis of anemia are a decrease in the concentration of hemoglobin and / or erythrocytes per unit volume of blood [9]. A reliable confirmation of the presence of IDA is the low level of serum ferritin [10]. Iron deficiency anemia, respectively, is formed as a consequence of iron deficiency.

Classic feature of the clinical blood test in case of IDA is traditionally microcytic, hypochromic anemia, low serum iron concentration, increased total iron-binding capacity of serum (TIBC) and low ferritin [11]. However, in clinical practice, the absence of laboratory manifestation of IDA (namely, a decrease in the concentration of erythrocytes and hemoglobin) does not always indicate the absence of the iron deficiency present in the latent stage of IDA [12]. Therefore, serum ferritin concentration analysis is used to verify such latent deficiency states. Serum ferritin has proven to be an excellent marker of total iron stores in the body, so in pathological conditions, the latter becomes an excellent indicator of the decrease of these reserves, namely hidden iron deficiency. According to the literature data, low serum ferritin content is detected in 19–33% of adult patients with celiac disease already at the time of diagnosis of

the disease [13]. Delayed detection of latent iron deficiency and absence of its correction forms iron deficiency anemia [14]. That is why the study of serum ferritin has caught our attention in this study.

In Ukraine, there are isolated studies, which included data on the frequency of detection of anemic syndrome in patients with celiac disease [15]. Studies of the presence or absence of iron deficiency, incl. hidden deficiency in patients with both celiac disease and NCGS, had not been conducted in our country before.

The purpose of the study: to investigate and analyze the general clinical and biochemical parameters of blood, reflecting the presence of anemia and / or iron deficiency in patients with celiac disease and NCGS present on GFD.

Tasks:

- To investigate the presence or absence of IDA in patients with celiac disease / NCGS on an GFD.
- To investigate blood counts in patients with celiac disease / NCGS on a GFD that reflects the presence of latent iron deficiency.
- To analyze the association and influence of GFD on blood counts of patients with celiac disease / NCGS.

Materials and methods

The study included 38 adult members of the Ukrainian Society of Celiac Disease, with previously diagnosed major types of cereals intolerance - celiac disease and NCGS. They were all on a diet with the complete exclusion of gluten.

Among the study participants were 9 (23.67%) men and 29 (76.33%) women; median age 41 years (IQR 21-50 years). The median maintained time at the GFD was 6.5 (IQR 4-12) years.

At first, all hematologic parameters were evaluated in the general group, and then all patients were divided into 2 groups according to their major disease: celiac disease and NCGS. The celiac group included 28 people, including 7 (25%) males and 21 (75%) females, with a median age of 36.5 (IQR 18.75-50.0) years and a median time on diet 8.75 (IQR 3-13) years. In the NCGS group - 10 people - 2 (20%) men and 8 (80%) women, with a median age of 42.0 (IQR 32.25-47.75) years and with a median time on a diet of 5.5 (IQR 5 -7) years. The groups did not differ significantly by age ($p = 0.528$), diet maintained time ($p = 0.326$), and gender ($p = 0.913$). Within all groups, there was significantly more quantity of women than men. And although the celiac group is predominant in the female population - in the NCGS group the number of women also dominated the number of men, with a ratio of 4: 1 (Table 1).

It should also be noted that clinically, all patients included in the study did not have sideropenic syndrome (patients did not notice hair loss, brittle nails, dry skin). The following hematologic parameters were determined for all subjects: erythrocyte content, hemoglobin, ferritin, TSIBC, iron and blood transferrin.

The following generally accepted laboratory criteria were used in our study: hemoglobin: - 135-172 g / l (for men), 120-156 g / l (for women); TSIBC 45.5-81.9 μmol / l; serum iron 9-21.5 μmol / l; ferritin 22-322 mcg / l for

Table 1. Gender composition of the study groups (n = 38)

Groups	Total	Women	Men	Ratio
Total	38	29	9	3,22 : 1
Celiac disease	28	21	7	3 : 1
NCGS	10	8	2	4 : 1

men and 10-291 mcg / l for women, transferrin 2.15-3.6 g / l). Depending on the combination of hematologic changes (ferritin, hemoglobin) we have distinguished the following pathological conditions: iron deficiency anemia (reduction of ferritin and hemoglobin), latent iron deficiency (isolated reduction of ferritin and normal hemoglobin) and iron deficiency (decrease in ferritin level regardless of other indicators).

Due to the difference in the reference values of ferritin by some laboratories, we performed an individualized calculation to decide whether or not iron deficiency was present.

Statistical processing of the results was performed using the EZR software package version 1.38 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is the graphical user interface of the software package R (The R Foundation for Statistical Computing, Vienna, Austria) [16]. Student's T-test was used to compare parametric criteria, and Mann-Whitney U-parameter was used for non-parametric criteria. The Spearman correlation coefficient (two-sided alternative hypothesis) was used to determine the correlation between the indicators. Fisher's exact Bonferoni correction for multiple comparisons was used to compare qualitative indicators.

Results

In the group of patients, we examined, the mean erythrocyte and hemoglobin values didn't differ from reference value. Other indicators of general blood test were also kept within normal limits. Serum ferritin levels, as a manifestation of possible iron deficiency in the general group, were also within the normal range of laboratory norms for adults almost in all cases. The mean values of TIBCS, serum iron, and transferrin in the general group didn't differ from reference value. The obtained result may, at first glance, indirectly indicate sufficient compensation for the investigated category of individuals, reflecting the long-term restoration of the structure of intestinal mucosa on the GFD. However, it represents only an average indicator, implying the possibility of deviation from the norm of these indicators in individuals.

Therefore, we analyzed the frequency of deviation of each of the above hematological parameters from the reference value. The results of the analysis are presented as a diagram in Fig. 1.

As can be seen from the diagram (Fig. 1), the reduced iron level of the general group is less common than normal level. A decreased level was found in 3 (23.08%) patients (NCGS n = 2; celiac n = 1; 15.39% and 7.69%, respectively).

The frequency of detection of altered (increased or decreased) transferrin levels in the general group of subjects was registered less frequently than normal level, and only in patients with celiac disease (15.38%, n = 2) n = 2. The TIBCS level was more often normal (92.3%, n = 12) (Fig. 1).

As mentioned above, erythrocyte indices didn't differ from reference value in the general study group. The same tendency persisted in the frequency analysis (Fig. 1). However, hemoglobin level was decreased in 5 (13.16%) patients, 3 of them were in the celiac group and 2 cases observed in NCGS group. Thus, 13.16% of the patients in the general group had IDA (a decrease in both ferritin and hemoglobin).

Because anemia was mild in the group of patients with established IDA, we hypothesized that there was latent iron deficiency that was not detectable according to the above studies. For this reason, our attention was drawn to the study of indicators of ferritin in the study group. As is known, the decrease in the ferritin concentration in the blood indicates the presence of hidden (latent) iron deficiency long before the formation of clinically pronounced IDA. Thus, we found a decrease in the concentration of ferritin in 12 (31.58%) of

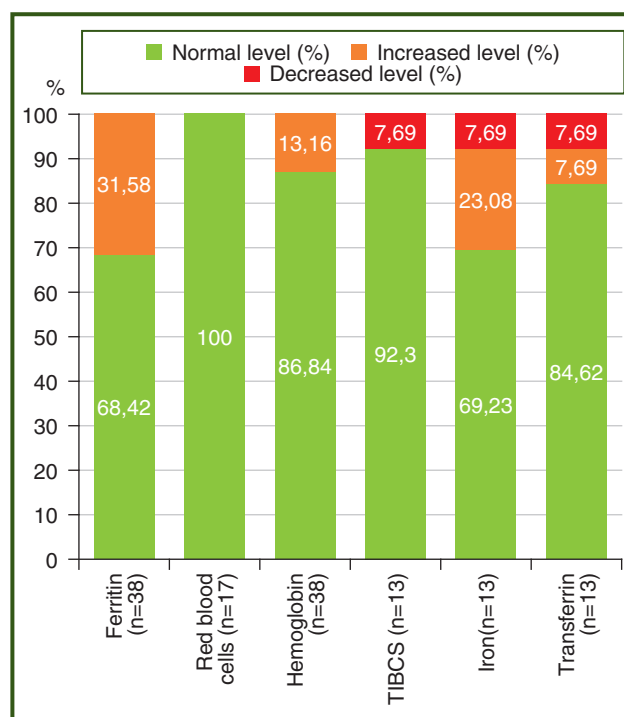


Fig. 1: Frequency of normal and altered blood counts in patients in the general group

patients. Of these, 26.32% (n = 10) were celiac patients and 5.26% (n = 2) had NCGS.

A more detailed analysis of the subgroup of patients with reduced ferritin is presented in Figs. 2.

Figure 2 presents data on the level of ferritin (a bar graph) in the general study population and analysis of the reduced ferritin group (pie chart). Of the entire surveyed population, 68.42% (n = 26) were individuals without iron deficiency, and 31.58% were patients with a decrease in ferritin level (n = 12). The analysis of indicators of erythrocytes and hemoglobin of blood in the reduced ferritin group determined that their average values were (M + SD): erythrocytes - 4.511 + 0.399 T / L (95% CI 4.306-4.717 T / L), hemoglobin 132.29 + 16, 42 g / l (95% CI 126.9-137.7 g / l). Thus we see that the red blood cell counts in the group of patients with iron deficiency remain normal, while hemoglobin appears to be close to the lower limit of the norm. Such a hematological picture becomes a frequent cause of insufficient attention of practitioners to slightly reduced and not always related levels of erythrocyte and hemoglobin concentrations.

An analysis of individuals with a deficiency in serum ferritin concentration (n = 12) showed that among them were 11 women (91.7%) and 1 man (8.3%); and patients with celiac disease - noticeably more (83.3%, n = 10) than with NCGS (16.7%, n = 2).

Among all individuals with reduced ferritin levels, 58% of patients had normal hemoglobin levels (n = 7, all from the group of celiac disease, 6 women and 1 man), which did not allow them to be diagnosed with IDA, but indicated an already existing latent iron deficiency. In 42% of individuals from the group with a reduced level of ferritin, in whom the hemoglobin level was reduced (n = 3 from the group of celiac disease and n = 2 from the NCGS, all women), the presence of IDA was detected (Fig. 2).

Thus, with a decrease in ferritin level in 31.58% of the total study group, the presence of mild IDA was noted in a third of them (13.16%), which indicates a more pronounced and unrecognized problem of latent iron deficiency in persons on GFD, not detected by routine general clinical analyzes (Fig. 3).

As follows from fig. 3, in the examined contingent, a normal level of iron is significantly more likely to occur than iron deficiency states. Despite this, we were able to identify patients with mild IDA - 13.16% and latent (latent) iron deficiency - 18.42%. After that, we decided to analyze the frequency of detection of IDA and LD in each group. Among 28 patients diagnosed with celiac disease, 10 (35.72%) showed a decrease in ferritin, and only 3 (10.71%) showed a simultaneous decrease in ferritin and hemoglobin (IDA); thus, latent iron deficiency was detected in 7 (25%) patients from the celiac disease group. Of the 10 patients with NCGS, ferritin was reduced in 2 (20%), and their hemoglobin was also reduced.

As a next step, we checked the presence of a correlation between the duration of patients' stay on the GFD and the obtained blood counts. A significant strong negative correlation was found between the duration of patients' stay on GFD and the content of red blood cells (correlation coefficient = -0.792, p = 0.006). Perhaps this is due to folic acid deficiency, which often develops in patients with celiac disease on GFD. This assumption requires further laboratory confirmation.

In a quantitative comparison of the studied parameters between the groups (celiac disease / NCGS), we did not find a statistically significant difference in any of them (p > 0.1) (Fig. 4).

The obtained data also indicate the need for an individualized examination with high-quality personalization of the results for each specific patient who is on GFD in order to identify hidden malabsorption disorders.

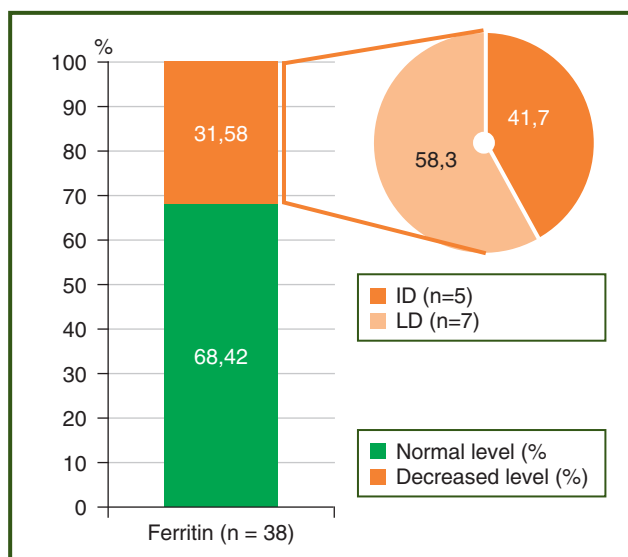


Fig. 2. The percentage of IDA and LD among patients with reduced levels of ferritin (n = 12)

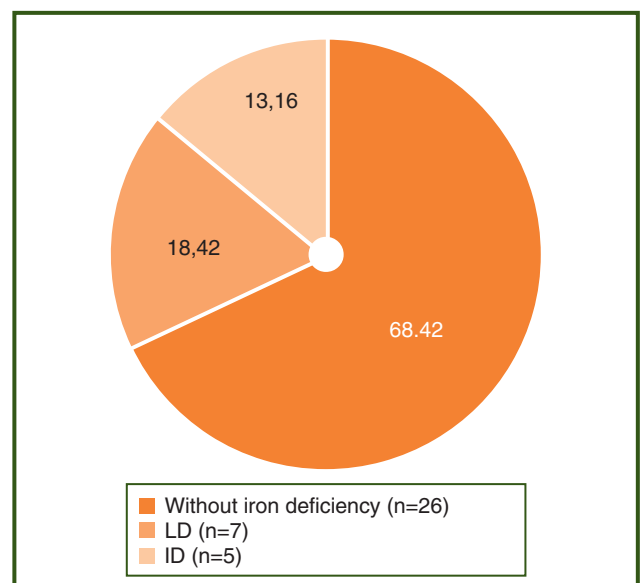


Fig. 3. Distribution of the studied population according to the degree of iron deficiency (n = 38)

Discussion

We have conducted a study of adult patients with celiac disease and NCGS who were on GFD for duration of 6.5 (IQR 4-12) years. Despite the long-term treatment of these gluten-dependent diseases, we have identified and recorded persistent malabsorption disorders associated with impaired iron metabolism in 31.58% of the examined. Moreover, mild IDA was recorded in 13.16% of patients. Despite a similar percentage of the revealed decrease in ferritin in the total number of studied, in the celiac group we didn't find statistically significant difference in the number of patients with reduced ferritin over the number of those with this indicator within normal limits. A part of the study was devoted to the presence of a possibly routinely undetectable latent iron deficiency, which remains unrecognized by traditional general clinical blood tests. In addition, the use of a statistical analysis of the average values of the studied blood parameters also does not allow revealing clinically significant difference in the groups. This required us to perform a qualitative analysis of all studied indicators.

Thus, our study demonstrated that celiac disease and NCGS, despite prolonged treatment and adherence to the requirements of GFD, are a continuing cause of iron deficiency conditions. Among the patients examined by us, IDA was recorded in 18.42% of the examined; a decrease in serum iron concentration was found in 23.08% of patients (15.39% with OBC, n = 2; and 7.69% with celiac disease, n = 1). A more detailed study of latent iron deficiency indicators according to the results of a study of the concentration of serum ferritin revealed its decrease in

31.58% of patients (35.7% of patients with celiac disease and 20% with NCGS).

All these data indicate that celiac disease and NCGS, even on the background of GFD, remain potential causes of micronutrient disorders. And the use of routine laboratory blood testing methods with analysis of red blood cell and hemoglobin levels, as well as serum iron concentrations, do not allow us to state with confidence that there is no latent iron deficiency, as well as sufficient restoration of the intestinal mucosa during treatment. Therefore, such patients need careful monitoring of blood counts using more subtle diagnostic tests, one of which should be the analysis of serum ferritin.

The literature data that we found indicate that the issues of restoring the micronutrient status of patients are still unresolved, especially for patients with NCGS (a new disease that occurs with latent malabsorption against the background of microscopic and submicroscopic changes in intestinal mucosa) [17]. Recent publications have increasingly shown that, despite the obvious benefits of GFD, in itself, a strict restrictive gluten-free diet often causes nutritional disorders, up to deficits. One of the most common deficits due to GFD is a deficiency of calcium, iron, magnesium and zinc. The 2018 review presents data on the effect of GFD on patients with celiac disease and gluten-dependent diseases, one of which is the NCGS [18]. In other similar articles, the authors find and emphasize the persistence of micronutrient deficiency in patients on a GFD [19, 20], especially iron [20]. In an article on possible ways to improve treatment outcomes against the background of GFD, the conclusion is drawn about the need to control B vitamins, microelements, es-

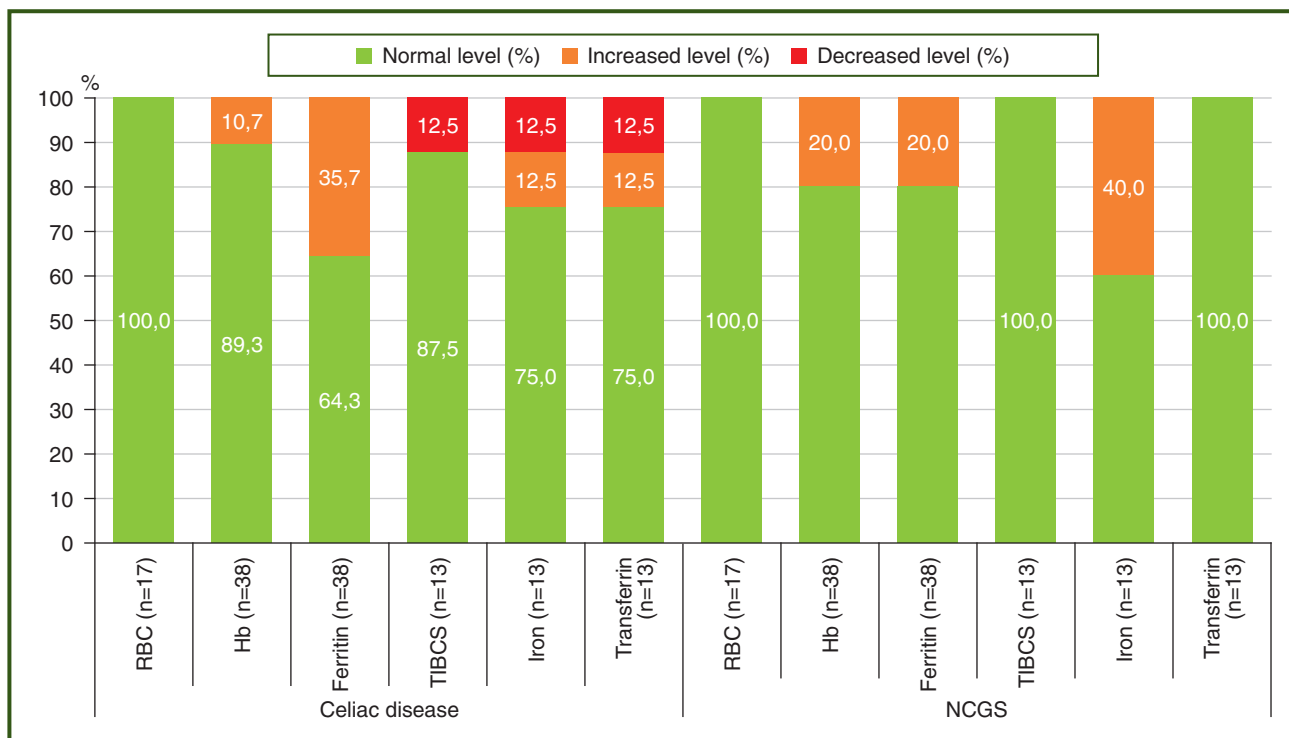


Fig. 4. The frequency of detection of altered blood count parameters of patients of celiac disease groups and gluten intolerance without celiac disease.

pecially iron, in individuals on GFD, and the need to fortify the widespread gluten-free commercial products with the mentioned micronutrients or individualized correction of laboratory-detected deficient conditions in such patients [2].

We also revealed a negative effect of GFD on the content of red blood cells in the blood - a strong negative relationship between these indicators. Perhaps the cause of this phenomenon is folic deficiency, which occurs due to a long diet with the exception of cereals, but this issue requires a separate study.

Conclusions

As a result of the examination of 38 patients of the Ukrainian public organization “Ukrainian Society of Celiac Disease”, on a gluten free diet lasting 6.5 (IQR 4-12) years, we revealed the presence of mild IDA in 13.16% of the examined, and latent iron deficiency was detected in 18.42% of the examined. A decrease in the concentration of iron in blood serum was observed in 23.08% of patients in the general group, but the frequency of iron deficiency (including latent) in the examined group by analysis of concentration of serum ferritin reached 31.58%, prevailing almost twice as much as the detection rate of IDA. Moreover, the content of red blood cells was normal in 100% of the examined. Among patients with iron deficiency, individuals with celiac disease are more likely to occur than among patients with NCGS (83.3% and 16.7%, respectively).

Thus, patients with celiac disease and NCGS, despite the following GFD, remain a risk group for the formation and persistence of hidden malabsorption micronutrient disorders (in particular, iron deficiency). The isolated indicator of red blood cells is not informative for the diagnosis of not only latent iron deficiency, but also IDA. Isolated serum iron levels are also not indicative of diagnosing these conditions. In this regard, it is ferritin that becomes the most significant marker of iron deficiency.

The data presented indicate the need for targeted monitoring of patients with celiac disease and NCGS on the background of GFD in order to identify hidden micronutrient disorders and the implementation of reasonable, individualized, correction of their consequences. Further studies are needed on a larger population of patients with celiac-dependent diseases for more accurate detection of associations between the studied parameters.

Conflicts of interests. Authors declare the absence of any conflicts of interests and their own financial interest that might be construed to influence the results or interpretation of their manuscript.

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Дослідження залізодефіцитних станів у хворих на глютензалежні захворювання, які перебувають на агліадиновій дієті

Резюме. Актуальність. Залізодефіцитна анемія (ЗДА) — це найпоширеніший позакишковий симптом при целиакії у дорослих. Досліджень наявності або відсутності залізодефіциту, зокрема прихованого, у пацієнтів, які мають целиакію, так само як і непереносимості глютену без целиакії (НГБЦ), раніше в нашій країні не проводилося. **Мета дослідження:** вивчити та проаналізувати загальноклінічні та біохімічні показники крові, що відображають наявність анемії та/або залізодефіциту у хворих на целиакію та НГБЦ на агліадиновій дієті (АГД). **Матеріали та методи.** У дослідження було включено 38 дорослих із діагнованими раніше видами непереносимості злаків — целиакією та НГБЦ. Усі вони перебували на дієті з повним виключенням глютену. Всім досліджуваним визначалися гематологічні показники: загальна залізов'язуюча здатність крові, вміст еритроцитів, гемоглобіну, феритину, заліза та трансферину крові. **Результати.** Внаслідок обстеження 38 пацієнтів, які перебували на агліадиновій дієті протягом 6,5 (IQR 4–12) року, ЗДА легкого ступеня була виявлена у 13,16 % обстежених, при цьому латентний залізодефіцит визначався у 18,42 % обстежених. Зниження концентрації сироваткового заліза крові відзна-

чено у 23,08 % хворих загальної групи, але частота залізодефіциту (зокрема латентного) в обстеженій групі за аналізом показників концентрації сироваткового феритину досягла 31,58 %, превалюючи над частотою виявлення ЗДА майже вдвічі. При цьому вміст еритроцитів був нормальним у 100 % обстежених. Серед пацієнтів із залізодефіцитом частіше зустрічаються особи з целиакією, ніж із НГБЦ (83,3 і 16,7 % відповідно). **Висновки.** Хворі на целиакію та НГБЦ залишаються в групі ризику з розвитку ЗДА, незважаючи на те, що вони знаходяться на агліадиновій дієті. Ізольований показник еритроцитів не є інформативним для діагностики не тільки прихованого залізодефіциту, але і ЗДА. Ізольований сироватковий рівень заліза також не є показовим для діагностики цих станів. Щодо цього саме феритин стає найбільш показовим маркером залізодефіциту. Необхідно цілеспрямовано спостерігати за цією групою хворих з метою виявлення прихованих форм мікронутрієнтного дефіциту, зокрема заліза, і здійснення обґрунтованої, індивідуалізованої корекції їх наслідків.

Ключові слова: целиакія; непереносимість глютену без целиакії; залізодефіцитна анемія; прихований залізодефіцит

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Исследование железodefицитных состояний у больных глютензависимыми заболеваниями, находящихся на аглиадиновой диете

Резюме. Актуальность. Железодефицитная анемия (ЖДА) — это наиболее частый внекишечный симптом при целиакии у взрослых. Исследований наличия или отсутствия железodefицита, в том числе скрытого, у пациентов, имеющих как целиакию, так и непереносимость глютену без целиакии (НГБЦ), ранее в нашей стране не проводилось. **Цель исследования:** изучить и проанализировать общеклинические и биохимические показатели крови, отражающие наличие анемии и/или железodefицита у больных целиакией и непереносимостью глютену без целиакии, находящихся на АГД. **Материалы и методы.** В исследование были включены 38 взрослых с диагностированными ранее основными видами непереносимости злаков — целиакией и НГБЦ. Все они находились

на диете с полным исключением глютену. Всем исследуемым определялись гематологические показатели: общая железосвязывающая способность сыворотки, содержание эритроцитов, гемоглобина, ферритина, железа и трансферрина крови. **Результаты.** В результате обследования 38 пациентов, находящихся на аглиадиновой диете продолжительностью 6,5 (IQR 4–12) года, выявлено наличие ЖДА легкой степени у 13,16 % обследованных, при этом латентный железodefицит выявлялся у 18,42 % обследованных. Снижение концентрации сывороточного железа крови отмечено у 23,08 % больных общей группы, но частота железodefицита (в том числе латентного) в обследованной группе по анализу показателей концентрации сывороточного ферритина достигла 31,58 %,

превалируя над частотой выявления ЖДА почти вдвое. При этом содержание эритроцитов было нормальным у 100 % обследованных. Среди пациентов с железодефицитом лица с целиакией встречаются чаще, чем среди пациентов с НГБЦ (83,3 и 16,7 % соответственно). **Выводы.** Больные целиакией и НГБЦ остаются в группе риска по развитию ЖДА, несмотря на то, что они находятся на агладиновой диете. Изолированный показатель эритроцитов не является информативным для диагностики не только скрытого железодефицита, но и ЖДА. Изолированный сывороточный уровень железа также не яв-

ляется показательным для диагностики данных состояний. В этом отношении именно ферритин становится наиболее показательным маркером железодефицита. Необходимо целенаправленное наблюдение за этой группой больных с целью выявления скрытых форм микронутриентного дефицита, в частности железа, и осуществления обоснованной, индивидуализированной коррекции их последствий.

Ключевые слова: целиакия; непереносимость глютена без целиакии; железодефицитная анемия; скрытый железодефицит
