

## Celiac disease in children and adolescents with Down syndrome

*Doença celíaca em crianças e adolescentes com síndrome de Down*

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### Abstract

**Objectives:** High prevalence rates of celiac disease in patients with Down syndrome have been reported in several countries. However, in Brazil there are no data regarding this association. In this study we report the prevalence of celiac disease in Down syndrome children and adolescents from southern Brazil.

**Methods:** Seventy-one patients (32 female and 39 male, 2-18 years) from Curitiba, Brazil, were studied. Eighty young people (42 male and 38 female, 2-19 years) were used as controls. All subjects were screened for the IgA-antiendomysium antibody (EmA) and IgA anti-tecidual transglutaminase (anti-tTG). EmA was measured by an immunofluorescence assay using umbilical cord as the substrate and anti-tTG by ELISA with tecidual transglutaminase as the antigen. The total IgA serum level was determined by turbidimetry.

**Results:** Five DS patients (7%) were positive for EmA-IgA, with titers from 1/5 to 1/80 and 14 (17.5%) for anti-tTG (21-340 units). All EmA positive patients also presented anti-tTG antibodies simultaneously. Clinical and histological findings of the intestinal mucosa confirmed celiac disease diagnoses in four patients. The other EmA positive patient was asymptomatic and was not submitted to duodenal biopsy. Patients only positive for anti-tTG presented borderline values (< 25 units) and were asymptomatic. None of the controls were positive for EmA or anti-tTG. No Down syndrome patients or controls presented IgA deficiency.

**Conclusions:** These data indicate a high prevalence (5.6%) of confirmed celiac disease in Down syndrome patients from southern Brazil.

*J Pediatr (Rio J). 2005;81(5):373-6: Antiendomysium antibodies, anti-transglutaminase antibodies, celiac disease, Down syndrome.*

### Resumo

**Objetivos:** Alta prevalência de doença celíaca em pacientes com síndrome de Down tem sido descrita em vários países. No entanto, no Brasil ainda não há relatos mostrando essa associação. O presente estudo teve como objetivo avaliar a prevalência de doença celíaca em crianças e adolescentes com síndrome de Down no sul do Brasil.

**Métodos:** Setenta e um pacientes (32 do sexo feminino e 39 masculino, 2-18 anos) provenientes de Curitiba, Brasil, foram estudados. Oitenta indivíduos (42 do sexo masculino e 38 feminino, 2-19 anos) foram utilizados como controles do estudo. Todas as amostras foram investigadas para anticorpo anti-endomísio (EmA) e anti-transglutaminase tecidual (anti-tTG). O EmA foi pesquisado através de imunofluorescência indireta usando cordão umbilical como substrato e o anti-tTG através da técnica de ELISA, utilizando transglutaminase tecidual como antígeno. As dosagens de IgA foram realizadas por turbidimetria.

**Resultados:** Cinco pacientes com síndrome de Down (7%) foram positivos para EmA-IgA, com títulos entre 1/5 to 1/80 e catorze (17,5%) para anti-tTG (21-340 unidades). Todos os pacientes positivos para EmA apresentaram simultaneamente positividade para o anti-tTG. Os achados clínicos e histológicos na mucosa intestinal confirmaram doença celíaca em quatro pacientes. O outro paciente EmA positivo não foi submetido a biópsia duodenal. Os pacientes positivos apenas para anti-tTG apresentaram valores limítrofes (< 25 unidades) e eram assintomáticos. Nenhum indivíduo do grupo controle foi positivo para EmA ou anti-tTG. Nenhuma amostra do estudo foi deficiente para IgA.

**Conclusões:** Os dados do presente estudo mostram alta prevalência (5,6%) de doença celíaca confirmada em crianças e adolescentes com síndrome de Down da região sul do Brasil.

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### Introduction

Down's syndrome (DS) is the most frequent chromosomal anomaly observed in the general population, affecting 1 in 800 newborns throughout the world and is the main genetic factor for moderate mental retardation.<sup>1</sup>

Different studies have shown that patients with DS present several immune dysfunctions,<sup>2,3</sup> which lead to increased frequency of recurrent infections, as well as autoimmune diseases. A high association between DS and autoimmune diseases has been already reported by several

authors,<sup>4-6</sup> with the most significant association with celiac disease (CD)<sup>7-11</sup> and autoimmune thyroiditis.<sup>12,13</sup>

CD affects individuals of all ages and is characterized by permanent gluten intolerance. In its classic form, CD appears with symptoms and signs of intestinal malabsorption. However, the disease may occur in a silent or latent form.<sup>14</sup> According to Marsh,<sup>15</sup> the duodenal mucosa can be normal or present changes ranging from mild alterations to severe atrophy in mucosal architecture. The treatment with a gluten-free diet results in clinical and mucosal recovery, with recurrence of the disease after the return of gluten to the diet.<sup>14</sup> The classic and symptomatic form of CD is the presence of diarrhea, vomiting and weight loss, which appears in a minority of cases. On the other hand, most of the patients present unspecific gastroenterologic symptoms such as dyspepsia, abdominal pain, flatulence and alteration of the intestinal rhythm. These characteristics often cause delay in CD diagnosis and the incorrect management of patients.<sup>14</sup>

In 1975, Bentley reported for the first time the association between CD and DS.<sup>16</sup> In the following years, several reports showed increased frequency of CD in individuals with DS in different populations, with a prevalence ranging from 3.2 to 10.3%.<sup>7-11</sup> In this study we investigate the prevalence of CD in children with DS from southern Brazil.

## Materials and methods

The institutional ethics committee approved the present investigation.

### Patients

Seventy-one consecutive DS patients (32 female and 39 male) with mean age 6.12 years (range 2-18 years) from the DS clinic of the *Hospital de Clínicas - Universidade Federal do Paraná* (UFPR) (Curitiba, Brazil) were studied. DS patients older than 18 years were excluded from the study. After formal consenting of DS children's first-degree relatives, 3 ml of blood were collected from each subject. Samples were centrifuged and the serum separated, aliquoted and immediately stored at -80 °C, until used. Only the EmA-IgA/anti-tTG positive patients were clinically evaluated and submitted to upper gastrointestinal endoscopy. Crypt hyperplasia and villous atrophy were classified as partial (PVA) or total (TVA), according to Marsh.<sup>15</sup>

### Controls

Blood samples of 80 healthy children of the same geographic area of the patients, matched with sex and age with the DS group (38 female and 42 male, mean age 8.02 years), range 2-19 years, were used as controls.

### EmA-IgA

All subjects were screened for EmA-IgA according to Volta *et al.*,<sup>17</sup> using immunofluorescence indirect assay, with human umbilical cord as substrate and FITC anti-IgA human as conjugate (INOVA, USA).

### Anti-tTG antibodies

Anti-tissue transglutaminase IgA antibodies (tTG) were determined by enzyme-linked immunosorbent assay (ELISA) with guinea pig liver tTG as antigen, using a commercial kit (INOVA Diagnostics Inc., San Diego, CA, USA), according to Dieterich *et al.*<sup>18</sup> Results were considered positive when higher than 20 units.

### Serum IgA

The total serum IgA levels was determined by turbidimetry (Behring, Germany).

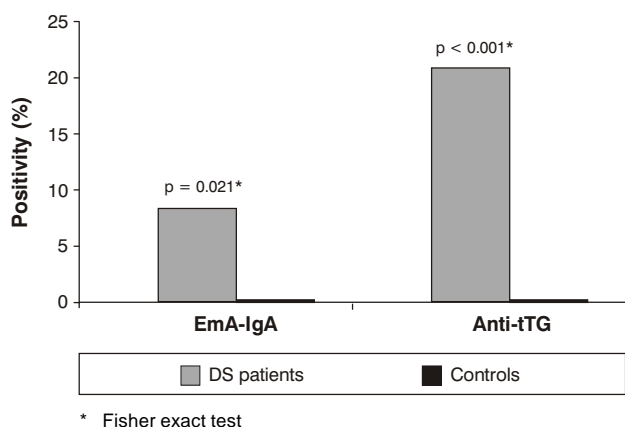
### Statistical analysis

Data were analyzed with Statistica (Microsoft, USA) software, using the Fisher exact test.

## Results

Serological screening for CD based on EmA and tTG antibodies was performed in 71 patients with DS and in 80 healthy children.

These results for DS patients and controls are shown in Figure 1. A highly significant positivity of EmA-IgA ( $p = 0.021$ ) and anti-tTG ( $p < 0.001$ ) was observed in the DS patients when compared to the controls. Five DS patients (7%; 5/71), four male and one female, were positive for EmA-IgA, with titers varying from 1/5 to 1/80. Fourteen patients (17.5%; 14/71), seven male and seven female, were positive for anti-tTG antibodies with values from 21 to 340 U/ml. Five of these patients were concomitantly positive for EmA-IgA and the other nine were only positive for anti-tTG antibodies, presenting borderline values (Table 1). None of the 80 control individuals was positive for EmA-IgA or anti-tTG.



\* Fisher exact test

**Figure 1** - Anti-endomysial (IgA-EmA) and anti-transglutaminase (tTG) antibodies in Down syndrome patients and controls

**Table 1** – Antiendomysium and anti-transglutaminase antibodies, symptoms and histologic aspects in DS patients

Patient	Sex	Age	EmA-IgA	Anti tTG* (U/ml)	Symptoms	Hystologic aspects
C.M.	M	6	POS 1/5	72	Symptom-free	ND
A.M.E.	M	8	POS 1/5	30	Dyepsia, growth failure, abdominal pain	Partial atrophy
R.D.	F	18	POS 1/80	198	Dyarrheia, abdominal pain	Total atrophy
R.K.	M	6	POS 1/80	176	Dyarrheia, growth failure	Total atrophy
R.S.A.	M	10	POS 1/80	340	Symptom-free	Total atrophy
S.R.O.	F	4	Negative	22	Symptom-free	ND
L.P.	F	12	Negative	21	Symptom-free	ND
R.I.	F	3	Negative	24	Symptom-free	ND
A.O.S.	F	15	Negative	23	Symptom-free	ND
E.H.S.	F	3	Negative	24	Symptom-free	ND
L.I.H.	M	3	Negative	24	Symptom-free	ND
D.M.S.	M	7	Negative	23	Symptom-free	ND
S.R.O.	F	5	Negative	31	Symptom-free	ND
J.B.	M	4	Negative	39	Symptom-free	ND

ND = Not done. \* Reference values: < 20 U/ml = Negative; 20 to 30 U/ml = weakly positive, > 30 U/ml = positive.

Among the five patients concomitantly positive for EmA-IgA and anti-tTG antibodies, three showed clinical symptoms such as diarrhea, abdominal pain, anemia and growth failure (Table 1). Four of them underwent an upper digestive endoscopy followed by duodenal mucosa biopsy. Histological aspects of duodenal mucosa compatible with CD were observed in all patients, three of them presented TVA and one PVA. Only one asymptomatic patient (EmA 1/5, anti tTG = 72 UI) did not undergo digestive endoscopy and duodenal biopsy. Thus, the prevalence of confirmed CD among the investigated DS patients was 5.6% (4/71).

Neither the DS patients nor the controls presented IgA deficiency.

## Discussion

This is the first report to show the prevalence of CD in children with DS in Brazil. Our findings confirmed the increased prevalence of CD among patients with DS reported by several studies in Europe, North America and Argentina.<sup>7-11</sup> The prevalence of CD in the general population in these countries ranges from 1:200 to 1:2,000, and among DS patients it presents a 20-200 fold increase. In Brazil, the prevalence for CD has been estimated to be 0.14% (1:687) in the central-western region<sup>19</sup> and 0.1% (1:1,000) in the south region.<sup>20</sup>

Although not all EmA positive patients in our study underwent an intestinal biopsy, the confirmed CD prevalence in DS patients was still very high (5.6%; 1:18), in comparison with the general population of the same geographical area (1:1,000). If we consider CD prevalence diagnosed by positive EmA, this prevalence would be 7%. The high

association of CD with DS has not yet been clarified, however both, CD and DS patients present more frequent dysfunction in their immune system and are more predisposed to autoimmune diseases, such as thyroid diseases, diabetes mellitus type 1, lupus and arthritis.<sup>4,5,6,12</sup> Besides that, this association may be related to shared common genetic markers. It has been shown that the DS patients with CD present the characteristic CD associated with high-risk human leukocyte antigens (HLA) DR3 and DQ2 alleles.<sup>21</sup> In addition, gene disorders caused by chromosomal imbalance may be related to the enhanced expression of CD in SD patients.

Different studies have shown that the most sensitive and specific serologic test for the diagnosis of CD is the assessment of EmA antibodies. During this investigation, five patients were positive for EmA-IgA (7%) and four of them underwent duodenal biopsy. All patients who underwent intestinal biopsy showed clinical manifestations and histological alterations of intestinal mucosa compatible with CD. The other patient both EmA-IgA and anti tTG positive was asymptomatic and did not undergo biopsy. It is possible that this patient had a latent form of CD, which needs to be followed-up. Thus EmA screening showed high sensitivity and specificity for the diagnosis of CD in the investigated patients. Our results corroborate the widely accepted concept that EmA-IgA is a specific screening test for CD.

EmA negative and anti-tTG positive patients, which did not undergo duodenal biopsy, will now be followed up. Nevertheless, anti-tTG antibodies have been shown to be positive in other diseases such as inflammatory bowel disease, chronic liver disease and diabetes mellitus.<sup>22</sup> Also, lower specificity of anti-tTG has been related to the use of

guinea pig liver extract as antigen in the ELISA kit. Recently, the use of human tissue transglutaminase as purified antigen has been shown to enhance the specificity of this assay.<sup>23</sup>

In the first 2 years of life, most of the CD patients present classic symptoms and when associated with DS this diagnosis is almost always delayed. Certain characteristics of DS children, such as a distended abdomen and the tendency to consider growth failure as natural consequences of the DS, can be responsible for the difficulty in diagnosing CD in these patients. Thus, several authors consider that the CD is underestimated as the cause of diarrhea, malnutrition or development deficit in patients with DS.<sup>9,24</sup>

The importance of the CD investigation in DS patients has been showed in recent studies, which recommended serologic tests every 2 years, since negative young individuals may be positive some years later.<sup>9</sup>

The data reported in the present study emphasize the value of investigating CD in DS patients, since the correct and early diagnosis will offer a better quality of life to the patient and his family.

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