THE IMPACT OF MORPHOLOGICAL CHANGES IN SMALL BOWEL MUCOSA ON IRON METABOLISM IN CHILDREN WITH CELIAC DISEASE

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A comprehensive study was carried out, which implied clinical and laboratory tests involving 164 children with morphologically proven celiac disease, of which 44 (26.8%) had iron-deficiency anemia (IDA) verified in them; 79 (48.2%) had latent iron deficiency (LID), and another 41 (25.0%) revealed no proven signs of iron deficiency (ID). The gastro-intestinal symptoms seen as the initial and most pathognomonic manifestation of celiac disease, are mostly typical of children with IDA. An analysis of the registered complaints and measurements shows that children with IDA have low body weight and height prevailing among them. The children with celiac disease accompanied with ID revealed higher levels of antibodies to tissue transglutaminase. Among children with IDA the severest atrophy of the small intestine mucosa (Stage 3C by Marsh – Oberhuber) was detected in 80.0% of cases, while among those with no ID only 21.1% had the same issue. During that, there was also correlation found between the stage of the atrophy and the serum levels of iron (r= –0.32, p<0.005) and serum ferritin (r= –0.50, p<0.001).

Key words: celiac disease, children, serological markers, iron deficiency anemia, latent iron deficiency, atrophy of mucosa

The current view on celiac disease, which is seen as a multifactor autoimmune issue in people with genetic predisposition, induced by gluten, and featuring lesion to the small bowel villi, offers a description not for clinical peculiarities alone yet also inevitable development of various micronutrient deficiency conditions in respective patients [1, 9].

Iron absorption in healthy bodies takes place through small bowel [2, 22, 24]. Atrophic changes observed in the small intestinal mucosa in case of celiac disease, flattening of the villi and a decrease in the number of highly differentiated enterocytes, as well deepening of crypts, will naturally be accompanied with reduced iron absorption [20]. This is the factor explaining why iron deficiency (ID) complicates the course of typical celiac disease, which manifests itself in early childhood as a rule [13, 21]. On the other hand, iron deficiency anemia (IDA) and latent iron deficiency (LID) are the most common extraintestinal manifestations of celiac disease in school-age children and adults [7, 8, 17].

In view of the controversial data to be found in literature and a sufficient stock of our own clinical and laboratory findings, we carried out an analysis of clinical, serological, and morphological issues related to celiac disease course in children with various types of ID.
Aim of study: to analyze the link between morphological and clinical-serological factors in children with celiac disease depending on the presence and type of ID.

Material and Methods. Retrospective analysis was carried out concerning case records for 164 children first diagnosed with celiac disease and aged 8 months – 17 years (mean age 4.5±0.3 yrs) who received in-patient treatment at the Philippsky Child Clinical Hospital Gastroenterology Department (Stavropol, Russia) at certain periods within 2001–2016.

The diagnosis was given subject to the ESPGHAN criteria (1990, 2012), which included typical clinical symptoms, from 2004 through 2010 – identification of specific antigliadin antibodies (AGA) of IgA and IgG classes; from 2011 – identification of tissue transglutaminase type 2 antibodies (anti-TG2) IgA and IgG, endomyosal antibodies (EMA), morphological study of the empty intestine mucosa (EIM) following the Marsh-Oberhuber classification (Stages 3A-3C). The exclusion criteria were negative titers of specific antibodies, lack of morphological study of the EIM.

Based on the clinical and anamnestic analysis, the typical disease was diagnosed in 148 (90.2 %) cases, while the number of atypical cases was 16 (9.8 %). The patients included 84 (51.2 %) girls and 80 (48.8 %) boys.

ID diagnostics included a hemogram test, detection of serum iron levels (SI), serum ferritin (SF), serum total iron-binding capacity (TIBC), and transferrin. The reference values for SI were 10.6–33.6 μmol/l, for TIBC – 40.6–62.5 μmol/l, for SF – 30–120 ng/ml, and 2.03–3.6 g/L for transferrin. The transferrin saturation (TSAT) is a value showing the absolute weight of SI in TIBC.

The manifesta-
tion (Stages 3А-3С). The exclusion criteria were negative titters of specific antibodies, lack of morphological study on the presence and the type of ID. The Group 1 included 44 (25.8 %) patients with IDA. The Group 2 had 79 (48.2 %) children with LID. The Group 3 involved 41 (26.8 %) patients with IDA. The Group 2 had 28 (63.6 %) children with no ID.

The patients were divided in three groups depending on the presence and type of ID. The Group 1 included 44 (25.8 %) patients with IDA, the Group 2 had 79 (48.2 %) children with LID, and the Group 3 involved 41 (26.8 %) patients with no ID.

The data were processed with statistical methods involving the software package ATTESTAT, Statistica 10.0. The parametric values at normal data distribution were assessed with Student’s t-test. For abnormal distribution, Mann-Whitney U-test was employed. Non-parametric values were calculated based on the χ² criterion. The relationship between the indices was evaluated through paired correlation coefficient of Pearson (r), Spearman. The difference was accepted as significant at p<0.05.

Results and Discussion. The rate of clinical-test proven ID in children in the period of celiac disease clinical manifestation was significantly above the population rate, while the ratio between IDA and LID remained the same, which was 1:1.9 in the group that underwent analysis. A detailed analysis revealed an increase in the ID degree along with an increase in the patient’s age and the duration of the malabsorption syndrome. The manifestation of celiac disease and first complaints expressed by children with IDA showed up at an average age of (X+sd) 2.3±0.5 yrs; in children with LID – at the age of 1.8±0.3 yrs, while in patients with no ID the same factor was 1.6±0.3 yrs (p<0.05). During that, the mean age for diagnosing IDA in children was 5.8±0.8, which is significantly above the age of diagnosis verification for both children with LID – 4.1±1.4 yrs, and patients with no ID – 4.0±0.5 yrs (p<0.05). The average duration for the latent period at IDA was 3.4±0.6 yrs; in children with LID it was 2.3±0.3 yrs (p<0.05), while for patients with no ID that index was 2.3±0.4 yrs (p<0.05). Naturally enough, a longer course of undiagnosed celiac disease and thus induced malabsorption of the major macro- and micronutrients in the small bowel increases the risk of developing ID.

Out of the patients with celiac disease complicated with IDA in its acute phase, 16 (36.4 %) were boys, and 28 (63.6 %) – girls; this balance was different in the group of children with LID – 46 (58.2 %) boys and 33 (41.8 %) girls (p<0.05), while in the group with no ID no significant gender differences were identified with 18 (43.9 %) boys and 29 (56.1 %) girls.

The frequency of complaints and clinical symptoms registered in patients with IDA, LID, and with no ID through the active stage of celiac disease can be seen in Table 1.

<table>
<thead>
<tr>
<th>Major symptoms</th>
<th>Abdominal distention</th>
<th>Diarrhea</th>
<th>Vomiting</th>
<th>Chronic abdominal pain</th>
<th>Decreased appetite</th>
<th>Low body weight</th>
<th>Low height</th>
<th>Irritability</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDA, n – 44</td>
<td>24 (54.5 %)</td>
<td>31 (70.5 %)</td>
<td>18 (40.9 %)</td>
<td>21 (47.7 %)</td>
<td>18 (40.9 %)</td>
<td>34 (77.3 %)</td>
<td>30 (68.2 %)</td>
<td>19 (43.2 %)</td>
</tr>
<tr>
<td>LID, n – 79</td>
<td>37 (46.8 %)</td>
<td>42 (53.2 %)</td>
<td>12 (15.2 %)</td>
<td>30 (38.0 %)</td>
<td>35 (44.3 %)</td>
<td>57 (72.2 %)</td>
<td>52 (65.8 %)</td>
<td>33 (41.8 %)</td>
</tr>
<tr>
<td>No ID, n – 41</td>
<td>13 (31.7 %)</td>
<td>17 (41.4 %)</td>
<td>5 (12.2 %)</td>
<td>15 (36.6 %)</td>
<td>19 (46.3 %)</td>
<td>23 (56.1 %)</td>
<td>17 (41.4 %)</td>
<td>12 (29.3 %)</td>
</tr>
<tr>
<td>Difference reliability (χ²)</td>
<td>&gt;0.05 &lt;0.05 &gt;0.05</td>
<td>&gt;0.05 &lt;0.01 &gt;0.05</td>
<td>&lt;0.001 &lt;0.001 &gt;0.05</td>
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</table>

Minor symptoms

<table>
<thead>
<tr>
<th>Constipation</th>
<th>Pain in bones</th>
<th>Tooth decay</th>
<th>Allergic rash</th>
<th>Headaches</th>
<th>Sleep disturbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDA, n – 44</td>
<td>2 (4.5 %)</td>
<td>2 (4.5 %)</td>
<td>2 (4.5 %)</td>
<td>9 (20.5 %)</td>
<td>5 (11.4 %)</td>
</tr>
<tr>
<td>LID, n – 79</td>
<td>11 (13.9 %)</td>
<td>2 (2.5 %)</td>
<td>2 (2.5 %)</td>
<td>25 (31.6 %)</td>
<td>5 (6.3 %)</td>
</tr>
<tr>
<td>No ID, n – 41</td>
<td>6 (14.6 %)</td>
<td>1 (2.4 %)</td>
<td>-</td>
<td>12 (29.3 %)</td>
<td>3 (7.3 %)</td>
</tr>
<tr>
<td>Difference reliability (χ²)</td>
<td>&gt;0.05 &gt;0.05 &gt;0.05</td>
<td>&gt;0.005 &gt;0.005 &gt;0.005</td>
<td>&gt;0.005 &gt;0.005 &gt;0.005</td>
<td>&gt;0.005 &gt;0.005 &gt;0.005</td>
<td>&gt;0.005 &gt;0.005 &gt;0.005</td>
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</tbody>
</table>

Celiac disease symptoms in children depending on degree and type of iron deficiency.

P₁ – significance of difference between groups of patients with IDA and LID;
P₂ – significance of difference between groups of patients with IDA and with no ID;
P₃ – significance of difference between groups of patients with LID and with no ID.

A comparative analysis revealed a number of important issues in celiac disease complicated with IDA. First, children with IDA, if compared to their counterparts with no ID, have diarrhea 1.7 times as often (p<0.01), abdominal distention 1.7 times as often (p<0.05), and a 3.4-time higher rate of complaints associated with vomiting (p<0.001). At the same time, the occurrence of poor appetite and chronic...
abdominal pain basically never depended on the presence and the type of ID (p>0.05).

On the other hand, quite natural was that children with celiac disease complicated with IDA manifested more prominent lagging in their physical development, due to which the number of patients who were underweight and of low height exceeded significantly that in the group with no ID. The rate of so-called minor symptoms of celiac disease in the groups under investigation revealed no substantial difference. Obviously, ID, as well as other deficiencies that result from celiac disease manifestation, will inevitably have a negative impact on the child’s physical growth and psycho-motor progress, thus leading to prominent asthenovegetative, and – more than seldom – to athenoneurotic symptoms, eventually deteriorating the patient’s life quality [4, 7, 9].

The Table 2 offers a view on the outcomes of a laboratory examination involving patients with celiac disease depending on the iron supply status.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Degree and type of ID</th>
<th>Reliability of difference (x²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDA, n = 44</td>
<td>LID, n = 79</td>
<td>No ID, n = 41</td>
</tr>
<tr>
<td>SI, μmol/l</td>
<td>5.4 ±0.3</td>
<td>8.6 ±0.4</td>
</tr>
<tr>
<td>SF, ng/ml</td>
<td>6.2 ±0.5</td>
<td>15.7 ±1.0</td>
</tr>
<tr>
<td>TIBC, μmol/l</td>
<td>79.7 ±0.5</td>
<td>71.7 ±0.5</td>
</tr>
<tr>
<td>Transferrin, g/l</td>
<td>3.9 ±0.1</td>
<td>3.3 ±0.1</td>
</tr>
<tr>
<td>TSAT, %</td>
<td>6.8 ±0.3</td>
<td>12.0 ±0.4</td>
</tr>
</tbody>
</table>

P₁ – significance of difference between groups of patients with IDA and LID;  
P₂ – significance of difference between groups of patients with IDA and with no ID;  
P₃ – significance of difference between groups of patients with LID and with no ID.

The data in Table 2 show that as sideropenia progresses in children with celiac disease, they reveal disturbed concentrations of iron-containing proteins as well as other laboratory signs of a decrease in the transport and tissue pool of iron, which are pathognomonic for ID.

A number of published items point at parallelism to be observed between anti-TG2, AGA and the severity degree of anemia [5, 19].

In our group of patients, the level of specific serological markers in patients with celiac disease, depending on the degree and the type of ID, was different. The average titer EMA (IgA, IgG) in children with IDA was 1213.3±221.9 U/l, which was significantly above that in patients with LID (637.8±123.8 U/l (p<0.05)) and with no ID (645.3±294.7 U/l (p<0.05)). There was a direct correlation detected between the antibody titer to endomysium and the level of transferrin (r= 0.45, p<0.01).

The differences in the anti-TG2 were rather insignificant. The average level of anti-TG2 IgA in the groups with IDA, LID, and with no ID, was 75.4±15.5 U/l, 77.4±10.4 U/l, and 56.8±10.0 U/l, respectively (p>0.05). The difference in the anti-TG2 IgG level was even less significant – 37.6±10.0 U/l, 25.0±3.8 U/l, and 23.9±3.8 U/l (p>0.05). Yet, correlation analysis revealed a reverse correlation between the anti-TG2 IgA level and SF (r= –0.28, p<0.02), as well as direct correlation between the anti-TG2 IgA and transferrin (r= 0.31, p<0.01).

Proof to close interrelation between malabsorption syndrome and the degree of anemia may be found in respective literature focusing on increasing ID rate along with atrophy progress in the small intestine [8, 13].

On Figure shows the structure of morphological changes in children with celiac disease depending on the degree and the type of ID. Patients with IDA had total atrophy of villi prevailing, which matched Stage 3C by Marsh, while Stage 3A was diagnosed in 5.0 % of cases only. In children with IDA, Stage 3C by Marsh was detected 2.0 times, and in children with no ID – 3.8 times less often compared to those with IDA. Correlation analysis helped reveal a reverse link between the stage of empty intestine atrophy and the hemoglobin level (r= –0.26, p<0.02), the level of SI (r= –0.32, p<0.005), the level of SF (r= –0.50, p<0.001). It is obvious that in patients with celiac disease, progressing atrophy of EIM leads not only to reduced transport pool of iron but also to deep depletion of its tissue reserve, which become complete with IDA development.

The occurrence rate of celiac disease in patients with IDA varies, according to different sources, falling in the range between 5–9 % to 21.3 %, while the list is topped by patients with refractory anemia (refractivity is defined as lack of increase in the hemoglobin level by 10 g/l and more after a 2-month treatment with peroral iron preparations) [12, 15]. N. Sharma et al. report that celiac disease was diagnosed (with serological and morphological methods) in 28 % of cases suffering from refractory anemia [23].

Literature offers often opposite ideas regarding the need for serological screening of celiac disease among patients with anemia [6, 14]. Despite lots of controversy in interpreting diagnostic value of anemia for celiac disease, which are due to employing serological methods with varying sensitivity and specificity, most researchers agree that very frequently developing ID and its manifest expression – IDA – stand an important pathogenetic component to the disease so a detailed laboratory examination of the transport and tissue pool of iron may serve a rather reliable predictor in verifying celiac disease [3, 6, 14, 20, 23]. The outcomes of our study, too, offer a piece of convincing proof to this stance.

Verification of IDA and LID in patients with no malabsorption syndrome is an indication for ferrotherapy, while its duration and the doses to be used shall depend not on the severity of anemia alone yet also on the iron preparations to be administered. The issue of the need and the duration of iron-medication treatment for patients with celiac disease after they are put on gluten-free diet
clinical, morphological and serological check-ups revealed a high rate of ID in children with celiac disease (75.0 %), while the balance of IDA and LiD, in view of no serious gender difference, appears as 1:1.9. The clinical manifestations complicated with IDA is associated with significant prominence of gastrointestinal symptoms and more delayed physical progress compared to children with no ID.

An analysis of morphological change shows that total atrophy in the empty intestine mucosa (matches Stage 3C by Marsh-Oberhuber) can be found in patients with IDA in 80.0 % of cases while in children with no ID this rate is only 21.1 %. Aggravation of atrophy in the small intestine that is to be found in children and adolescents in case of undiagnosed celiac disease comes along with gradual depletion of the transport, and then of the tissue iron pool.

Therefore, identifying IDA in children going through an active stage of celiac disease will serve a reliable criteria witnessing serious atrophy in the small bowel; it also is associated with prominent delay in physical progress and takes monitoring of iron metabolism including indicators of transport and markers of tissue iron reserve through sticking the course of a gluten-free diet.

References