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# Celiac Disease Diagnosis in Misdiagnosed Children

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## ABSTRACT

Antiendomysial antibodies (EMA) are today considered the most sensitive and specific serological marker of celiac disease (CD). The aim of the present study was to assess the occurrence of EMA of IgG isotype in EMA IgA negative children with clinical suspicion of malabsorption and their relationship with CD. Serum EMA IgG1 determination was performed on 30 EMA IgA negative children with clinical suspicion of CD. Total serum IgA levels were further investigated. Sixty children with gastroenterological diseases other than CD were used as control disease patients and 63 healthy children were evaluated as the control group. Eighteen out of 30 children in the study showed EMA IgG1 positivity in sera and a villous height/crypt depth ratio <3:1 as index of intestinal atrophy. It is noticeable that a selective IgA deficiency was present in only 9 of 18 EMA IgG1 positive children. In addition, clinical symptoms, EMA IgG1, and mucosal atrophy disappeared after 8–10 mo on a gluten-free diet. Neither EMA IgA nor EMA IgG1 were detected in the children in the control groups. The other 12 children in study group showed no histologic abnormalities and were EMA IgG1 negative. In this study, we reveal a group of EMA IgG1 CD children without IgA deficiency. The diagnosis was based on the presence of gluten-dependent typical serological and histologic features of CD. Our data suggest that EMA IgG1 determination could be a new tool in the diagnostic workup of CD, useful in avoiding possible misdiagnosis. **(*Pediatr Res* 48: 590–592, 2000)**

**Abbreviations**

**CD,** celiac disease
**EGDS,** esophagogastroduodenoscopy
**EMA,** antiendomysial antibodies
**AGA,** antigliadin antibodies
**IgG1,** immunoglobulins, G1 subclass
**GFD,** gluten-free diet

CD is a lifelong gluten intolerance characterized by villous flattening with crypt hyperplasia of the small bowel mucosa and improvement or normalization of the mucosal architecture after a GFD (1). The presence of circulating EMA, highly sensitive and specific for CD (2–5), and their disappearance after a GFD confirms the diagnosis (6). Therefore, EMA IgA are today accepted as a reliable and noninvasive screening test for CD. Moreover, a recent study reported the presence of EMA of IgG isotype only in celiac children with selective IgA deficiency (7). These authors reported a low prevalence of AGA in these celiac children, suggesting the need to use different serological tests to reveal CD in the presence of IgA deficiency.

The aim of our study was to evaluate the occurrence of EMA IgG and their relationship to CD in a group of EMA IgA negative children with total or subtotal intestinal atrophy.

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## MATERIALS AND METHODS

***Subjects.*** Thirty EMA IgA negative children (12 males and 18 females, mean age 10.6, range 2–16 y) with clinical suspicion of CD were enrolled in the study as the patient group.

Sixty children (27 males and 33 females, mean age 11.3, range 4–16 y) with gastroenterological diseases other than CD (17 lactose intolerance, 3 cow's milk intolerance, 15 esophagitis, and 25 infectious diarrhea) were selected as the disease control group.

Sixty-three healthy children (30 males and 33 females, mean age 10.5, range 3–16 y) consisting of 25 patients with irritable bowel syndrome and 38 first-degree relatives of children with CD, were also enrolled in the study.

All the patients at the time of admission in the study were on a gluten-containing diet.

Informed consent was obtained from all subjects under study. The study was approved by the applicable institutional review board.

***Detection of EMA.*** EMA were sought in sera diluted 1:5 by means of indirect immunofluorescence analysis on cryostat sections of monkey esophagus (Eurospital SpA, Trieste, Italy).

FITC-conjugated anti-human IgG1 (Sigma Chemical Co., St. Louis, MO, U.S.A.) were used diluted 1:100. Positive EMA results were identified by reticulin-like staining of smooth-muscle bundles (Fig. 1).

***Detection of AGA.*** AGA were measured in sera, diluted 1:100 for AGA IgA and 1:500 for AGA IgG, by an in-house ELISA (8). Alkaline-phosphatase-conjugated goat anti-human IgA and IgG (Dako, Copenhagen, Denmark), diluted 1:1000, were used. OD values were obtained by enzyme immunoassay (EIA) reader at 405 nm.

AGA values were expressed as OD sample/OD pool normal serum + 3 SD. The cut-off values, 0.9 for AGA IgA and 1.1 for AGA IgG, were identified by using a receiver operating characteristic analysis (8).

***Detection of total IgA.*** Total IgA immunoglobulins were measured by a radial immune-diffusion method (Diffuplate, Bioscentifica, Buenos Aires, Argentina) and results were evaluated by referring to a standard curve. Normal values ranged between 90 and 220 mg/dL and, in accordance with the manufacturer's instructions, 90 mg/dL was used as a cut-off point to identify IgA deficiency.

***Histologic analysis.*** Two biopsy specimens of duodenal mucosa were obtained from each child in study by EGDS, for diagnostic purposes. The villus height/crypt depth ratio was measured and values below 3:1 were considered pathognomonic of CD.

***Follow-up.*** All of the EMA IgG1 positive children were kept on a GFD, and compliance with the diet was checked by a monthly serum EMA detection. When EMA IgG1 positivity and clinical signs and symptoms disappeared, in accordance with European Society for Pediatric Gastroenterology and Nutrition criteria (9), a second EGDS for histologic analysis was performed.

## RESULTS

Eighteen of the 30 EMA IgA negative patients with clinical suspicion of CD showed total or subtotal intestinal mucosa atrophy with a villus height/crypt depth ratio below 3:1. No histologic abnormalities were found in the other 12 children in the patient group (Table 1).

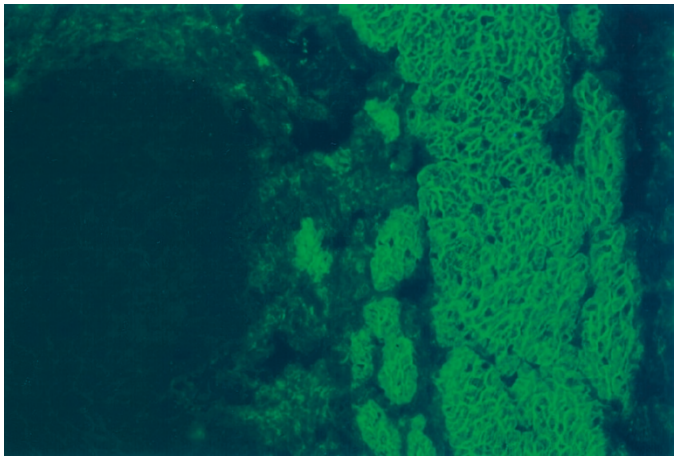

**Figure 1.** Serum EMA IgG1 positive. A feature of EMA is a honeycomb-like fluorescence pattern along muscolaris mucosae of monkey esophagus.

**Table 1.** *Histological, serum EMA, and AGA positive results*

| Subjects | Intestinal atrophy* | EMA positivity | | AGA positivity | |
|---|---|---|---|---|---|
| | | IgA | IgG1 | IgA | IgG |
| Patients group ($n$ = 30) | | | | | |
| CD patients ($n$ = 18) | 18/18 | 0/18 | 18/18 | 4/18 | 6/18 |
| Negative patients ($n$ = 12) | 0/12 | 0/12 | 0/12 | 4/12 | 5/12 |
| Control groups | | | | | |
| Disease control group ($n$ = 60) | — | 0/60 | 0/60 | — | — |
| Healthy children ($n$ = 63) | — | 0/63 | 0/63 | — | — |

* Villus height/crypt depth ratio <3:1.

EMA of IgG1 isotype were found in all of the 18 EMA IgA negative children presenting with total or subtotal intestinal mucosa atrophy. On the other hand, no EMA, IgA, or IgG1 were detected in the 12 children with clinical suspicion of CD who did not show intestinal mucosa atrophy, in the 60 disease controls, or in the 63 healthy children (Table 1).

AGA were measured in all 18 EMA IgG1 positive children and in all 12 children with EMA IgA and who were EMA IgG1 negative. AGA of IgA class were positive in 4 of 18 children with intestinal mucosa atrophy and in 4 of 12 EMA negative children, whereas AGA of IgG class was positive in 6 of 18 children with intestinal mucosa atrophy and in 5 of 12 EMA negative children (Table 1).

Sensitivity and specificity of AGA IgA determination were 22.2% and 66.7%, respectively, whereas for AGA IgG determination, sensitivity and specificity were 33.3% and 58.3%, respectively.

Normal levels of total serum IgA were found in 9 of 18 EMA IgG1 positive children (50%), therefore, 9 children were considered as having serum IgA deficiency.

In all 18 EMA IgG1 positive children studied, EMA IgG1 disappeared within 4–6 mo on a GFD, and a complete clinical remission was observed within 8–10 mo of gluten withdrawal. The second EGDS, performed when EMA IgG1 were not more detectable, showed in all these patients a complete recovery of the mucosal villi architecture after a GFD.

## DISCUSSION

CD, defined as a permanent intolerance of the small intestine to gluten, is characterized by gluten-dependent changes in villous morphology and/or signs of immunologic activation of the lamina propria with serum antiendomysial antibodies (10, 11). Although EMA are today considered the most specific serological marker of CD, with a specificity approaching to 100%, their sensitivity ranges between 74% and 100% (2–5). Nonetheless, although EMA IgG have been reported in the occurrence of selective IgA deficiency (7), EMA currently used in the diagnostic work-up of CD are of the IgA class. Therefore, data emerging from this report reveal a group of children with symptoms typical of CD and intestinal atrophy presenting EMA IgG1 in the absence of EMA IgA positive results. Anti-human IgG1 detection was selected in relation to the known distribution of IgG subclasses in humans (12, 13). Furthermore, unlike to the observations reported in the literature (7), our data showed that EMA IgG1 occurred also in the

absence of IgA deficiency. Therefore, the presence of EMA IgG1 is not always linked to impaired IgA production but is probably due to a different isotypic switch leading to the development of B-cell clones producing EMA IgG1 instead of IgA.

As previously reported in the literature (7), our study further showed that AGA had very low sensitivity and specificity in the population of EMA IgG1 positive children. This observation confirms that EMA IgG1 determination is necessary to reveal subjects already misdiagnosed using other serological tests. Furthermore, the absence of EMA IgG1 in the control groups determines a high value for the specificity of EMA IgG1 test in CD.

It is noticeable that in all of these EMA IgG1 positive children, intestinal atrophy was found. Moreover, clinical symptoms, EMA IgG1 positivity, and mucosal atrophy disappeared within 8–10 mo of a correct GFD, showing their strict gluten dependency and therefore suggesting that EMA IgG1 patients have CD.

CD has been likened to an iceberg because of the large number of people suffering from this condition who elude correct diagnosis (14). Our data identifies a group of EMA IgG1 CD patients, without IgA deficiency but with the same characteristics described for the typical EMA IgA positive CD patients (1), which could be representative of the submerged part of that iceberg. In fact, EMA IgG1 evaluation could increase the sensitivity of EMA testing, allowing the identification of CD patients who may be missed using EMA IgA test alone. Thus, we suggest the introduction of EMA IgG1 determination as new serological tool useful in the diagnostic work-up of CD.

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