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Clinical and biological correlations in celiac disease in children: the prospective single experience of a romanian tertiary center

A case-control study (Strobe-Compliant study)

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Abstract

Celiac disease—a chronic inflammatory disease of the intestine—is triggered by gluten or associated protein consumption.

The aim of our study was to assess the sensitivity, specificity of the combined anti-transglutaminase 2 (TG_2)/deamidated gliadin peptide antibodies (DGP), and antiendomisium antibodies (EMA), to determine the distribution of HLA-DQ_2/DQ_8 for the 140 tested patients, and also to evaluate the clinical and laboratory characteristics of patients admitted with the suspicion of celiac disease (CD). Children included in the study were divided into: group 1, patients with confirmed CD; group 2, patients with "potential" CD; group 3, control group, patients without CD. We assessed the standard laboratory data, the level of TG_2/DGP and EMA antibodies, as well as the distribution of HLA molecules in the selected patients. Histopathological examination was considered the criterion standard for diagnosis in most cases.

The sensitivity of TG_2/DGP was 85% and the specificity 92%. EMA showed a sensitivity of 82% and a specificity of 98%. The vast majority of patients diagnosed with CD were either HLA-$DQ_{2.5}$ (encoded by DQA_1*05 & DQB_1*02) positive (87.5%) or HLA-DQ_8 (encoded by DQB_1*03:02) positive (12.5%). One patient showed a positivity only for HLA-$DQ_{2.2}$ (encoded by DQA_1*02 & B_1*02).

Our study showed that the genetic risk for CD was present in more than one-third of the cases without a confirmed diagnosis of CD. Therefore, the awareness of genetic susceptibility for CD is essential because of the fact that these individuals can develop the disease at any point of their lives. The sensitivity of TG_2/DGP and EMA were very similar, whereas EMA presented a higher specificity as that of TG_2/DGP.

Abbreviations: ALT = alanin aminotransferase, AST = aspartat aminotransferase, CD = celiac disease, DGP = deamidated gliadin peptides, EMA = anti-endomisium antibodies, Hgb = hemoglobin, HLA = human leucocyte antigen, Htc = hematocrit, LDH = lactate dehydrogenase, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume, PLT = number of platelets, RBC = number of red blood cells, SD = standard deviation, TG_2 = tissue transglutaminase type 2.

Keywords: celiac disease, genetic testing, serology

1. Introduction

Celiac disease (CD) is a systemic immune-mediated disorder caused by gluten and other prolamins manifested in genetically susceptible individuals (HLA-DQ_2 or DQ_8 haplotypes).[1] Immune reaction in the small intestine causes intraepithelial leukocyte infiltration, villous atrophy, and crypt hyperplasia.

Clinical manifestations vary from mild to severe, and the disease may be silent for many years. "Classic" symptoms include: chronic diarrhea, weight loss, malabsorption, and

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Research involving Human Participants and/or Animals: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Tirgu Mures (approval no.13/18.07.2011).

Informed consent was individually obtained from all children's legal tutors of participants included in the study.

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iron-deficiency anemia.[2–4] In addition to gastrointestinal symptoms, the disease can have extraintestinal manifestations, such as osteoporosis, dermatitis herpetiformis, neurological problems, liver disorders, and arthritis.[5]

The etiology of CD is multifactorial, but genetic factors have a major influence, as documented in twin studies[6] and studies showing a strong dependence of HLA-DQ_2 and HLA-DQ_8 haplotypes.[7] Ninety-five percent of patients with CD have the HLA-DQ_2 heterodimer, which is encoded by alleles DQA_1 *05 and DQB_1 *02, either cis configuration or trans, and most of the remainder have the HLA-DQ_8 heterodimer, encoded by the DQB_1 * 0302 allele. The expression of these molecules, HLA-DQ_2 and HLA-DQ_8, is necessary but not sufficient to cause the disease because approximately 30% to 40% of the white population is HLA-DQ_2-positive and only 1% develops CD. Individuals who lack heterodimers HLA-DQ_2 or HLA-DQ_8 can be ruled out from the group at risk to develop CD. The higher or lower risk of developing the disease depends on the presence or absence of these alleles, their combination, and the number of copies.[8]

The DQ_2 molecule presents gluten-derived peptides, modified by tissue transglutaminase, to CD_4+ T cells. The DQ_8 molecule is capable of binding to a different set of gluten-derived peptides with different affinities than the DQ_2 molecule. This finding could explain the minor association between the DQ_8 molecule and the development of CD.[9]

The role of environmental factors and non-HLA alleles in CD development are well known.[10,11] One study found an involvement of rotavirus in the development of CD, a finding that requires confirmation by further studies.[12] The age at which gluten is introduced to the diet has been reported to affect the risk of CD autoimmunity in genetically predisposed children.[13] Other studies have shown that the delayed introduction of gluten did not modify the risk of CD among at-risk infants.[14]

The prevalence of CD in different populations is about 1%,[15,16] but there are countries with a higher or lower prevalence.[17] Although it is a relatively frequent disease, it remains underdiagnosed because of the great variation of symptoms and the diagnosis can be delayed for many years.[18]

Very few data are available on the epidemiology of CD in Romania. A study published by Dobru et al[19] in 2003, conducted on a group of 2436 adult patients found a prevalence of 2.22% for CD. Popp et al,[20] in a study on 148 asypmptomatic adult family members of patients with CD, found a prevalence of 8.7%.

The diagnosis of CD is based on determining specific antibodies: anti-transglutaminase (TG_2), anti-endomysium (EMA), and deamidated gliadin antibodies (DGP) as well as interpreting the histological changes in duodenal biopsies (villous atrophy, crypt hyperplasia).[1] Anti-EMA antibodies are directed against the extracellular TG_2.[21] With the exception of anti DGP, these antibodies are usually of the IgA class. In patients with IgA deficiency, the same type of antibodies, but of IgG class, can be detected.[22] TG_2 appears in abundance in the gut and it functions by deamidation of proteins and peptides, including fragments of gliadin which increase the reactivity of T cells in patients with CD.[23] Immunofluorescence assays, such as EMA, require microscopic evaluation and may be subject to interobserver variability. The specificity of the EMA test results is 98% to 100% in specialized laboratories.[24,25] Positive titers of anti-TG_2 and EMA are associated with a high probability of CD in children and adolescents.[21,25] Despite this fact, high levels of anti-TG_2 have been described in a series of illnesses other than CD, such as hepatic disorders, psoriasis, autoimmune diseases, infections, tumors, and myocardial lesions.[26–28] These diseases are not associated with positive EMA results, which explains the better reliability of EMA for the diagnosis of CD.

The aims of our study were to assess the genetic risk for CD in a pediatric population from Romania with suggestive clinical symptoms for CD, taking into account the clinical and laboratory parameters of the patients, and to establish the diagnostic value of the serological markers used for diagnosing CD.

2. Materials and method

A cross-sectional prospective study was performed on 173 pediatric patients with the clinical suspicion of CD hospitalized in the Pediatric Clinic of the Emergency County Hospital Targu Mures, Romania, Pediatric Gastroenterology Department, between 2009 and 2015. The parents of only 156 of the patients agreed to the inclusion of their children in our study and from them, only 140 patients remained after a selection according to sex and age to comply with the pair method. Clinical, demographic, laboratory, and histopathological data were obtained from the medical records of the patients.

Patients were divided into 3 groups: patients with confirmed CD—28 children; patients with genetic susceptibility for CD, which do not meet all the diagnostic criteria but they were proved to carry a genetic risk of CD (HLA DQ_2 or DQ_8 positive)—49 patients; and the control group consisting of patients without any proved criteria for the diagnosis of CD and without any genetic risk for CD (HLA-DQ_2- and DQ_8-negative)—63 patients.

We assessed the following data: demographic information (sex, age at diagnosis), symptoms, clinical data (weight, clinical examination), laboratory data (the level of anti-TG_2 antibodies, anti-DGP antibodies, anti-EMA antibodies, HLA-DQ_2/DQ_8 testing, blood count, iron, liver enzymes, lactate dehydrogenase [LDH], albumins, total proteins), and histopathological data: the results of the duodenal biopsies. These data were assessed at the moment of diagnosis and at a subsequent reevaluation at 6 months, to appreciate the clinical and paraclinical changes determined by the gluten-free diet.

The diagnosis of CD was established according to the ESPGHAN criteria[1]: a positive duodenal biopsy (Marsh 2/3) or a titer of TG_2 >10 times the upper limit of normal, combined with positive EMA and genetic testing.

The inclusion criteria were: pediatric patients (1–18 years) admitted to the Pediatric Gastroenterology Department between 2009 and 2015 with the suspicion of CD based on clinical symptoms, such as: chronic, intermittent diarrhea or chronic constipation, recurrent abdominal pain, abdominal distension, recurrent or persistent vomiting, chronic fatigue, weight deficit, height deficit, and/or failure to thrive.

The exclusion criteria were: incomplete clinical or laboratory data, age <1 year and >18 years, associated autoimmune or chronic disorders, signs of acute infection at the moment of admission, previously introduced gluten-free diet, and patients whose parents refused to sign the informed consent.

HLA-DQ_2/DQ_8 testing was performed using a polymerase chain reaction amplification followed by hybridization technique: CeliacStrip-OPEGEN from OPERON (Zaragoza, Spain). We indicated as HLA-$DQ_{2.5}$-positive the individuals who carried the *DQA_1*05* and *DQB_1*02 alleles*. The individuals with *DQB_1*0302* and *DQA_1*03* alleles were indicated as DQ_8-positive and those carrying the *DQA_1*02 allels* and *DQB_1*02* as $DQ_{2.2}$-positive.

EMA IgA antibodies were measured by indirect imunofluorescence on a substrate of monkey esophagus (NOVA LITE Endomysial Antibody, INOVA Diagnostics, San Diego, CA). The EMA is the fine blade of connective tissue between the smooth muscle fibers of the muscular layers of the esophagus. If EMA antibody is present, it will bind to the connective tissue and will present a greenish fluorescence. An EMA positive-result was defined by the presence of a characteristic pattern of fluorescence at a dilution ≥1/5.

Serum TG_2/DGP was measured using a determination kit via the enzyme-linked immunosorbent assay (ELISA) method: QUANTA Lite h-tTG/DGP Screen (INOVA Diagnostics, San Diego, CA). This kit allows a semiquantitative determination of IgA and IgG anti-TG_2 and DGP. The antigens used were: human tissue transglutaminase and synthetic deamidated gliadin peptides. The patients' sera were diluted at 1:101. We considered positive antibody titers >20 U/mL, according to the manufacturer's recommendations.

Duodenal biopsies were obtained by upper gastrointestinal endoscopy. We have taken 3 to 4 biopsies from different places of D_3. The preparation of the biopsy fragments was performed using standard histopathological techniques. The fragments were examined after staining with hematoxylin-eosin, Pas-Alcian, and Giemsa. Intraepithelial lymphocytosis was evidenced by imunomarking with CD_3.

For this study, we have obtained the consent of the ethics committee of the University of Medicine and Pharmacy in Targu Mures (approval no.13/18.07.2011). The parents of the pediatric patients signed an informed consent on agreeing to the processing of personal data, and an informed consent on agreeing to perform upper gastrointestinal endoscopy.

Statistical analysis was performed with the programs Excel 2007 and GraphPad Instat. To assess the normality of continuous variables, the Kolmogorov-Smirnov test was applied. Quantitative variables were compared using *t* test, Mann-Whitney *U* test, Wilcoxon test, analysis of variance test, or Kruskal–Wallis test, when appropriate. We interpreted all the tests against a $P = .05$ significance threshold, and statistical significance was considered for *P* values below the significance threshold.

3. Results

From the 28 patients with confirmed CD at which the TG_2/DGP antibodies were determined, 4 patients had negative results, <20 UI/mL (sensitivity = 85%). Seven of 28 patients with confirmed CD at which the anti-EMA antibodies were determined had negative results, for 2 of which the results were positive at reevaluation (sensitivity = 82%).

In the control group, 5 of 63 tested patients presented false-positive anti-TG_2/anti DGP titers (specificity = 92%). The anti-EMA antibodies showed a slight positive result in 1 patient of 63 tested (specificity = 98%).

The positive predictive value for the TG_2/DGP combined determination was 82.7%, whereas the negative predictive value was 93.5%. The positive predictive value for EMA determination was 95.8%, and the negative predictive value was 92.5%.

In the celiac group, 87.5% of the patients were HLA-$DQ_{2.5}$ (DQA_1*05 & DQB_1*02)-positive and 12.5% HLA-DQ_8 (DQB_1*03:02)-positive. In group 2, of 49 tested patients, 69.4% were HLA-$DQ_{2.5}$-positive and 44.9% HLA-DQ_8-positive.

In the celiac patients group, the male/female ratio was approximately one-fourth and the mean age at diagnosis 9 years. The main signs and symptoms at the time of diagnosis as well as the secondary diagnoses of the patients in group 1 are illustrated in Figure 1.

For the celiac patients, duodenal biopsies were collected in 15 patients, and for the remaining patients, the diagnosis of CD was based on the TG_2 values (>10× normal) coupled with a positive anti-EMA result and an increased genetic risk for CD. The biopsy results were: Marsh III in 14 cases (2 cases Marsh 3B, 3C Marsh 10 cases, unspecified in 2 cases) and Marsh II in 1 case (Fig. 2).

On comparing the values of TG_2/DGP between groups, we obtained a statistically significant difference between patients with CD and controls ($P < .0001$) and between patients with CD and potential ($P < .01$) using the Kruskal-Wallis (Dunns) test; the values of TG_2/DGP in the group with CD were higher (Table 1).

Table 1 illustrates the data obtained by comparing the laboratory data of the patients from each group. We obtained statistically significant differences for hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, red blood cell number, iron, AST, and ALT. The differences between the laboratory data of celiac patients at the first admission and at reevaluation, respectively, are included in Table 2. We obtained statistically significant differences for hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, red blood cell number, iron, TG_2/DGP, and ALT.

4. Discussions

The present study is one of the few to study the sensitivity and specificity of serological diagnostic methods and the distribution

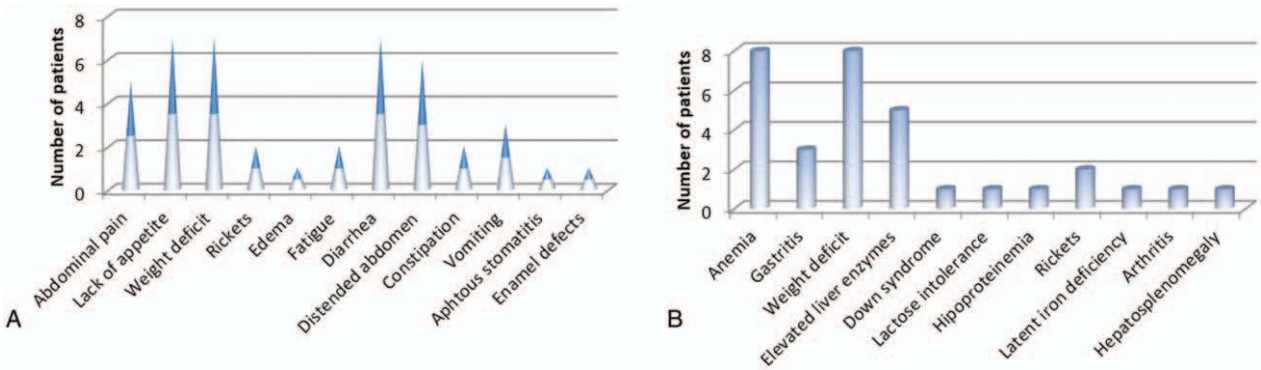

Figure 1. (A) Signs and symptoms of patients with celiac disease. (B) Secondary diagnoses of patients with celiac disease.

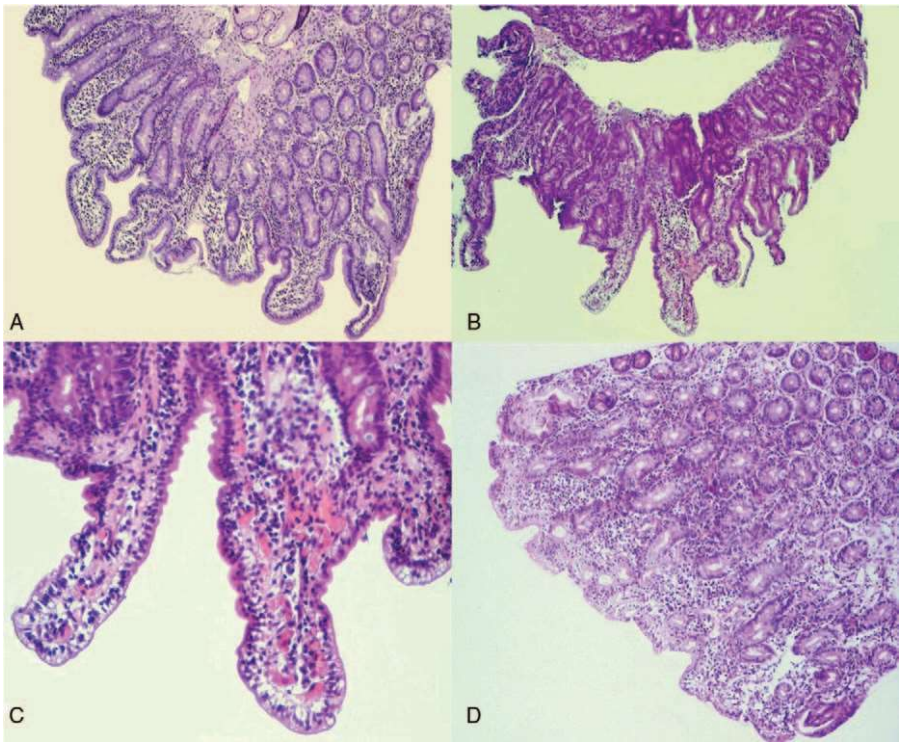

Figure 2. (A) Crypt hyperplasia, normal villi, Marsh II, hematoxylin and eosin (HE), 4×. (B) Marked villous atrophy and crypt hyperplasia, Marsh IIIB, HE, 4×. (C) Intraepithelial lymphocytes, Marsh III B, HE, 10×. (D) Complete villlous atrophy and crypt hyperplasia, Marsh IIIC, HE, 4×.

of HLA molecules, in addition to clinical and laboratory characteristics of patients with CD on a population from Romania.

We found the sensitivity of TG_2/DGP and EMA to be very close (85% vs. 82%), whereas EMA presented a higher specificity as that of TG_2/DGP (98% vs. 92%). We also assessed the positive and negative predictive values of the 2 serological diagnostic methods. The positive predictive value for the TG_2/DGP combined determination was 82.7%, much lower than that of EMA determination, which was 95.8%. The negative predictive values for the 2 methods were very close, 93.5% for TG_2/DGP and 92.5% for EMA.

In a meta-analysis of 16 studies on the accuracy of serological tests for the diagnosis of CD, Giersiepen et al[29] achieved a sensitivity ≥90% and a specificity of 98.2% for EMA. EMA test is based on an indirect immunofluorescence technique using a substrate which can be either monkey esophagus or human umbilical cord, the accuracy of the test is the same for each

Table 1

Comparison between the laboratory tests of the patients in the 3 groups.

Parameters	Celiac group 1 (n=28)	Potential group 2 (n=49)	Control group 3 (n=63)	*P*
Hgb, g/dL (mean±SD)	11.46±1.71	12.41±1.26	12.44±1.92	.02 group 1 vs. 3
Htc (%) (mean±SD)	35.73±3.84	35.73±3.84	36.53±4.71	.61
MCV, fL (mean±SD)	74.94±7.32	79.31±4.97	78.65±4.61	.003 group 1 vs. 2 and group 1 vs. 3
MCH, pg (mean±SD)	24.58±3.29	27.37±2.14	27.05±2.77	.004 group 1 vs. 2 and group 1 vs. 3
RBC, $\times 10^6$ cells/mm^3 (mean±SD)	4.92±0.36	4.58±0.40	4.64±0.51	.01 group 1 vs. 2 and group 1 vs. 3
PLT, $\times 10^3$ cells/mm^3 (mean±SD)	395.2±126.2	329.4±113.2	347.6±127.4	.10
Iron, μmol/L (mean±SD)	7.47±2.57	13.55±6.65	12.57±5.27	.008 group 1 vs. 2 and group 1 vs. 3
AST, U/L, median (min–max)	33.0 (12.6–309)	27.9 (16.1–73.0)	35.1 (16.1–137)	.02 group 2 vs. 3
ALT, U/L, median (min–max)	22.1 (8.1–356)	16.4 (5.7–82.0)	16.3 (8.8–236)	.03 group 1 vs. 2
LDH, U/L (mean±SD)	238.9±53.8	298.0±102.4	263.5±96.3	.12
Albumin, g/dL (mean±SD)	4.16±0.57	4.62±0.43	4.54±0.40	.24
Proteins, g/dL (mean±SD)	6.92±0.73	6.95±0.60	7.05±0.43	.70
TG_2/DGP, median (min–max)	133.00 (1.2–565)	1.10 (0.40–1.80)	1.99 (0.33–38.1)	Group 1 vs. group 2: $P<.01$; group 1 vs. group 3: $P<.0001$; group 2 vs. group 3: $P=.62$

Data were expressed mean±standard deviation (analysis of variance test) and median (min–max) (Kruskal-Wallis test).

ALT=alanin aminotransferase, AST=aspartat aminotransferase, DGP=deamidated gliadin peptides, Hgb=hemoglobin, Htc=hematocrit, LDH=lactate dehydrogenase, MCH=mean corpuscular hemoglobin, MCV=mean corpuscular volume, PLT=number of platelets, RBC=number of red blood cells, TG_2=transglutaminase.

Table 2

Comparison between the data on initial evaluation and on reevaluation of patients with celiac disease.

Parameters	Examination	Mean ± SD	*P*
Hgb, g/dL	Initial	11.46 ± 1.71	.01*
	Reevaluation	12.75 ± 1.28	
Htc (%)	Initial	35.73 ± 3.84	.96*
	Reevaluation	35.64 ± 8.95	
MCV, fL	Initial	74.94 ± 7.32	.02*
	Reevaluation	80.24 ± 6.40	
MCH, pg	Initial	24.58 ± 3.29	.01*
	Reevaluation	27.15 ± 1.96	
RBC, $\times 10^6$ cells/mm^3	Initial	4.92 ± 0.36	.05*
	Reevaluation	4.66 ± 0.39	
PLT, $\times 10^3$ cells/mm^3	Initial	395.2 ± 126.2	.70*
	Reevaluation	379.4 ± 141.9	
Iron, μmol/L	Initial	7.47 ± 2.57	.003*
	Reevaluation	16.34 ± 7.27	
AST, U/L	Initial	33.0 (12.6–309)	.21†
	Reevaluation	31.1 (12.0–53.6)	
ALT, U/L	Initial	22.1 (8.1–356)	.03†
	Reevaluation	15.4 (10.9–25.0)	
LDH, U/L	Initial	238.9 ± 53.8	.43*
	Reevaluation	218.8 ± 62.90	
TG_2/DGP, UI/mL	Initial	133.0 (1.2–565)	.02†
	Reevaluation	13.45 (1.0–201)	

ALT = alanin aminotransferase, AST = aspartat aminotransferase, DGP = deamidated gliadin peptides, Hgb = hemoglobin, Htc = hematocrit, LDH = lactate dehydrogenase, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume, PLT = number of platelets, RBC = number of red blood cells, TG_2 = transglutaminase.

* Student pair test.

† Wilcoxon test.

substrate.[29–32] Studies have demonstrated a sensitivity and specificity for IgA EMA of 95% and 99%.[33] Despite the high specificity of the determination, there are certain aspects of this test that may limit its use in clinical practice. It is semiquantitative, operator-dependent, expensive, and takes a long time for processing. IgA EMA testing can be useful if the anti-TG_2 IgA test result is equivocal. A positive IgA EMA test is strong evidence of the presence of CD in patients without bowel atrophic lesions.[34] A further indication of the determination of IgA EMA is to support the diagnosis of CD in symptomatic children with a high titer of IgA tTG (>10× ULN) without the need for a duodenal biopsy according to the ESPGHAN 2012 criteria.[1]

In the ESPGHAN report on the CD-specific antibodies, the specificity of anti-TG_2 antibodies measured by ELISA was lower than the anti-EMA and varied according to the kit used.[29] It was not possible to obtain overall performance estimates of sensitivity and specificity because of the heterogeneity of studies evaluated, but in 11 of 15 studied populations, the sensitivity has reached 90%, and the specificity in 13 of 15 population was estimated at 90%.[1]

The performance of anti-DGP antibodies is lower than that of TG_2 and EMA.[35] In a meta-analysis of studies comparing the performance of DGP antibodies and TG_2, Lewis and Scott achieved a sensitivity of 87.8% versus 93% and a specificity of 94.1% versus 96.5%.[24]

There have been few published studies on the combined determination of anti-TG_2/DGP.[36–39] Agardh et al conducted a study that included 119 children with CD, 57 children with other diseases, and 398 patients in the control group, yielding a sensitivity of 100% and a specificity of 89% in children with other diseases, and 97% in the group control.[36] Jaskowski et al,[37] in a study including 111 pediatric patients with suspected CD and 130 adults with dermatitis herpetiformis, achieved a sensitivity of 92.6% and a specificity of 96.1% in the pediatric cohort. A similar result was obtained by Porcelli et al[39]; they report a sensitivity of 100% and a specificity of 91.12% of the combined test, in a study on 59 pediatric patients. Given these excellent results, the authors recommended the combined screening as first-line determination to identify CD in children.

Few studies from Romania addressed the performance of the serological diagnostic tools for CD. In a study published in the *International Journal of Celiac disease* by Belei et al,[40] 368 children were tested for IgA TG_2, EMA, and IgA/IgG-TG_2/DGP combined assay. The authors found a sensitivity of 95.4% and a specificity of 100% for the combined IgA/IgG-TG_2/DGP assay, whereas EMA showed a sensitivity of 91% and a specificity of 100%. The positive predictive value for the combined assay was 100% and the negative predictive value 99.7%. The positive predictive value for EMA was 100%, whereas the negative predictive value was 99.4%. These results are similar to our own; the differences may occur because of the difference in the size of the study group.

In a study conducted on 26 adults with CD, Tărmure et al found a sensitivity of 100% for TG_2 and of 92% for EMA in patients with total villous atrophy, whereas in patients with subtotal villous atrophy, 43% were negative for EMA and 50% were negative for TG_2.[41] Samaşca et al[42] studied the IgA anti-TG_2 levels in a group of 890 children and found a sensitivity of 77.3%, positive predictive value of 55.2%, a specificity of 93.1%, and a negative predictive value of 97.3%. However, the study compared the anti TG_2 levels with those of EMA as criterion standard, and the diagnosis was not confirmed through histopathological examination.

In our study, we found that the majority (93.8%) of patients diagnosed with CD were either HLA-$DQ_{2.5}$ ($DQA_1{}^*05$ and $DQB_1{}^*02$)-positive (87.5%) or HLA-DQ_8 ($DQB_1{}^*03{:}02$)-positive (12.5%). One patient had none of the antigens, instead showed a positivity for HLA-$DQ_{2.2}$ ($DQA_1{}^*02$ and $B_1{}^*02$). Our results correspond to The European Genetics Cluster on Celiac Disease, which observed less DQ_2 positive patients in Southern Europe than in Northern (84.5% vs. 90.2%).[43]

Studies showed that 90% of patients with CD present the HLA-$DQ_{2.5}$ heterodimer, and most of the remainder are HLA-DQ_8-positive.[44,45] The discovery that the predisposition for CD is virtually given by just these 2 heterodimers resulted in considering patients HLA-$DQ_{2.5}$ and DQ_8-negative without risk of CD. However, there are numerous studies that show a frequency of 3% to 5% of patients diagnosed with CD, which are HLA-DQ_8- and $DQ_{2.5}$-negative.[46–48] Many of these patients were, as in our case, HLA-$DQ_{2.2}$-positive. Thus, to avoid misdiagnosis, it is important to determine HLA-$DQ_{2.2}$ as well.

In our study, the main signs and symptoms of patients diagnosed with CD were diarrhea, weight-stature deficit, and lack of appetite (the same frequency, 31.8%), followed by a distended abdomen (27%), and abdominal pain (23%). The literature suggests a higher incidence of diarrhea (up to 50%) and abdominal pain (up to 90% of children in Canada[49]) among the symptoms at the time of diagnosis.

The main diagnoses associated with CD were: anemia (44%), low weight (44%), and modified liver enzymes (27%). These consequences of CD are well known: studies showed a presence of anemia in 3% to 12% of patients, children and adults,[50,51] low weight in 50% to 90% of children,[19] and modified liver enzymes in 5% of patients.[3] Two patients had Down syndrome,

and arthritis, respectively, which are described in the literature as associated to CD in 0.3% and 1.5% of cases.[5,51]

Comparing laboratory data from patients with CD at first evaluation and on reevaluation, we obtained statistically significant differences for hemoglobin, MCV, MCH and iron and RBC; in all cases, the average is higher on reevaluation. Secondary diagnosis and therapeutic intervention increase hemoglobin and red blood cell parameters, thus solving anemia. We observed a statistically significant decrease in ALT, a decrease in AST and LDH, increased protein and albumin, and an increase in body weight between diagnosis and reassessment, but without obtaining a statistically significant difference. We achieved a statistically significant difference between the values of TG_2 at the moment of diagnosis and after a period of time after starting the gluten-free diet, which confirms the favorable role of dietary interventions on both the symptoms and laboratory parameters.

Certain limitations of this study should be mentioned. It included a small number of patients diagnosed with CD, which decreased the statistical power of the study. However, very few studies on CD, which include genetic testing have been published in Romania.[52] It is an expensive determination, which is available in few hospitals, despite its great utility for the diagnosis. Some patients included in the study did not receive genetic testing due to parental refusal; however, the diagnosis was made certain using the histopathological examination. Nevertheless, to our best knowledge, this is the first study from Romania that aimed to assess the genetic risk for CD among pediatric patients together with the diagnostic values of serological markers used for diagnosing CD.

5. Conclusions

Our study showed that the genetic risk for CD was present in more than one-third of the cases without a confirmed diagnosis of CD. Therefore, the awareness of genetic susceptibility for CD is essential due to the fact that these individuals can develop the disease at any point of their lives. The sensitivity of TG_2/DGP and EMA were very similar, whereas EMA presented a higher specificity as that of TG_2/DGP. Laboratory parameters, such as hemoglobin, iron, and liver transaminases, shower to be significantly abnormal in patients diagnosed with CD, but their values improved at 6 months after the initiation of gluten-free diet.

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