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Anemia in pediatric celiac disease: association with clinical and histological features and response to gluten-free diet

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Figure: 1

Tables: 4

Abstract

Goals: To compare clinical, serological and histological manifestations between children with anemia and without anemia at celiac disease (CD) diagnosis.

Background: Despite being a common finding, the association between the presence of anemia and clinico-histopathological presentation of CD in children remains obscure.

Study: 455 celiac disease patients <18 years of age were divided into those with anemia and those without anemia at diagnosis. The groups underwent comparisons of a variety of clinical, serological and laboratory parameters and severity of small-bowel mucosal damage. Further, adherence and clinical and serological response to the gluten-free diet (GFD) were compared.

Results: Anemia was detected in 18.0% of the patients. Children with anemia had higher values for transglutaminase 2 antibodies (120.0 U/l vs. 88.0 U/l, $p<0.001$) and, by definition, lower values for hemoglobin (10.5 g/dl vs. 12.8 g/dl, $p<0.001$) and other iron parameters. They were also less often screen-detected (13.4% vs 34.6%), had more severe histological damage ($p=0.048$) and poorer dietary adherence (78.3% vs 87.5%, $p=0.035$) than the non-anemic patients. Anemia recovered in 92% after a median of one year on a GFD, but hemoglobin values remained significantly lower compared with the non-anemic group (12.5 g/dl vs. 13.2 g/dl, $p=0.045$). There was no difference between the groups in the clinical and serological response to the GFD ($p=0.318$).

Conclusions: Anemia at CD diagnosis is associated with more severe histological and serological presentation in children. Further, low hemoglobin may not fully recover even after a median of one year on a strict GFD.

Keywords: Celiac disease, children, anemia, serology, histology

What is known

- Celiac disease (CD) is one of the most common chronic diseases in children
- A very common manifestation in untreated CD is anemia
- The association between the presence of anemia and clinico-histopathological presentation of CD is unclear

What This Study Adds

- Anemic children with CD have more severe clinical, serological and histological disease than non-anemic children
- Low hemoglobin levels may not fully recover even after one year on a gluten-free diet
- Early diagnosis of anemia at CD diagnosis is important

Introduction

Several recent screening studies have revealed that, with a prevalence of up to 2%, celiac disease (CD) is one of the most common lifelong disorders in children.[1,2] Simultaneously the condition has been found to have a very heterogeneous clinical presentation, including classical gastrointestinal symptoms such as diarrhea and abdominal pain, and a variety of extraintestinal symptoms such as arthralgia, dermatitis herpetiformis, poor growth and hypertransaminasemia.[3-6] Arguably one of the most common manifestations of CD in children is iron-deficiency anemia, of which the prevalence in untreated patients has varied from 16 % up to 84 % depending on the study.[7-12] Even mild or subclinical anemia can be detrimental to health since, besides the direct effects of reduced oxygen transport capacity, it predisposes children for example to poor cognitive and psychomotor development and to impaired immune defense.[13-15] Despite being such a prevalent finding, however the association between anemia and the clinico-histopathological presentation of CD remains poorly established. Interestingly, a recent study has shown adult CD patients with anemia as a prominent symptom to have more severe clinical and histological disease than those presenting with diarrhea.[16] Evidently, this issue should also be carefully investigated in children, in whom anemia may have markedly different causes and consequences compared with adults. Here we sought to address this issue by comparing a variety of clinical, histological, serological and laboratory findings and dietary response between children presenting with and those without anemia at CD diagnosis.

Materials & Methods

Patients and study design

The study was carried out at the Department of Pediatrics, Tampere University Hospital and at the Tampere Center for Child Health Research. Data on the clinical, histological and

serological findings were collected systemically from the medical records of all children (age less than 18 years) with biopsy-proven CD diagnosed at the Department of Pediatric Gastroenterology from the year 2000 onwards. From the year 2014 onwards most of the study children have been enrolled prospectively. After data collection and preliminary analysis children were divided into those with and those without anemia at the time of CD diagnosis, and all study variables (see below) were compared between these two groups. Anemia at CD diagnosis was defined as a Hb value lower than the age- and sex-specific reference.[17] Moreover, follow-up data regarding adherence and clinical response to treatment with a gluten-free diet (GFD) were collected as available.

The Ethics Committee of The Pirkanmaa Hospital District approved data collection from the medical records and prospective patient recruitment. In addition, all children and/or their parents recruited prospectively gave written informed consent.

Data analyses

Clinical characteristics

The following clinical information was gathered on all study children: demographic and anthropometric data, presence of CD associated or other co-morbidities (e.g. type 1 diabetes, Down's syndrome, asthma, allergies) and CD in the family. The type of the clinical presentation at diagnosis was classified as gastrointestinal, extraintestinal or screen-detected, and severity of presentation as no symptoms, mild symptoms (occasional, somewhat bothersome gastrointestinal or extraintestinal symptoms) or moderate/severe symptoms (frequent and/or severe symptoms significantly disturbing daily life). Gastrointestinal symptoms were further sub-categorized into abdominal pain, constipation, diarrhea, vomiting

and other, and extraintestinal symptoms and signs into aphtous ulcers, joint symptoms, liver abnormalities, neurological symptoms, poor growth, skin symptoms and other.

Serology and laboratory parameters

Serum IgA-class transglutaminase 2 antibodies (TG2-ab) were measured in the hospital laboratory either by traditional ELISA (Phadia, Uppsala, Sweden, before 2011) or by an automatized recombinant-based EliA assay (Phadia). In our settings values ≥7.0 U/l for TG2-ab are considered positive and the maximum provided value is 120.0 U/l. Serum IgA-class endomysial antibodies (EmA) were measured in the Tampere Center for Child Health Research by a well-defined immunofluorescence method as described elsewhere.[18] EmA titer 1:5 was considered positive and further diluted up to 1:4000 or until negative. In case of a selective IgA-deficiency the corresponding IgG antibodies were determined.

The following iron-related laboratory parameters measured as a part of routine clinical practice were recorded for each child when available: blood hemoglobin (Hb) (g/dl), erythrocyte mean corpuscular volume (MCV) (fl), plasma total iron (µmol/l), plasma transferrin receptor 1 (TfR1) (mg/l) and plasma ferritin (µg/l). Further, plasma alanine aminotransferase (ALT) (U/l), plasma alkaline phosphatase (ALP) (U/l), plasma albumin (g/l), plasma thyroid-stimulating hormone (TSH) (mU/l) and plasma thyroxine (pmol/l) were recorded in order to further elucidate the overall severity of the disease. For consistency we decided to accept only laboratory values taken in our hospital laboratory at the day of endoscopy for the baseline comparisons between the groups. Further, values other than Hb were started to be taken systemically only during the latter part of the study period.

Histology

In our clinical practice a minimum of four distal duodenal biopsies are taken upon upper gastrointestinal endoscopy in all cases with CD suspicion. From the year 2011 onwards 2-3 additional biopsies have been routinely taken from the anatomical duodenal bulb as recommended in recent guidelines.[19] The biopsies are processed in the hospital pathology unit and only well-oriented and representative cuttings are accepted for further microscopic analyses.[20] The severity of small-bowel mucosal damage is systemically graded by pathologist into partial (PVA), subtotal (SVA) and total villous atrophy (TVA). These grades correspond approximately Marsh-Oberhuber grades IIIa, IIIb, and IIIc, respectively.

Adherence and response to the gluten-free diet

All children/parents received personal guidance for the GFD from a dietitian after the celiac disease diagnosis. Adherence to the diet was evaluated after 6-12 months, and was considered strict if only a few minor lapses were reported; lapses less than once in a month were rated as "occasional lapses"; and more lapses as "no GFD". Good clinical response was defined as disappearance of symptoms, negative seroconversion or marked decrease in CD autoantibodies and improvement in possible abnormalities in growth and laboratory values. In addition, the EmA, tTG2-ab and hemoglobin values measured after a median 12 months on GFD were recorded and compared between the study groups.

Statistical analysis

The variables are presented either as medians with lower and upper quartiles or as percentage distributions. In addition, the figure for available data in each variable is reported in the tables. Non-parametric variables were compared with Mann-Whitney U test and categorical variables were examined by cross tabulation with χ^2-test. A p-value <0.05 was considered

significant in all analyses. All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS Inc. Chicago, IL, USA).

Results

The final study cohort comprised 455 children of whom 82 (18.0 %) were found to have anemia and 373 (82%) had normal hemoglobin levels at CD diagnosis. Children with anemia were significantly older and had fewer CD in the family compared with the non-anemic patients (Table 1). No differences were seen between the study groups either in gender or in the prevalence of any of the CD associated and other co-morbidities (Table 1).

The main clinical presentation at diagnosis was more often extraintestinal and less often screen-detected in the anemic children than those in the non-anemic group (Figure 1A). Of specific gastrointestinal symptoms the anemic patients suffered less from vomiting, and there was also a non-significant trend towards more constipation and less diarrhea (Table 2). In contrast, there were no differences between the groups in the prevalence of specific extraintestinal symptoms (Table 2). Anemic children also had a somewhat higher percentage of moderate/severe symptoms, though the difference was not significant (Figure 1B). The results remained unchanged when adjusted by age (data not shown).

The median values for both TG2-ab and EmA were higher in the anemia group at diagnosis (Table 3). Furthermore, by definition, Hb and all iron-related variables were poorer in the anemic children, while there were no differences between the groups in the other laboratory parameters (Table 3). Anemic children also evinced more severe small-bowel mucosal damage, the difference being caused by the higher proportion of total villous atrophy and the lower proportion of partial villous atrophy (Figure 1C).

Children in the anemia group showed significantly poorer adherence to the GFD at follow-up (78.3% vs 87.5%, p=0.035). However, in all cases there were only occasional lapses and none reported unrestricted gluten consumption. After a median of 12 months on GFD celiac autoantibody and Hb values were significantly improved (median change anemia group 2.0g/dl (16% increase) and non-anemic group 0.4g/dl (3% increase), respectively) in both groups compared with the baseline (Table 4). Anemia recovered in 92% of the children, but the anemia group still yielded significantly lower median Hb levels than the non-anemic group (Table 4). All four (8%) subjects with persistent anemia (baseline Hb values 6.5 g/dl, 9.7 g/dl, 11.2 g/dl and 11.6 g/dl) were on a strict GFD and showed good clinical and serological response. Altogether 25 anemic children (30 %) initiated oral iron supplementation after the CD diagnosis, including three out of the four with persistent anemia. Clinical response to the GFD was seen in 94.3% of anemic and 96.6% of non-anemic children (p=0.318).

Discussion

The main finding in the present study was that children with anemia at the time of CD diagnosis had more severe disease in terms of serology and small-bowel mucosal histology compared with those presenting without anemia. In addition, the anemic children showed partly incomplete recovery of Hb values even after a median of one year on a GFD.

Altogether 18% of the CD patients here had anemia, which is a relatively low figure compared with most previous studies conducted in children.[7-9,11] For example, de Vizia and colleagues[21] observed anemia to be present in 34% and Demir and colleagues[22] in 53% of pediatric CD patients at diagnosis, and the highest prevalences of anemia in untreated CD children, up to 85%, have been reported in studies from India.[8,9] However, in some of the

more recent studies figures closer to ours have been reported. In a British study[23] the prevalence of anemia in CD children was 20%, in a U.S. study[24] it was 20%, and in a previous smaller study from Finland[10] 25%. This global variation in anemia prevalences possibly reflects differences in clinical presentation, as in many developed countries the previously common severe infantile CD has almost disappeared, while in less developed countries the classical malabsorptive disorder still prevails.[8] Moreover, multiple nutritional defects, including iron deficiency, are very common in the third world nations and may further contribute to the high prevalence of anemia. In contrast, anemia is very rare in native Finnish pediatric population, and even the 18% seen in celiac patients here was a clear overrepresentation[25], in particular as there were no immigrants in our study cohort.[26] Interestingly, we have recently shown that the prevalence of anemia as an initial presentation of CD could be again slightly increasing in developed countries.[27] This somewhat surprising phenomenon might be attributable to the increasing activity of clinicians to seek out CD in children with unexplained anemia.

Perhaps the most conspicuous difference between the groups here was the presence of more severe histological damage among the anemic children. Our results are in agreement with those of a recent study conducted among adult CD patients from the USA by Abu Daya and colleagues.[16] The authors compared patients presenting with diarrhea to those presenting with anemia and, as here, observed more advanced mucosal atrophy in the latter group. Moreover, in both the present and the U.S. study anemic patients had higher CD autoantibody values at diagnosis. This is in line with previous CD findings showing a correlation between the results of serology and histology[28-30], and further demonstrates the presence of more advanced disease in anemic patients. It seems logical that severe enteropathy should predispose to iron-deficiency anemia in view of the reduced absorptive surface of the intestine.[7] On the other

hand, one would also assume more diarrhea and other gut-related symptoms, this being the case neither here nor in the U.S. study.[16] Also, despite the more severe mucosal damage in the anemic patients, in the present study there was no difference between the groups in the prevalence of poor growth. Evidently, the eventual clinical picture in CD is a result of a much more complicated process than only a secondary effect of the mucosal damage.[31] Interestingly, previous results obtained by Matysiak-Budnik and colleagues[32] indicate that anemia might not be merely an outcome but also an active player in the CD pathogenesis. The authors showed that gluten may enter the body via enterocyte transferrin receptors, whose expression increases during iron deficiency.[33] As a result, anemia could further promote gluten influx, leading to a kind of self-perturbing pathogenic loop. The iron metabolism is further complicated by the presence of mucosal inflammation, which may again affect the iron intake via hepcidin upregulation.[34,35] Obviously, more studies are needed to elucidate these intriguing issues, but in any case our results indicate that an early detection of anemic CD patients is important to prevent possible permanent complications related to advanced CD.

Both groups here showed excellent clinical and serological response to the GFD, further confirming the overall good dietary adherence of the study children. Although, for some obscure reason, adherence was still somewhat lower in the anemia group, none of the patients had major dietary lapses and the few with ongoing anemia maintained a strict GFD and had good clinical response. Thus, neither the persistent anemia nor the lower median Hb value in the anemia group on follow-up seem to be fully explained by compliance problems.[36] Previously Annibale and colleagues[37] have reported anemia to recover in 94% of adult patients after 12 months on diet, and in a pediatric study by Catal et al.[38] this was seen in 94% after a median of 48 months. In contrast, in a study by Bergamaschi et al.[39] recovery was seen

in 70% of patients after 12 months, and in a study by Pulido et al.[40] in only 55% after one and in 78% after five years. Annibale et al. reported no additional iron supplementation, in the study by Catal et al. supplementation was given in all anemic patients, while the other reports did not provide this information. Besides supplemental iron, another factor potentially affecting these variable recovery results is dietary adherence, on which no precise definition was reported in the aforementioned studies. It is also possible that some CD patients have a genetic predisposition to low Hb and thus both fulfil the criteria for anemia more easily and also evince seemingly incomplete recovery.[41,42] In any case, when designing the follow-up of these children, clinicians should bear in mind that the recovery of anemia may be protracted even on a strict GFD.

Major strengths of the present study are the well-defined and large cohort of children with histologically confirmed CD, and the large number of different variables investigated. On the other hand, limitations are the retrospective design and lacking long-term follow-up results. In addition, even the short-term follow-up results were missing from quite many patients, and we did not have detailed data of dietary habits possibly affecting to the iron intake and hemoglobin values. Another limitation is lacking values of vitamin B12 and folate, which are not routinely taken in our clinical practice. Further, many laboratory parameters were not taken systemically during the whole study period and were thus missing from a substantial part of the children. Also, in order to reduce possible bias caused by variation in time and place of the Hb measurement a part of these values were omitted from the Table 3. It must also be emphasized that the study children were very often screen-detected and diagnosed in a single tertiary center, and thus may not fully represent the wide spectrum of CD seen in everyday clinical practice. CD is well-known among physicians and gluten-free products are

easily available in Finland; the follow-up results might thus be better compared with countries where awareness is lower and the diet more difficult to maintain.[43]

To conclude, our results demonstrate that the presence of anemia at the diagnosis of CD is associated with more advanced clinical and histological presentation. Therefore, in order to prevent excessive burden and possible permanent complications caused by ongoing untreated CD, it is important to identify and treat these anemic children as early as possible. Clinicians should also be aware that the recovery of anemia may take a rather long time despite a strict GFD, and thus special emphasis should be placed on the follow-up of this patient group.

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Figure legends

Figure 1. The main clinical presentation (A), severity of the symptoms (B) and degree of small-bowel mucosal atrophy (C) in children presenting with or without anemia at CD diagnosis. The total number of study patients was 82 in the anemia group and 373 in the non-anemia group, but some of them were omitted from the figure owing to unclear or contradictory medical information.

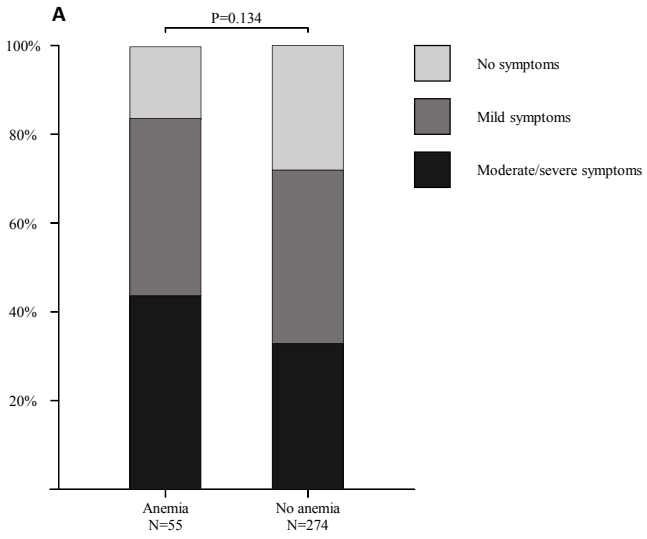

Table 1. Demographic data, presence of celiac disease in the family and co-morbidities in 82 children with anemia and 373 children without anemia at celiac disease diagnosis.

	Anemia n=82		No anemia n=373		p value
Variable	n	%	n	%	
Girls	58	70.7	241	64.6	0.307
Celiac disease in the family[1]	15	27.3	104	43.5	0.033
Co-morbidities					
Type 1 diabetes	4	5.1	33	9.0	0.276
Thyroidal disease	2	2.5	5	1.4	0.361
Down´s syndrome	0	0.0	3	0.8	1.000
Epilepsy	0	0.0	2	0.5	1.000
Asthma	4	5.1	35	9.5	0.273
Any allergy	18	22.0	86	23.1	0.885
Other chronic illness[2]	8	9.8	43	11.5	0.645
Age, median (Q_1, Q_3), years	8.5 (5.0, 13.4)		7.4 (4.7, 11.0)		0.038

Data were available in >95% of the patients in each variable except [1]in 55 and 239 patients, respectively

[2]E.g. Asperger's syndrome, ulcerative colitis, migraine, systemic juvenile idiopathic arthritis, chronic neutropenia, anorexia nervosa

Q_1, Q_3, lower and upper quartiles

Table 2. Distribution of different gastrointestinal and extra-intestinal symptoms in 82 children with anemia and 373 children without anemia at celiac disease diagnosis.

	Anemia n=82		No anemia n=373		p value
Variable	n	%	n	%	
Gastrointestinal symptoms					
Diarrhea	16	19.5	112	30.0	0.060
Abdominal pain	38	46.3	166	44.5	0.383
Constipation	16	19.5	58	15.5	0.063
Vomiting	0	0.0	10	2.7	0.021
Other[1]	7	10.0	39	11.3	0.838
Extra-intestinal symptoms[2]					
Poor growth	19	23.2	85	22.8	1.000
Rash	5	6.1	21	5.6	0.796
Neurological symptoms	1	1.2	9	2.4	1.000
Joint symptoms	3	3.7	21	5.6	0.594
Aphtous ulcers	2	2.4	8	2.1	0.698
Liver abnormalities	3	3.7	8	2.1	0.426
Other[3]	4	4.9	23	6.2	0.800

[1]E.g. flatulence, abnormal/bloody stools, gastroesophageal reflux, abdominal distension
[2]Excluding anemia
[3]E.g. dental enamel defects, fatigue, alocipea, muscle pains, brittle nails

Table 3. Baseline laboratory parameters and anthropometric data in 82 children with anemia and 373 children without anemia at celiac disease diagnosis.

	Anemia n=82		No anemia n=373		p value
Variable	n[1]	Median (Q_1, Q_3)	n[1]	Median (Q_1, Q_3)	
EmA, titer	64	1:500 (1:500, 1:4000)	249	1:500 (1:100, 1:1000)	<0.001
TG2-ab, U/l[2]	63	120.0 (80.7, 120.0)	271	88.0 (29.0, 120.0)	<0.001
Hemoglobin, g/dl	76	10.5 (9.3, 11.5)	264	12.8 (12.1, 13.3)	<0.001
MCV, fl	68	74.0 (68.0, 79.0)	227	81.0 (78.0, 84)	<0.001
TfR1, mg/l	34	6.2 (4.4, 12.0)	52	3.8 (3.0, 4.8)	<0.001
Ferritin, µg/l	39	7.0 (3.5, 13.0)	70	15.0 (9.8, 26.0)	<0.001
Total iron, µmol/l	18	5.6 (3.7, 11.9)	24	14.1 (10.0, 19.9)	0.002
ALT, U/l	41	22.0 (16.0, 29.0)	106	19.5 (15.5, 24.5)	0.172
ALP, U/l	25	212.0 (137.0, 242.0)	81	196.0 (165.0, 235.0)	0.861
Albumin, g/l	24	37.5 (35.8, 39.3)	70	39.0 (37.0, 41.0)	0.128
TSH, mU/l	40	2.8 (1.4, 3.6)	122	2.5 (1.7, 3.2)	0.650
Thyroxine, pmol/l	16	14.7 (13.6, 16.0)	48	14.4 (13.0, 16.3)	0.561
Height, SD	42	-0.1 (-0.8, 1.0)	210	0.1 (-0.7, 1.0)	0.659
Weight, SD	33	-0.3 (-1.5, 0.5)	160	-0.4 (-1.2, 0.4)	0.728

[1]Data available; [2]Upper limit of the assay is 120.0 U/l
Q_1, Q_3, lower and upper quartiles; EmA, endomysial antibodies; TG2-ab, transglutaminase 2 antibodies; MCV, mean corpuscular volume; TfR1, transferrin receptor 1; ALT, alanine aminotransferase; ALP, alkaline phosphatase, TSH, thyroid-stimulating hormone; SD, standard deviation

Table 4. Recovery of serum celiac disease autoantibodies and hemoglobin after a median of 12 months on a gluten-free diet (GFD) in children with anemia and those without anemia at celiac disease diagnosis

	Anemia		No anemia		p value
	n[1]	Median (Q_1, Q_3)	n[1]	Median (Q_1, Q_3)	
TG2-ab, U/l[2]					
At diagnosis	54	120.0 (120.0, 120.0)	204	120.0 (30.0, 120.0)	0.002
On a GFD	54	6.3 (2.5, 14.0)	204	4.4 (2.3, 9.5)	0.093
EmA, titer					
At diagnosis	46	1:1000 (1:500, 1:4000)	164	1:200 (1:100, 1:1000)	< 0.001
On a GFD	46	1: <5 (1: <5, 1:5)	164	1: <5 (1: <5, 1:5)	0.144
Hemoglobin, g/l					
At diagnosis	50	10.5 (9.0, 11.5)	158	12.8 (12.1, 13.3)	< 0.001
On a GFD	50	12.5 (12.1, 13.4)	158	13.2 (12.5, 13.6)	0.045

[1]Value available both at diagnosis and on GFD; [2]Upper limit of the assay is 120.0 U/l
The change within the groups on GFD was significant ($p<0.001$ in each) in all variables