СРПСКИ АРХИВ
ЗА ЦЕЛОКУПНО ЛЕКАРСТВО
SERBIAN ARCHIVES
OF MEDICINE

Address: 1 Kraljice Natalije Street, Belgrade 11000, Serbia
☏ +381 11 4092 776, Fax: +381 11 3348 653
E-mail: office@srpskiarhiv.rs, Web address: www.srpskiarhiv.rs

Paper Accepted* **ISSN Online 2406-0895**

Original Article / Оригинални рад

Nedeljko Radlović[1,†], Zoran Leković[2,3], Marija Mladenović[4], Vladimir Radlović[2], Biljana Vuletić[5, 6], Siniša Dučić[2,3], Zoran Golubović[2,3], Dejan Nikolić[2,3], Meho Mahmutović[7], Snežana Petrović-Tepić[8]

Frequency, severity and type of anemia in children with classical celiac disease

Учесталост, тежина и тип анемије код деце са класичном целијачном болешћу

[1]Serbian Medical Society, Academy of Medical Sciences, Belgrade, Serbia;
[2]University Children's Hospital, Belgrade, Serbia;
[3]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[4]Valjevo Medical Centre, Valjevo, Serbia;
[5]Kragujevac Clinical Center, Pediatric Clinic, Kragujevac, Serbia;
[6]University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia;
[7]Novi Pazar General Hospital, Novi Pazar, Serbia;
[8]University of Banja Luka, School of Medicine, Republic of Srpska, Bosnia and Herzegovina

Received: December 3, 2018
Accepted: February 15, 2019
Online First: March 18, 2019
DOI: https://doi.org/10.2298/SARH181203021R

*__Accepted papers__ are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the *Serbian Archives of Medicine*. They have not yet been copy edited and/or formatted in the publication house style, and the text may be changed before the final publication.

Although accepted papers do not yet have all the accompanying bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: the author's last name and initial of the first name, article title, journal title, online first publication month and year, and the DOI; e.g.: Petrović P, Jovanović J. The title of the article. Srp Arh Celok Lek. Online First, February 2017.

When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.

†**Correspondence to:**
Nedeljko RADLOVIĆ
Serbian Medical Society, Džordža Vašingtona 19, 11000 Belgrade, Serbia
E-mail: **n.radlovic@botel.net**

Frequency, severity and type of anemia in children with classical celiac disease

Учесталост, тежина и тип анемије код деце са класичном целијачном болешћу

SUMMARY

Introduction/Objective Anemia is the most common extraintestinal manifestation of celiac disease (CD) in children. The aim of this study was to determine the frequency, severity and type of anemia in children with a classical CD, as well as the differences between anemic and non-anemic patients in their age, duration of illness, percentile body length or height, percentage of body weight (BW) deviation compared to ideal and degree of damage to the small intestine mucosa.

Methods The study was based on a sample of 90 children, 56 females and 34 males, ages 7-90 (18.23±12.70) months with a classical CD. The diagnosis of the CD is based on the ESPGHAN criteria from 1990 and 2012, and the anemia at the 2011 WHO reference values..

Results Anemia was found in 47 (52.22%) patients, of which in 23 cases mild (Hb 100-109 g/L) and 24 moderately severe (Hb 70-99 g/L), respectively, in 34 (72.34%) of them microcitic (MCV <70 fl) and in 13 normocytic (MCV 70-87 fl). Low serum iron levels (<10.7 μmol/L) were found in 68 (75.56%), and hypoferritinemia (<16 ng/ml) in 77 (85.56%) patients. Except for a greater deficit of BW in patients with anemia compared to those without anemia (-14.64±9.60 vs. -8.56±11.87%, p <0.01), differences in other defined features were not significant.

Conclusion Mild or moderate iron deficiency anemia occurs in slightly more than half of children with a classical type CD. In anemic compared to non-anemic patients, there is a significantly higher BW deficit, while differences in other characteristics typical for this type of disease are not significant.

Keywords: classical celiac disease, children, anemia

САЖЕТАК

Увод/Циљ Анемија је најчешћа екстраинтестинална манифестација целијачне болести (ЦБ) у дечјој доби. Циља рада је био да се утврди учесталост, тежина и тип анемије код деце са класичном ЦД, као и разлике између анемичних и неанемичних болесника у узрасту, дужини трајања болести, перцентилу телесне дужине или висине, проценту одступања телесне тежине (ТТ) у односу на идеалну и степену оштећења слузнице танког црева.

Метод Студијом је обухваћен узорак од 90 деце, 56 женског и 34 мушког пола, узраста 7-90 (18,23±12,70) месеци са класичним ЦД. Дијагноза ЦД је базирана на *ESPGHAN* критеријумима из 1990. и 2012. године, а анемије на референтним вредностима *WHO* из 2011. године.

Резултати Анемија је констатована код 47 (52,22%) болесника и то код 23 лака (*Hb* 100-109 *g/L*) и код 24 средње тешка (*Hb* 70-99 *g/L*), при чему код 34 (72,34%) микроцитна (*MCV* <70 *fl*) и код 13 нормоцитна (*MCV* 70-87 *fl*). Снижен ниво гвожђа у серуму (<10,7 *μmol/L*) утврђен је у 68 (75,56%), а феритина (<16 *ng/ml*) у 77 (85,56%) пацијената. Изузимајући већи дефицит *BW* код болесника са анемијом у односу на оне без анемије (-14,64±9,60 односно -8,56±11,87%, *p* <0,01), разлике у другим дефинисаним обележјима између анемичних и неанемичних испитаника нису биле значајне.

Закључак Лака или умерено тешка сидеропенијска анемија се јавља код нешто више од половине деце са ЦБ. Код анемичности у поређењу са неанемичним болесницима регистрован је значајно већи дефицит ТТ, док разлике у другим карактеристикама типичним за ову врсту болести нису биле значајне.

Кључне речи: класична целијачна болест, деца, анемија

INTRODUCTION

Anemia is the most common extraintestinal manifestation of celiac disease (CD) [1-5]. Depending on the study, it is found in 16% to 84% of newly detected patients, more often and more pronounced in severe and prolonged forms of the disease [4-8]. A key role in the

pathogenesis of anemia in CD, both in children and adults, is iron deficiency, while lack of folic acid, vitamin B12, copper and protein results in a lesser expression [4, 9, 1-12]. In a significant number of cases anemia can be the main, and often the only sign of the disease [9, 13-19]. This clinical presentation of CD is commonly seen in adults and adolescents, although it is not rare in school and preschool children [9]. According to the results of some studies, CD as the etiological factor of sideropenic anemia participates with a prevalence of 6-21.3% [9, 17-19]. Hence, some authors recommend that all patients with siderophenic anemia of an unclear cause, especially those resistant to oral iron therapy, should be tested on the CD [13, 17, 19].

OBJECTIVE

The aim of our study was to determine the frequency, severity and type of anemia in children with a classical CD. In addition, there are analyzed the differences between anemic and non-anemic patients in the age of diagnosis of basic disease and its previous duration, percentile body length (BL) or height (BH), percentage of body weight deviation (BW) compared to the ideal and the degree of damage of small intestine mucosa.

METHODS

The objectives of the study were analyzed on a sample of 90 children (56 female and 34 male) ages 7-90 (18.23±12.70) months with classical CD, ie type of the disease followed by chronic diarrhea (>2 weeks) and failure to thrive. The study protocol was approved by the local ethics committee. Diagnosis of CD was based on the European Society for Pediatric Gastroenterology, Hepatology and Nutrition guidelines published in 1990 and 2012 [20, 21].

Diagnosis was preceded by a detailed medical history, complete clinical examination and appropriate laboratory tests. The study protocol was approved by the local ethics committee.

In the history of the disease, for each patient, exact data related to the onset, duration and severity of the underlying disease According to the data from parents, all respondents had optimally progressed and had normal blood counts before the onset of the disease. During the clinical examination, each of them was measured BL/BH and BW and the obtained values were compared with the standard for the appropriate age and gender. The values of BL/BH are expressed in percentages, and deviations in BW in relation to the ideal in percent.

In accordance with modified Marsch criteria, small intestinal mucosal damage is classified into infiltrative (I), infiltrative-hyperplastic (II), destructive (III), and hypoplastic (IV) [22]. Depending on the degree of destruction of villi, destructive enteropathy are additional differentiated on the partial (IIIa), subtotal (IIIb) and total (IIIc).

Blood count and serum iron and ferritin concentrations were determined by standard laboratory methods from a blood portion taken in the morning and before breakfast. The diagnostic criterion for anemia was level of the hemoglobin (Hb) for children up to 5 years below 110 g/L, and for children 5-11 years below 115 g/L [23]. The Hb value of 100-109 g/L was classified as a slight anemia, from 70 to 99 g/L moderate, and below 70 g/L severe [23]. The reference value for red blood cells count (RBCs) was 3.90-5.10 x 10^{12}/L, for mean cell volume (MCV) 70-87 fl, for mean cell Hb (MCH) 25-31 pg, and for iron serum concentration 10.7-31.3 μmol/L of ferritin 16-100 ng/ml [24]. Differentiation of anemia types is based on the values of MCV, MCH and serum iron concentration.

The differences between the anemic and non-anemic groups of children in the age of diagnosis and the duration of the underlying disease were tested by Oneway ANOVA, (on-the-clock analysis of variance), in gender by $\chi 2$ testom, in the degree of small intestinal

mucosal damage by Kruskal-Wallis and Mann-Whitney's test, and in the percentile BL/BH and the percentage of BW deviation compared to the ideal by Student's T-test.

RESULTS

Anemia with Hb values of 71-109 (96.62±9.33) g/L was observed in 47 of 90 or 52.22% of patients. None of them had severe anemia, while the incidence of mild and moderately severe anemia was almost the same (24 vs. 23). The number of RBCs in the blood in the whole group of subjects varied from 2.56-5.19 (4.29±0.73) x 10^{12}/L, while the MCV value was 50.5-88.0 (64.76±9.18) fl, serum iron concentrations of 2.1-15.5 (5.96±3.32) μmol/L and ferritin 2-18 (7±4.20 ng/ml. In the group of children with anemia, the number of RBCs was low in 15 (31.91%) of them, normal in 27 (57.45%), and elevated in 5 (5.11-5.70 x 10^{12}/L). In the same group of patients, MCV was decreased in 34 (72.34%) and normal in 13, while MCH was low in 35 (74.47%) and normal in 12. In the whole group of subjects, low serum iron levels was determined in 68 (75.56%) cases, and ferritin in 77 (85.56%). Granulocyte and platelet counts in the blood was normal in all.

The duration of symptoms before the diagnosis was 1-6 (2.21±1.48) months. The majority, 50 (55.56%), were at the age of 1-2 years, 28 younger than 1 year and 12 over 2 years. The values of percentile BL/BH ranged from 5-90 (37.62±26.26), and the percentage deviation BW compared to the ideal for the appropriate age and gender from +18.5 to -33 (-11.58±10.80). In all patients, a destructive enteropathy (type III) was found, of which in 7 of them partial (IIIa), in 41 subtotal (IIIb) and in 42 total (IIIc).

Differences in age and duration of disease, gender, percentile BL/B, percentage of deviation BW in relation to ideal and degree of damage of the obtained small intestine

samples among patients with anemia and without anemia are shown in Table 1. As can be seen, with the exception of significantly higher deficit BW in patients with anemia than in those without anemia, other differences were not significant.

DISCUSSION

Anemia in CD is primarily caused by iron deficiency, but also by the lack of other nutritional factors necessary for normal erythropoiesis, such as folic acid, vitamin B12, proteins and copper [10, 12, 25, 26]. Hence, viewed pathogenically, it belongs to a group of nutritive or hypoproliferative anemia [10]. Deficit of iron, protein and copper results in insufficient Hb synthesis and causes anemia of hypochromic and microcitic type, where the number of RBCs can be normal and elevated, while folic acid and vitamin B12 deficiency block normal regeneration of RBCs and give macrocytic anemia [27]. In the state of a combined deficit of a factor of essential importance for normal erythropoiesis, anemia gets normocytic features [10]. Folic acid deficiency, in addition to a smaller number of Er and low Hb and the number of reticulocytes, is characterized by high values of MCV and MCH and a reduced number of granulocytes and platelets [10]. An identical hematological image also has a lack of vitamin B12, but it is, except in heavy form of classic CD, rarely seen [4, 27, 28].

The basis of the deficit of the factors necessary for erythropoiesis is the absorption disorder caused by the inflammation of the small bowel mucosa [29]. The morphological and functional damage of the small intestine mucosa to the CD is most pronounced in its proximal part, i.e. in the segment where most of the nutrients are absorbed [29]. Negative nutritional balance in the classic type of CD is also significantly contributed by insufficient intake caused by anorexia and vomiting [30]. As with other inflammatory diseases, additional involvement in iron malabsorption also has a suppressive effect of hepcidin [12, 30].

The consequences of the disease are more pronounced in children in the first 2 years of age, i.e. in the period of the most intensive growth and development, especially in the cases of its prolonged duration [4, 9, 29]. The age of our patients was 18.23±12.70 months, and the length of the symptoms until the diagnosis was 2.21±1.48 months, resulting in a significant deficit of BW (-11.58±10.80%), reduced percentage of BL/BH (37.62±26.26) and high representation of subtotal and total enteropathy (92.22%). In accordance with these facts, the prevalence of anemia in our patients was high (52.22%). The mean Hb value in anemic patients was 96.62±9.33 g/L. None of them had severe anemia (Hb <70 g/L), while the incidence of mild and moderate anemia was almost the same (24 vs. 23). According to morphological features, anemia was microcitic and hypohromous in three quarters of cases and normocytic and normochromic in others. In the whole group of subjects, low serum iron levels were determined in 68 (75.56%) cases, and ferritin in 77 (85.56%).

Patients with anemia compared to non-anemic had a significantly higher deficit of BW. However, differences in the age of diagnosing the underlying disease, its previous duration, the percentile of BL/BH, and the severity of the histological lesion of the small intestine mucosa were not significant. The explanation for this finding is probably in severe clinical expression of the underlying disease and/or before its onset in lower values of Hb, RBCs and iron reserves in anemic patients compared to non-anemic patients. In support of the second hypothesis is the fact that the length of symptoms to diagnosis in this sample of patients was almost twice shorter than the average life of RBCs (2:21±1:48 vs. 4 months).

CONCLUSION

Mild or moderate iron deficiency anemia occurs in slightly more than half of children with a classical type CD. In anemic compared to non-anemic patients, there is a significantly

higher BW deficit, while differences in other characteristics typical for this type of disease, such as its duration, age of diagnosis, percentile of BL/BH and the degree of damage of the small intestine mucosa, are not significant.

Conflict of interest: None declared

REFERENCES

1. Branski D, Troncone R, Fasano A. Celiac disease (Gluten-sensitive enteropathy). In: Kligmena RM, Stanton BF, St Geme III JW, Schor NF, eds. Nelson Textbook of Pediatrics, 20th ed. Philaderlphia:Elsevier. 2016. p. 1835-8.
2. Fisgin T, Yarali N, Duru F, Usta B, Kara A. Hematologic manifestation of childhood celiac disease. Acta Haematol. 2004;111(4):211-4. DOI:10.1159/000077568.
3. Sanseviero MT, Mazza GA, Pullano MN, Oliveiro AC, Altomare F, Pedrelli L, et al. Iron deficiency anemia in newly diagnosed celiac disease in children. Minerva Pediatr. 2016;68(1):1-4. PMID:26864718.
4. Ertekin V, Selimoglu MA, Altinkaynak S. Celiac disease in childhood: evaluation of 140 patients. Eurasian J Med. 2009;41(3):154-7. PMID:25610093
5. Rajalahti T, Repo M, Kivelä L, Huhtala H, Mäki M, Kaukinen K, et al. Anemia in pediatric celiac disease: Association with clinical and histological features and response to gluten-free diet. J Pediatr Gastroenterol Nutr. 2017;64(1):e1-e6. doi: 10.1097/MPG.0000000000001221.
6. Çatal F, Topal E, Ermiştekin H, Yildirim Acar N, Sinanoğlu MS, Karabiber H, et al. The hematologic manifestations of pediatric celiac disease at the time of diagnosis and efficiency of gluten-free diet. Turk J Med Sci. 2015;45(3):663-7. PMID:26281336.
7. Deora V, Aylward N, Sokoro A, El-Matary W. Serum vitamins and minerals at diagnosis and follow-up in children with celiac disease. J Pediatr Gastroenterol Nutr.2017;65(2):185-9. doi: 10.1097/MPG.0000000000001475.
8. Singh P, Arora S, Makharia GK. Presence of anemia in patients with celiac disease suggests more severe disease. Indian J Gastroenterol. 2014;33(2):161-4. doi: 10.1007/s12664-013-0423-1.
9. Celiac disease: Working Group Report of the First World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr. 2002; 35: S78-S88. PMID:12192175.
10. Halfdanarson TR, Litzow MR, Murray JA. Hematologic manifestation of celiac disease. Blood 2007; 109(2): 412-21. DOI:10.1182/blood-2006-07-031104.
11. Fernández-Bañares F, Monzón H, Forné M. A short review of malabsorption and anemia. World J Gastroenterol. 2009;15(37):4644-52. PMID:19787827.
12. Freeman HJ. Iron deficiency anemia in celiac disease. World J Gastroenterol. 2015;21(31):9233-8. doi: 10.3748/wjg.v21.i31.9233.
13. Mody R, Brown P, Wechler D. Refractory iron deficiency anemia as the primary clinical manifestation of celiac disease. J Pediatr Hematol Oncol. 2003; 25(2): 169-72. PMID:12571473.
14. Fisgin T, Yarali N, Duru F, Usta B, Kara A. Hematologic manifestation of childhood celiac disease. Acta Haematol. 2004; 111(4):211-4. DOI:10.1159/000077568 → glavna ili jedina manifestacija CD
15. Abu Daya H, Lebwohl B, Lewis SK, Green PH. Celiac disease patients presenting with anemia have more severe disease than those presenting with diarrhea. Clin Gastroenterol Hepatol. 2013;11(11):1472-7. doi: 10.1016/j.cgh.2013.05.030.
16. Annibale B, Capurso G, Chistolini A, D'Ambra G, DiGiulio E, Monarca B, et al. Gastrointestinal causes of refractory iron deficiency anemia in patients without gastrointestinal symptoms. Am J Med. 2001;111(6):439-45. PMID:11690568.

17. Ertekin V, Tozun MS, Küçük N. The prevalence of celiac disease in children with iron-deficiency anemia. Turk J Gastroenterol. 2013;24(4):334-8. PMID:24254265.
18. Mandal AK, Mehdi I, Munshi SK, Lo TC. Value of routine duodenal biopsy in diagnosing coeliac disease in patients with iron deficiency anaemia. Postgrad Med J. 2004; 80(946): 475-7. DOI:10.1136/pgmj.2003.014670.
19. Grisolano SW, Oxentenko As, Murray JA, Burgart LJ, Dierkhising RA, Alexander JA. The usefulness of routine small bowel biopsies in evaluation of iron deficiency anemia. J Clin Gastroenterol. 2004; 38(9): 756-60. PMID:15365400.
20. Walker-Smith JA, Guandalini S, Schmitz J, Shmerling DH, Visakorpi JK. Revised criteria for diagnosis of coeliac disease. Report to working group of European Society of Paediatric Gastroenterology and Nutrition. Arch Dis Child. 1990;65(8):909–11. doi: 10.1136/adc.65.8.909.
21. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, ESPGHAN Working Group on Coeliac Disease Diagnosis. ESPGHAN Gastroenterology Committee et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012;54(1):136–60. doi: 10.1097/MPG.0b013e31821a23d0.
22. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol. 1999;11(10):1185–94. doi: 10.1097/00042737-199910000-00019.
23. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.1)
24. Belton NR. Biochemical and physiological tables and reference ranges for laboratory tests. In: McIntosh N, Helms PJ, Smyth RL, eds. Forfar&Arneil's Textbook of Pediatrics. Edinburgh: Churchill Liv. 2003. p. 1879-916.
25. Hershko C, Patz J. Ironing out the mechanism of anemia in celiac disease. Haematologica. 2008;93(12):1761-5. doi: 10.3324/haematol.2008.000828. patog
26. Stein J, Connor S, Virgin G, Ong DE, Pereyra L. Anemia and iron deficiency in gastrointestinal and liver conditions. World J Gastroenterol. 2016;22(35):7908-25. doi: 10.3748/wjg.v22.i35.7908. petog
27. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr. 2004;24:105-31. DOI: 10.1146/annurev.nutr.24.012003.132306
28. Leffler DA, Green PH, Fasano A. Extraintestinal manifestations of coeliac disease. Nat Rev Gastroenterol Hepatol. 2015;12(10):561-71. doi: 10.1038/nrgastro.2015.131.
29. Radlović N. Celiac disease. Srp Arh Celok Lek. 2013;141(1-2):122-6. PMID:23539923.
30. Janus J, Moerschel SK. Evaluation of anemia in children. Am Fam Physician. 2010;81(12):1462-71. PMID:20540485.

Table 1. Differences in the age of diagnosis of CD, duration of symptoms, percentile BL/BH, BW percentage deviation compared to the ideal and the degree of damage of the small small intestine mucosa in patients with anemia and without anemia

Observed features	Patients with anemia (No 47)	Patients without anemia (No 43)	Statistical significance
Age (months)	7.5-60 (16.42±10.72)	7.5-90 (16.52±5.96)	n.s.
Duration of symptoms (months)	1-6 (2.37±1.54)	1-6 (2.03±1.42)	n.s.
Percentil of BL/BH	5-90 (40.0±26.37)	5-90 (35.25±16.22)	n.s.
% deviation of BW	+9 do -33 (-14.64±9.60)	+18.5 do -28 (-8.56±11.87)	p <0.01
Enteropathy (No) IIIa : IIIb : IIIc	2 : 21 : 24	5 : 20 : 18	n.s.

BL – body length; BH – body height; BW – body weight; ns – not significant

