

ORIGINAL ARTICLE

The celiac iceberg: from the clinical spectrum to serology and histopathology in children and adolescents with type 1 diabetes mellitus and Down syndrome

ROSANE COSTA GOMES¹, JUSSARA CERQUEIRA MAIA¹, RICARDO FERNANDO ARRAIS¹, CARLOS ANDRÉ NUNES JATOBA², MARIA AUXILIADORA CARVALHO ROCHA², MARIA EDINILMA FELINTO BRITO¹, ANA LAISSA OLIVEIRA NAZION¹, CLARISSA MARQUES MARANHÃO¹ & HÉLCIO DE SOUSA MARANHÃO¹

¹Department of Pediatric and ²Department of Anatomy Pathology, Federal University of Rio Grande do Norte, Natal, Brazil

ABSTRACT

Objective: The objective of this study is to investigate the occurrence of gastrointestinal (GI) and extraintestinal symptoms in children and adolescents with type 1 diabetes mellitus (DM1) and Down syndrome (DS) and their association with specific antibodies and histopathology of celiac disease (CeID), representing its clinical forms in the iceberg. **Material and methods:** Cross-sectional study (November 2009–December 2012) conducted at an outpatient care facility in Northeast Brazil including patients [DM1 ($n = 111$); DS ($n = 77$)] aged 10 months–18 years old. Measurement of anti-endomysial (EmA) and anti-tissue transglutaminase (anti-tTG) IgA antibodies was performed, as was that of anti-tTG-IgG in the cases with low serum IgA. The patients with antibody positivity were subjected to small intestine biopsy. **Results:** GI symptoms occurred in 53.7% of the sample, extraintestinal symptoms in 4.3%, and antibody positivity in 28.2% ($n = 53$). Of those who underwent biopsy ($n = 40$), histopathological findings of CeID were found in 37.5% [DM1 = 5/111 (4.5%), DS = 10/77 (13.0%)]. GI symptoms were associated with antibody positivity, but not with the histopathology. The GI (32.5%), silent (5.0%), and potential (62.5%) forms of disease were detected. **Conclusions:** The prevalence of GI symptoms was high in groups DM1 and DS, and the occurrence of such symptoms was associated with antibody positivity. The lack of association between the symptoms and histopathological findings points to the inconsistency of the former as indicators of CeID. Although the GI form predominated among the cases with active CeID, its contribution to the celiac iceberg was smaller compared with the potential form, which determined the large and submerged base of the iceberg representing the high-risk groups investigated.

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Introduction



Celiac disease (CeID) is an immune-mediated systemic disease triggered by gluten in genetically susceptible individuals of any age and ethnic group. It is characterized by the presence of specific antibodies, enteropathy, and a wide clinical spectrum ranging from forms with gastrointestinal (GI) and/or extraintestinal symptoms to the silent, latent, and potential forms, which may be represented as an iceberg [1–3].

The role of gluten in other conditions is increasingly emphasized, as many diseases, the autoimmune ones in particular, are associated with CeID, including type 1 diabetes mellitus (DM1) and chromosomal abnormalities, such as Down syndrome (DS) [2,4–6]. In several countries (including Brazil), the prevalence of CeID in

DM1 varies from 3.0% to 16.0% [2,5,7–12] and in DS from 4.0% to 17.0% [2,6,13–17], being considerably higher compared with the overall population (0.5–1.0%) [1,2,18].

From the clinical point of view, most individuals with DM1 and DS are believed to exhibit the silent form of CeID [2,7,14]. However, a diagnosis based on more sensitive and specific serologic screening has allowed the retrospective identification of signs and/or symptoms that were formerly not considered, thus calling attention to the emergence of other clinical profiles [10,13,15,19–23].

The GI and/or extraintestinal symptoms exhibited by patients with DM1 and DS may be considered appropriate to those conditions and thus not taken into account

Correspondence: Prof. Rosane Costa Gomes,  Department of Pediatric, Federal University of Rio Grande do Norte, Natal, 59012-310 Brazil.
 rcostagomes31@gmail.com

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in the CeID diagnosis, which is thus delayed, consequently impairing the quality of life in patients and hindering the prevention of disease complications [10,19,21,24].

Within the current scenario, the clinical presentation of CeID in DM1 and DS is the focus of much interest, necessitating studies that assess concomitantly clinical symptoms, more than one serological marker, histopathology of the small intestine, and their associations to identify CeID in the aforementioned high-risk groups.

In 2009, the Brazilian Health Ministry published a national protocol for investigating CeID. However, the recommendations made are still not satisfactorily met because of the difficulty of their implementation by the Brazilian Public Health System. Thus, CeID screening has not yet been included in the monitoring routine of patients with DM1 and DS in most public health services, and therefore, the presence of signs and symptoms should serve as a warning for the need to investigate CeID [25].

The aim of the present study was to investigate the presence of GI and extraintestinal symptoms suggestive of CeID in children and adolescents with DM1 and DS and their association with specific antibodies and histopathology of disease, thereby visualizing its clinical presentation in the iceberg.

Methods

The present cross-sectional study was conducted from November 2009 to December 2012 at the pediatric outpatient clinic of a university hospital (Federal University of Rio Grande do Norte [Universidade Federal do Rio Grande do Norte – UFRN]), which is a regional reference for pediatric specialties, located in Natal, the capital of Rio Grande do Norte (RN) State, Brazil. The state has estimated population of 3.1 million inhabitants, being 77.8% of urban area and 34.2% aged below 19 years. The pediatric outpatient clinic serves around 4000 referrals a month. There is no regional statistics on the percentage of DM1 and DS in this age group. The study population was represented by the universe of children and adolescents enrolled in programs of care for DM1 (group DM1) and DS (group DS), who were consecutively recruited. Individuals from both genders aged 10 months–18 years residing in the capital and the interior of RN, who had regularly ingested gluten within the previous 3 months, independent of the presence or absence of clinical symptoms, were included in the study. The procedures comprised clinical and laboratory assessments and small intestine biopsy (SIB) through upper GI endoscopy (UGIE). The patients with

heart disease from group DS were subjected to a cardiological assessment before UGIE, which was not performed in the cases rated as high risk. The patients without laboratory data were excluded from the study.

The study was approved by the research ethics committee of the Onofre Lopes University Hospital, ruling no. 226/08. The participants' parents or guardians and the patients in group DM1 aged ≥ 12 years signed an informed consent form.

Clinical assessment (interview, physical examination, and anthropometric assessment)

A questionnaire was applied by four of the authors, who were previously trained to perform this procedure. The data collected included participant's identification, gender, current age, age at diagnosis of DM1 (if applicable), GI symptoms (abdominal pain, constipation, diarrhea, abdominal distension, vomiting, flatulence, and unsatisfactory weight gain/weight loss), extraintestinal signs and symptoms (reported unsatisfactory growth or any other extraintestinal complaint), age at the onset of symptoms and their duration, length of exclusive breastfeeding, age at the first introduction of gluten into the diet, and occurrence of other diseases.

All the participants underwent a physical examination. The anthropometric assessment in group DM1 was based on the analysis of the height-for-age (HA) and body mass index (BMI)-for-age ratios in percentiles using the programs ANTHRO or ANTHRO PLUS for children under and over 5 years old, respectively; those programs use the World Health Organization (WHO) standard curves (2006/2007) [26] as a reference. For group DS, the percentile curves formulated by Cronk et al. (1988) [27], which are specific for the growth assessment of children with the syndrome, were used to analyze the weight-for-age (WA) and HA ratios.

Laboratory assessment (anti-tTG-IgA/IgG, EmA-IgA and total IgA)

The levels of anti-tissue transglutaminase (anti-tTG) and anti-endomysial (EmA), immunoglobulin (Ig)A antibodies, and serum total IgA were measured. Anti-tTG-IgG was also assessed in the cases with low total IgA to avoid the interference of IgA deficiency. Venous blood samples (5 mL) were collected during the visits in anticoagulant-containing tubes, and sent to the UFRN laboratory for clinical analysis, where they were centrifuged, distributed into aliquots, and stored at -20°C until analyzed. The anti-tTG (IgA and IgG) antibodies were investigated through an enzyme-linked immunoassay (ELISA) using human recombinant antigen and the cutoff point

(≥ 10 U/mL) recommended by the kit manufacturer. EmA-IgA was measured by the indirect immunofluorescence technique using monkey esophageal tissue as the substrate; uniform fluorescence at a 1:5 dilution was considered positive. Both tests were performed with the commercial kit *Orgentec Diagnostika* (Mainz, Germany) [28].

The serum IgA levels were analyzed by immunoturbidimetry. The reference values per age were used; values two standard deviations below the mean (< -2.0 SD) were considered indicative of IgA deficiency, with total deficiency being defined by levels ≤ 7 mg/dL and partial deficiency above this [29].

Histopathological assessment

Only the participants with positive anti-tTG-IgA or IgG and/or EmA were subjected to SIB through UGIE, which was performed in the operating room. Three specimens were taken from the duodenal bulb and four from the second part of the duodenum, fixed with 10% formalin and sent to the UFRN Anatomic Pathology Laboratory, where they were processed in paraffin and stained with hematoxylin–eosin. Two pathologists independently performed the histopathological analysis under an optical microscope, and a third was called in to solve eventual discrepancies. The Marsh [30] classification later modified by Oberhuber et al. [31] was used, whereby the results were graded as follows: Marsh 0, normal mucosa; Marsh 1, infiltrative pattern; Marsh 2, crypt hyperplasia; and Marsh 3, which delineates increasing grades of villous atrophy (3a, 3b, and 3c). Diagnosis of CelD was established as the presence of Marsh 2 or 3 [1].

CelD classification

CelD was classified following the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [1] as GI, extraintestinal, silent, and potential forms. The occurrence of GI symptoms and exclusively extraintestinal symptoms characterized the GI and extraintestinal forms, respectively, and the absence of symptoms corresponded to the silent form; all this with screening and enteropathy consistent with CelD. The potential form was defined as positive serology, with or without symptoms, and the absence of enteropathy in the duodenal specimens. The clinical forms were represented in the celiac iceberg.

Statistical analysis

The quantitative variables were described as the mean \pm SD and the categorical variables as the frequency and

percentage. The mean values of the variables with normal distribution (Kolmogorov–Smirnov test) were compared by Student's *t* test, and the values without a normal distribution by the Mann–Whitney test. Pearson's Chi-square or Fisher's exact test analyzed the association between independent categorical variables and outcome (dependent variables), and the prevalence ratio was calculated. The significance level was set as alpha value = 0.05 and a 95% confidence interval; *p*-values < 0.05 were considered significant.

Results

In total, 188 children and adolescents were included for analysis. The mean age was 8.95 ± 4.74 years (range: 10 months–18 years old), 102 (54.3%) were female, and 95 (50.5%) resided in the interior of RN. The distribution of the sample was as follows:

Group DM1: 111 patients; mean age: 10.84 ± 4.48 years old; an average length of DM1: 3.83 ± 3.31 years. Thirty-two patients exhibited positive markers [EmA IgA = 17/111 (15.3%), anti-tTG-IgA = 12/111 (10.8%), anti-tTG and EmA (3/111(2.7%)); 25 (78.1%) underwent SIB, the children's guardians did not give consent in the other seven cases; and histopathological findings of CelD were found in five (4.5%) patients.

Group DS: 77 patients; mean age: 5.97 ± 3.66 years old. A total of 21 patients exhibited positive markers [EmA IgA = 10/77(13.0%), anti-tTG-IgA = 5/77(6.5%), anti-tTG and EmA (6/77(7.8%)); 15 (71.4%) underwent SIB, the children's guardians did not give consent in three cases and the cardiological assessment was unfavorable in an additional three cases. Histopathological findings of CelD were found in 10 (13.0%) patients.

The means of the following biodemographic variables exhibited significant difference: current age (in years) ($p < 0.01$); age (in years) at the onset of symptoms [DM1 = 8.34 ± 3.90 ; DS = 3.70 ± 3.16 ($p < 0.01$)]; age (in months) at the first introduction of gluten into the diet [DM1 = 9.35 ± 4.47 ; DS = 7.78 ± 4.82 ($p = 0.02$)]; and length (in months) of exclusive breastfeeding [DM1 = 4.67 ± 3.11 ; DS = 3.75 ± 3.04 ($p = 0.03$)]. Statistical significance was not found relative to the mean duration of symptoms (in years) [DM1 = 2.40 ± 2.21 ; DS = 2.46 ± 3.07 ($p = 0.60$)].

GI symptoms were present in 101 (53.7%) patients [DM1 = 52 (46.8%); DS = 49 (63.6%); $p = 0.02$]. The frequency of these symptoms per group is described in Table I.

Extraintestinal symptoms were present in 30/188 (16.0%) patients, in eight cases (4.3%) alone and in 22

Table I. Frequency of gastrointestinal symptoms in children and adolescents with type1 diabetes mellitus (DM1 group) and Down syndrome (DS group) at the Pediatric Hospital/UFRN, Natal/Brazil.

Gastrointestinal symptoms	DM1 group <i>n</i> = 111		DS group <i>n</i> = 77		<i>p</i> value ^a	PR (CI 95%)
	abs	rel%	abs	rel%		
Abdominal pain	34	30.6	24	31.2	0.937	0.99 (0.76–1.28)
Constipation	12	10.8	33	42.9	0.001	2.38 (1.76–3.22)
Abdominal distension	32	28.8	09	11.7	0.005	1.45 (1.16–1.81)
Poor weight gain	29	26.1	06	7.8	0.001	1.54 (1.25–1.90)
Flatulence	13	11.7	18	23.4	0.034	1.54 (1.07–2.21)
Diarrhea	14	12.6	06	7.8	0.292	1.21 (0.88–1.66)
Vomiting	10	9.0	05	6.5	0.531	1.14 (0.78–1.66)

^aPearson's Chi-square test.

PR, prevalence ratio; CI, confidence interval.

Table II. Gastrointestinal symptoms and their association with serological markers and histopathological findings of celiac disease in children and adolescents with type1 diabetes mellitus (DM1 = 111) and Down syndrome (DS = 77) at the Pediatric Hospital/UFRN, Natal/Brazil.

Gastrointestinal symptoms	Serological markers				<i>p</i> PR/CI 95%	Histopathological findings				<i>p</i> PR/CI 95%
	<i>n</i> = 53		<i>n</i> = 135			<i>n</i> = 15		<i>n</i> = 25		
	Positive DM1 = 32 DS = 21	Negative DM1 = 79 DS = 56	Present DM1 = 5 DS = 10	Absent DM1 = 20 DS = 5		abs	rel%	abs	rel%	
Abdominal pain	28	52.8	30	22.2	<0.01 ^a 2.51/1.61–3.90	10	66.7	11	44.0	0.16 ^a 1.81/0.75–4.34
Constipation	11	20.8	34	25.2	0.522 ^a 0.83/0.46–1.47	05	33.3	03	12.0	0.12 ^b 2.00/0.95–4.20
Abdominal distention	19	35.8	22	16.3	<0.01 ^a 2.00/1.28–3.11	06	40.0	08	32.0	0.60 ^a 1.23/0.55–2.76
Poor weight gain	17	32.1	18	13.3	<0.01 ^a 2.06/1.32–3.22	03	20.0	09	36.0	0.47 ^b 0.58/0.20–1.69
Flatulence	08	15.1	23	17.0	0.747 ^a 0.90/0.47–1.71	02	13.3	03	12.0	1.00 ^b 1.07/0.33–3.42
Diarrhea	10	18.9	10	7.4	0.02 ^a 1.95/1.17–3.24	04	26.7	03	12.0	0.39 ^b 1.71/0.76–3.82
Vomiting	06	11.3	09	6.7	0.28 ^a 1.47/0.75–2.86	04	26.7	01	4.0	0.05 ^b 2.54/1.32–4.91

^aPearson's Chi-squared test.^bFisher's exact test.

PR, prevalence ratio; CI, confidence interval.

(11.7%) associated with GI symptoms. The distribution of the extraintestinal symptoms was as follows: acquired hearing loss (1), epileptic seizures (1), chronic fatigue (1), and unsatisfactory growth (17) in group DM1; arthralgia (1), arthritis (1), epileptic seizures (1), and unsatisfactory growth (7) in group DS.

In group DM1, short stature was found in 16 (13.4%) patients, underweight in two (1.8%), and overweight in 21 (18.9%). Of the five children in group DM1 with CeID, one exhibited short stature, two normal weight, and three overweight. In group DS, short stature was found in three (3.9%) patients, underweight in two (2.6%), and overweight in five (6.5%). All 10 children in group DS with CeID exhibited adequate body weight and height.

The screening found positive serology results in 53/188 (28.2%) patients; upon considering each marker, a significant difference was not found between the

groups, DM1 = 32 (28.8%) and DS = 21 (27.3%) [*p* = 0.81]. Among the 53 patients, 38 (71.7%) exhibited GI symptoms. There was an association of GI symptoms with positive serology results (Table II), which did not occur in the case of the extraintestinal symptoms (*p* = 0.47). Low serum IgA levels were found in 23 (12.2%) patients, a finding indicative of partial IgA deficiency. Anti-tTG-IgG was assessed in 18/23 patients, and the titers were negative in all of them. The other five had previously shown positive anti-tTG-IgA titers.

In total, 40/53 (75.5%) patients with positive serological markers underwent biopsy. CeID was confirmed in 15/40 (37.5%) of these patients, whose duodenal mucosa exhibited Marsh-Oberhuber stages 3a and 3b. In the other 25 patients, the duodenal mucosa exhibited Marsh-Oberhuber stages 0 or 1. An association was not

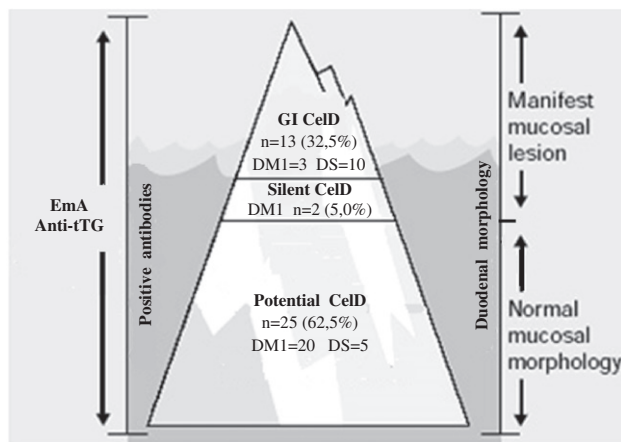


Figure 1. Diagram of celiac disease (CeID) iceberg originating from the clinical forms of children and adolescents with type 1 diabetes mellitus (DM1) and Down syndrome (DS) which were subjected to biopsy ($n = 40$). The visible portion is represented for the gastrointestinal (GI) form and the submerged portion for the silent and potential forms of CeID.

observed between histopathological findings of CeID and GI (Table II) or extraintestinal ($p = 0.90$) symptoms.

The proportion of patients with histopathological findings of CeID was larger in group DS (13.0%) compared with DM1 (4.5%) [$p = 0.03$]. Among the 10 patients with CeID in group DS, one exhibited autoimmune thyroiditis and alopecia areata and another already had clinical remission from acute lymphocytic leukemia. None of the five patients with CeID from group DM1 exhibited any other associated autoimmune disorder.

The GI, silent, and potential forms of CeID were found in the investigated population and represented in the iceberg (Figure 1). No case of extraintestinal form was identified.

Discussion

The present investigation reports on the clinical presentation of CeID in patients with DM1 and DS from specialized care. Up to the time of study inclusion, they had never undergone screening for CeID.

Analysis of the biodemographic characteristics showed that the time elapsed since the onset of symptoms was similar between groups DM1 and DS, leading to the assumption that the children's first visit to the specialized service was already late. Weaning from breastfeeding was too early, and gluten was first introduced into the children's diet after the age of 6 months, both of which are considered risk factors for CeID [32–34]. Because DM1 and DS are high-risk groups for CeID, their presence presumably increases the risk of developing the disease. However, the hypothesis that

breastfeeding and the age at first introduction of gluten behave as preventive factors against CeID is not supported by recent scientific evidence [35,36].

The frequency of symptoms was high in the population assessed. GI symptoms are known to occur in both DM1 and DS. In the case of DM1, symptoms may be the result of GI motility disorders, changes in visceral sensitivity and secretion of neurotransmitters, in addition to diseases affecting the GI system, such as CeID [37]. These facts accounted for the high prevalence of abdominal pain in group DM1, as was previously reported in some studies [10,21,23]. Clinical symptoms of CeID are estimated to occur in 50% of individuals with DM1, with abdominal pain in 16% of the cases [19]. Reduced intestinal muscle tone, which occurs in individuals with DS, and the possible occurrence of previously undiagnosed CeID may contribute to the higher frequency of constipation and flatulence in this group of patients [13,15].

The positive serology results found in the present study was higher than that in other studies conducted in Brazil, which varied from 2.5% in Rio de Janeiro [9] to 21.0% in Recife [12] for patients with DM1 and was 7.0% among children and youths with DS in Southern Brazil [17]. The histopathological assessment confirmed the high prevalence rate of CeID in groups DM1 and DS, being comparable with the rates found in some European countries [5,6]. In Brazilian studies with patients with DM1, such as the investigations conducted by Baptista et al. [11] and Whitacker et al. [10], the prevalence rate was 4.8% and 4.0%, respectively, similar to that found in the present study (4.5%). For DS, the prevalence of CeID was the same in the studies by Lobe et al. [16] and Nisihara et al. [17] (5.6%) and lower than that detected in the present study (13.0%).

The high frequency of positive serological markers yielded a spectrum, one end of which comprised 15 patients with positive antibodies and CeID proven biopsy and the other end of which comprised 25 patients with positive antibodies and normal biopsy results, most of whom were from group DM1, indicating the potential form of CeID. It is believed that screening through high-sensitivity and high-specificity markers contributed to the extension of these findings, which would not have been possible had single and isolated techniques been used. Thus, it seems reasonable to suggest that in the case of high-risk groups, screening for CeID is more reliable and efficacious when association of antibodies are investigated, in addition to the analysis of serum IgA in the patients with negative serological markers who use this fraction.

The cross-sectional design of the study might have influenced the frequency of the potential form of CeID.

Such patients are at high risk for subsequently developing villous atrophy, and thus, monitoring their clinical condition and serological markers (associated or not with additional biopsies) is crucial for defining the diagnosis. Barera et al. [38] monitored a large population of diabetic children with positive serology for CeID over 6 years and found that 60% were seropositive by the time DM1 was diagnosed, whereas the remaining 40% were diagnosed with CeID in the following 4 years.

Other studies also found potential CeID in patients with DM1, such as Franzese et al. [39], who detected it in 12.2% of the participants in their multicenter study in Italy. In Brazil, potential CeID was found in 3.8% of patients with DM1 [11].

It should be observed that although the behavior of specific antibodies in the overall population of celiac patients is satisfactorily known, there is little evidence relative to such behavior in specific high-risk groups, in which case, dysregulation of the immune system may contribute to the production of autoantibodies and the consequent increase in their positivity. Thus, the possibility of false-positive results for CeID antibodies found in this study should be kept in mind, i.e., their detection is not necessarily associated with present or future occurrence of CeID. Sardy et al. [40] observed that false-positive reactions for anti-tTG can occur without any relationship to CeID in patients with autoimmune diseases.

The presence of GI symptoms might have contributed to the selection of patients with positive markers, inasmuch as such symptoms allegedly reflect the presence of CeID. The association found between them was significant for abdominal pain, abdominal distension, diarrhea, and unsatisfactory weight gain (Table II), whose signs and symptoms are mainly related to malabsorption phenomena. However, this association should be viewed with caution because, although the symptoms may sometimes be profuse, the subjectivity of certain complaints should be considered, as it may lead to mistaken interpretations and limitations in establishing the relevance of such a relationship. Therefore, there is no basis to infer that a given symptom may indicate the positivity of a certain marker.

The lack of an association between the presence of symptoms and histopathological findings of CeID reinforces the inconsistency of symptoms as disease indicators, as they can also represent manifestations of DM1 and DS. However, the undervaluation of symptoms may delay the CeID diagnosis and increase the duration of exposure to gluten. Thus, GI and extraintestinal symptoms appear to behave more as confounding than as elucidative variables in the aforementioned high-risk groups.

The clinical presentation of CeID was variable and heterogeneous in groups DM1 and DS. The GI form prevailed among the cases with active disease, which was detected in all the group DS patients and in most of the group DM1 patients, thus contributing to the visible portion of the iceberg. When all forms of the disease were analyzed in combination, the higher prevalence of potential CeID was largely determinant of the large and submerged base of the iceberg (Figure 1), which indicates that the silent form may not be the most frequent, as indicated by previous studies [2,7,14,19,21]. The disease profile might have changed after the advent of more sensitive and specific serological markers, such as EmA and anti-tTG, which allow identifying patients with positive antibodies, but still without compatible histopathological findings.

In conclusion, the frequency of the GI form was high among the cases with active CeID, particularly in group DS, while the potential form of disease was the most prevalent, especially in group DM1, thereby demonstrating the emergence of other clinical profiles. The contribution of the GI form to the celiac iceberg was smaller compared with the potential form. The lack of an association between symptoms and histopathological findings of CeID stresses the inconsistency of the former as disease indicators, even though they were associated with antibody positivity.

The high frequency of the potential form of CeID reinforces the critical need to perform systematic serological screening in high-risk groups such as patients with DM1 and DS. The follow-up of such patients with respect to the future development of active CeID is recommended. The high prevalence of CeID in DM1 and DS reinforces the importance of serological screening in the diagnostic approach in specialized services and should call attention to the need to introduce such screening within Brazil's public healthcare system.

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