

# Serum antigliadin antibody levels as a screening criterion before jejunal biopsy indication for celiac disease in a developing country

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## Abstract

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The objective of the present study was to determine the efficacy of detection of antigliadin immunoglobulins G and A (IgG and IgA) for the diagnosis of celiac disease in a developing country, since other enteropathies might alter the levels of these antibodies. Three groups were studied: 22 patients with celiac disease (mean age: 30.6 months), 61 patients with other enteropathies (mean age: 43.3 months), and 46 patients without enteropathies (mean age: 96.9 months). Antigliadin IgG and IgA ELISA showed sensitivity of 90.9 and 95.5%, respectively. With the hypothetical values of prevalence ranging from 1:500 to 1:2000 liveborns, the positive predictive value varied from 8.5 to 2.3% for IgG and from 4.8 to 1.1% for IgA. Considering the patients without enteropathies, specificity was 97.8 and 95.7% for IgG and IgA, respectively. In patients with other enteropathies, specificity was 82.0 and 84.1%, respectively. When patients with and without other enteropathies were considered as a whole, specificity was 88.8 and 91.6%, respectively. The specificity of positive IgG or IgA was 93.5% in children without enteropathies and 78.7% in the presence of other enteropathies. The negative predictive value for hypothetical prevalences varying from 1:500 to 1:2000 liveborns was 99.9%. Thus, even in developing countries where the prevalence of non-celiac enteropathies is high, the determination of serum antigliadin antibody levels is a useful screening test prior to the jejunal biopsy in the investigation of intestinal malabsorption.

### Key words

- Celiac disease
- Enteropathies
- Antigliadin antibodies

## Introduction

Currently, the criteria for the diagnosis of celiac disease require the demonstration of typical changes in the small intestinal (jejunal) biopsy followed by clinical improve-

ment on a gluten-free diet. According to established criteria for the confirmation of the diagnosis of celiac disease, three consecutive intestinal biopsies are needed (1). This invasive procedure requires trained personnel and is unpleasant for patients. More-

over, the total period of time for the diagnostic investigation is more than two years.

Valuable experience has been obtained with the quantitation of antigliadin antibodies as an additional diagnostic method in several developed countries (2-9). This method helps to prevent the lack of detection of cases of celiac disease and also to avoid many unnecessary jejunal biopsies. However, despite important advances in the immunological methods for the diagnosis of celiac disease, the high incidence of non-celiac enteropathies, that may induce the synthesis of antigliadin antibodies, may limit their feasibility in developing countries (10). Consequently, there is controversy about the usefulness of antibody detection in developing countries.

The aim of the present study was to assess the profile and to determine the sensitivity and specificity of the enzyme-linked immunosorbent assay (ELISA) for the determination of antigliadin immunoglobulins G and A (IgG and IgA) antibodies in Brazilian children with celiac disease, with other enteropathies and with no gastrointestinal symptoms.

## Patients and Methods

### Patients

During the 1991-96 period, three study groups were selected at the Gastroenterology Clinics of the University Hospital, Federal University of Minas Gerais. The groups consisted of children with a clinical picture suggestive of intestinal malabsorption such as chronic gastroenterologic symptoms or low stature (below the 3rd percentile), who were submitted to jejunal biopsy and blood sampling as part of the routine clinical evaluation. Informed consent to participate in this research was obtained from the children's parents. The project was approved by the Research Ethics Committee of the Federal University of Minas Gerais.

### Study groups

*Celiac disease group.* Twenty-two patients with intestinal malabsorption, presenting severe jejunal villous atrophy and cuboidal epithelial surface with many intraepithelial lymphocytes.

*Other enteropathy group.* Sixty-one patients with gastroenterologic symptoms such as abdominal distension or pain or chronic diarrhea, with normal or nonspecific changes in the intestinal mucosa.

*Control group.* Forty-six patients submitted to low stature evaluation, without hormonal or gastroenterologic symptoms and with a normal jejunal biopsy.

The general description of the patients is presented in Table 1. The mean age and consequently the weight and height were higher in the control group than in the other groups which were included in the study for the etiological diagnosis of low stature, usually defined after three years of age.

### Methods

*Antigliadin IgG and IgA detection.* Serum obtained by centrifugation from 5 ml of blood drawn from each patient was stored at -20°C until use. ELISA for antigliadin antibody detection was performed by the method of Huff et al. (11), with small modifications. Briefly, ELISA plates were coated with 100 µl per well of 50 µg/ml crude gliadin (Sigma, St. Louis, MO, USA) in carbonate buffer at 4°C for 12 h and blocked with 150 µl per well of 2% bovine albumin in PBS at 37°C for 60 min. Sera (100 µl per well) diluted 1:100 (for IgA detection) or 1:500 (for IgG detection) were then left to stand at 37°C for 60 min in duplicate according to a plate protocol that included positive and negative controls. One hundred microliters per well of peroxidase-linked anti-human IgG or IgA antibody (Sigma) was then added and the plates were incubated at 37°C for 60 min. Development of color was achieved using

100 µl per well of ABTS (2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid); Sigma). The reaction was interrupted after 10 min with 10% SDS (Sigma) and absorbance was read at 405 nm using an ELISA reader. After each of the above described steps, the plates were washed five times with PBS containing 5% Tween 20. The cutoff point was defined as the mean plus two standard deviations for a group of 20 normal children, being 0.022 for IgA (mean = 0.0065, SD = 0.0076) and 0.103 for IgG (mean = 0.0393, SD = 0.032).

### Jejunal biopsy

Jejunal biopsies were obtained by the oral route using the pediatric Carey capsule placed at the angle of Treitz, and visualized by X-ray. The mucosal fragments were mounted on a Millipore filter with the villous surface facing up, and immediately immersed in 10% formalin solution, embedded in paraffin, stained with hematoxylin-eosin and periodic acid Schiff and examined according to the method of Pereira et al. (12). The following aspects were considered for the definition of celiac pattern: absent or vestigial villi, cuboidal and basophilic epithelial surface containing many interepithelial lymphocytes (12).

### Statistical analysis

Data were analyzed using the statistical EPI INFO v.6.0 and SPSS software (13). Proportions were compared by the chi-square test and means were compared by the Kruskal-Wallis test. Analysis of variance (ANOVA) was used to compare means and the Scheffé test was used to identify the groups responsible for the differences. The 95% confidence interval (95% CI) was defined for the determinations of sensitivity, specificity and predictive values. The level of significance was set at 5%.

For the calculation of the predictive values, hypothetical incidences of 1:500, 1:1000 and 1:2000 were used based on literature data from other countries (14-17).

## Results

### Antigliadin antibodies

Antigliadin IgA antibodies were detected in 21 of 22 patients with celiac disease, resulting in a sensitivity of 95.5% (95% CI: 75.1 to 99.8%). Specificity was 88.5% when considering patients from the other enteropathy group, since 7 of 61 patients presented positive anti gliadin IgA antibodies.

Table 1. Sex, age, weight and height distribution of patients in the groups with celiac disease (CD), other enteropathies (OE) and control (CO).

Characteristic	Study groups			$\chi^2$ (P)
	CD (N = 22)	OE (N = 61)	CO (N = 46)	
Sex, N (%)				
Male	10 (45.5)	25 (41)	30 (65.2)	CD vs CO (P = 0.121)
Female	12 (54.5)	36 (59)	16 (34.8)	OE vs CO (P = 0.013)
Age (months)				
Mean $\pm$ SD	30.6 $\pm$ 28.8	43.3 $\pm$ 38.1	96.9 $\pm$ 48.5	CD vs CO (P = 0.010)
Median (range)	19.3 (6.0-135.6)	27.9 (4.8-156.5)	92.6 (12.2-170.8)	OE vs CO (P = 0.000)
Weight (kg)				
Mean $\pm$ SD	10.9 $\pm$ 5.9	14.3 $\pm$ 8.9	19.5 $\pm$ 7.3	CD vs CO (P = 0.000)
Median (range)	9.7 (6.0-35.0)	11.2 (5.0-49.5)	18.9 (6.5-36.7)	OE vs CO (P = 0.001)
Height (cm)				
Mean $\pm$ SD	85.8 $\pm$ 16.1	94.1 $\pm$ 23.0	110.0 $\pm$ 19.6	CD vs CO (P = 0.000)
Median (range)	81.5 (68.0-136.0)	87.8 (61.0-160.0)	111.3 (68.0-148.0)	OE vs CO (P = 0.000)

Table 2. Serum antigliadin IgG and IgA in the groups with celiac disease (CD), other enteropathies (OE) and control (CO).

Antigliadin antibodies	CD (N = 22)	OE (N = 61)	CO (N = 46)
IgG	0.367 ± 0.225 (0.046-1.090)	0.065 ± 0.081 (0-0.310)	0.039 ± 0.032 (0-0.144)
IgA	0.293 ± 0.280 (0-1.149)	0.009 ± 0.015 (0-0.067)	0.007 ± 0.008 (0-0.036)

Data are reported as means ± SD and range of ELISA absorbance values. IgG antibodies: ANOVA:  $F = 81.69$ ;  $P = 0.0000$ . Scheffé test ( $P < 0.05$ ):  $CD > OE$ ;  $CD > CO$ . IgA antibodies: ANOVA:  $F = 56.24$ ;  $P = 0.0000$ . Scheffé test ( $P < 0.05$ ):  $CD > OE$ ;  $CD > CO$ .

Figure 1. ELISA absorbance values of antigliadin IgG antibodies in celiac disease (CD, N = 22), control (CO, N = 46) and other enteropathy (OE, N = 61) groups. The dotted line represents the cutoff value. The solid lines represent the mean of the absorbance values for each group.

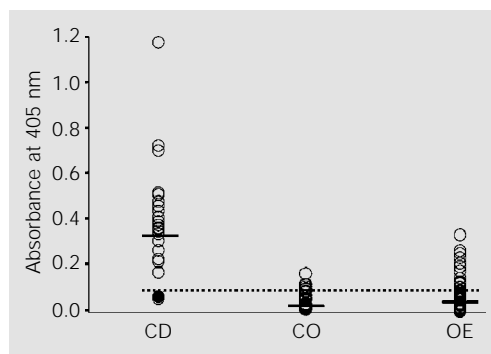


Figure 2. ELISA absorbance values of antigliadin IgA antibodies in the celiac disease (CD, N = 22), control (CO, N = 46) and other enteropathy (OE, N = 61) groups. The dotted line represents the cutoff value. The solid lines represent the mean of the absorbance values for each group.

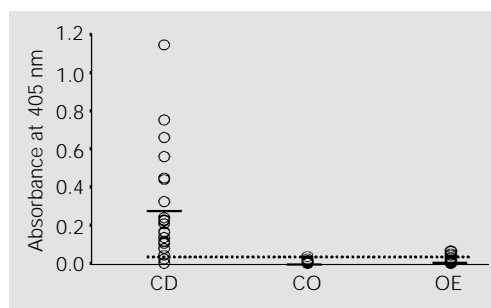


Table 3. Positive (PPV) and negative predictive values (NPV) reported as percent of antigliadin IgG and IgA antibodies based on hypothetical values of prevalence.

Prevalence	IgG		IgA	
	PPV	NPV	PPV	NPV
1:500	8.5	99.9	4.8	99.9
1:1000	4.4	99.9	2.0	99.9
1:2000	2.3	99.9	1.1	99.9

When these antibodies were evaluated in the control group, only 2 of 46 patients were positive, with a specificity of 95.7% (95% CI: 84.0 to 99.2%). When the other enteropathy and control groups were considered together, 9 of the 107 patients were positive for antigliadin IgA antibodies, with an overall specificity of 91.6%.

Antigliadin IgG antibodies were positive in 20 of 22 patients with celiac disease, resulting in a sensitivity of 90.9% (95% CI: 69.4 to 98.4%). The specificity was 82% when considering patients from the other enteropathy group, since 11 of 61 patients presented positive antigliadin IgG. When these antibodies were evaluated in the control group, only one of 46 patients was positive, resulting in a specificity of 97.8% (95% CI: 87.0 to 99.9%). Taken together, 12 of the 107 patients that comprised the other enteropathy and control groups had positive antigliadin IgG, with an overall specificity of 88%.

The absorbance values for antigliadin IgG and IgA are shown in Table 2. A significant difference was observed in the mean absorbance values recorded for the three study groups for IgA (Figure 1) and IgG (Figure 2) using ANOVA. Using the Scheffé test, the difference was attributed to the differences between the control group and other enteropathy and celiac disease groups versus control. No difference was observed between the other enteropathy and control groups.

Predictive positive and negative values were calculated considering hypothetical values of incidence of celiac disease ranging from 1:500 to 1:2000 liveborns. The positive predictive values were very low, ranging from 8.5 to 2.3% and from 4.8 to 1.1% for IgG and IgA, respectively. However, a high negative predictive value of 99.9% was found for IgG and IgA (Table 3).

## Discussion

The main objective of this study was to

evaluate the usefulness of antigliadin antibody quantitation in the screening for jejunal biopsy indication during the investigation of the causes of enteropathies and low stature, especially in those children with ambient enteropathies.

In the last two decades, the determination of serum antibodies has proved to be a useful tool for diagnosis and follow-up of patients on a gluten-free diet (2,3,18-21). Nevertheless, most of the studies refer to populations from developed countries, raising questions about the utility of these tests in nondeveloped countries, where the high frequency of so-called ambient enteropathies may produce false-positive results for antigliadin antibodies (10).

In the present study, high sensitivity for the IgG and IgA antigliadin antibody determination, 90.9 and 95.5%, respectively, was observed in children subjected to jejunal biopsy on the basis of routine clinical indications, with typical pathological findings of celiac disease.

Two other tests represent alternative serological tools for the celiac disease diagnostic screening test. The determination of anti-endomysium antibodies (22-24) and anti-tissue transglutaminase (25,26), both presenting high sensitivity and specificity, requires more in-depth studies in order to establish its usefulness in developing countries. Grodzinski (27) suggests that antigliadin antibodies should be used as a screening test and the anti-endomysium antibodies as a confirmatory test before the intestinal biopsy. However, several investigators have pointed out the possibility of false-negative antibody detection (9,28,29). The advantages of the detection of antigliadin antibodies are the feasibility of the ELISA technique and

the low cost.

Khoshoo et al. (30), in India, reported that IgG and IgA antigliadin antibodies were significantly higher in children with celiac disease than in children with other enteropathies.

In Brazil, antigliadin antibodies were investigated in children with celiac disease, showing a sensitivity of 90.4% for IgG and of 64.2% for IgA and a specificity of 87.0% for IgG and 92.1% for IgA. The authors consider that the positive antibodies associated with the characteristic histological findings confirm the diagnosis of celiac disease. However, a small number of patients were tested for IgA by Medeiros (31): 14 of 29 patients with celiac disease, 49 of 106 with other enteropathies, 27 of 45 with protracted diarrhea, and 26 of 56 control patients.

Many studies have used groups of adults as control, some of them without a jejunal biopsy (4,32-36). In general, antigliadin IgA has proven to be more specific for celiac disease. Tucker et al. (36) observed a higher specificity for IgG, in agreement with the present results.

Although the frequency of celiac diseases in our country has not been estimated, the hypothetical values permit us to conclude that if a child has no IgG and no IgA antigliadin antibodies he has a 99.9% chance of not having celiac disease, with no need to be submitted to a jejunal biopsy.

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