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Isolated positive anti-gliadin immunoglobin-A antibody in children with gastrointestinal symptoms

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Background/aims: *The use of immunoglobulin G and A anti-gliadin antibodies for celiac disease screening has decreased due to higher specificity and sensitivity of tissue transglutaminase and endomysial antibodies. Greater values of immunoglobulin-A anti-gliadin antibody have been associated with more severe mucosal damage in proven and probable celiac disease patients. The aim of this study was to determine whether anti-gliadin antibody immunoglobulin A has any clinical importance in diagnosing celiac disease in children. Children with a chronic history of vomiting, abdominal pain, diarrhea, or constipation in the outpatient clinic were evaluated for celiac disease.* ***Materials and Methods:*** *Tissue transglutaminase and anti-gliadin antibody immunoglobulin A in serum were determined by ELISA test and endomysial antibodies immunoglobulin A by indirect immunofluorescence. Most of these children with isolated positive anti-gliadin antibody immunoglobulin A were further evaluated by performing proximal gastrointestinal biopsies.* ***Results:*** *Sixteen children had isolated positive anti-gliadin antibody immunoglobulin A (negative tissue transglutaminase and endomysial antibodies immunoglobulin A). Eight were male (mean age: 9.7 years). None had immunoglobulin A deficiency. Thirteen underwent an upper endoscopy with multiple small bowel biopsies. Two patients had villous atrophy and slightly increased intraepithelial lymphocytes (Marsh 3a), which could make the diagnosis of celiac disease likely. These two patients had high titers of anti-gliadin antibody immunoglobulin A above 70 Units.* ***Conclusions:*** *An isolated positive anti-gliadin antibody immunoglobulin A result in the absence of positive tissue transglutaminase and endomysial antibodies immunoglobulin A should raise the suspicion of the diagnosis of celiac disease. This could be a non-specific phenomenon that could be found in other gastrointestinal conditions, latent celiac disease, or gluten hypersensitivity. A longitudinal clinical follow-up is recommended in these children to confirm the diagnosis.*

Key words: Isolated positive anti-gliadin immunoglobin A antibody, negative tissue transglutaminase and endomysial antibodies, celiac disease, Marsh classification, gastrointestinal symptoms, gluten hypersensitivity

Gastrointestinal semptomları olan çocuklarda izole anti-gliadin immunglobulin-A antikor pozitifliği

Amaç: *Çölyak hastalığı taramasında, anti-gliadin immunglobulin G ve A antikorlarının kullanımı, doku transglutaminaz ve endomisyal antikorların daha yüksek olan duyarlılık ve özgüllüğü nedeniyle giderek azalmaktadır. Kanıtlanmış ve muhtemel çölyak hastalığı olgularında, yüksek anti-gliadin immunglobulin A değerleri daha ciddi mukozal hasarla ilişkilidir. Çalışmanın amacı, anti-gliadin immunglobulin A'nın çocuklarda çölyak hastalığı tanısı konmasında klinik önemi olup olmadığını tespit etmektir.* ***Gereç ve Yöntem:*** *Polikliniğe kronik kusma, karın ağrısı, ishal veya kabızlık hikayesiyle başvuran çocuklar, çölyak hastalığı açısından değerlendirildi. Serum doku transglutaminaz ve anti-gliadin immunglobulin A düzeyleri, ELİSA testiyle, endomisyal antikor immunoglobulin A ise indirek immunofloresans yöntemiyle tespit edildi. Anti-gliadin immunglobulin A sonucu pozitif olan çocukların çoğuna ileri tetkik olarak proksimal gastrointestinal biyopsi yapıldı.* ***Bulgular:*** *Onaltı çocukta, izole anti-gliadin immunglobulin A pozitifliği (negatif doku transglutaminaz ve endomisyal antikor immunoglobulin A) saptandı. Sekiz tanesi erkekti (ortalama yaş, 9.7). Hiçbirisinde immunoglobulin A yetmezliği yoktu. Onüç tanesinden, üst endoskopi ile çok sayıda ince barsak biyopsisi alındı. İki hastada villöz atrofi ve intraepitelial lenfosit sayısında hafif artış (Marsh 3a) ile muhtemel çölyak hastalığı tanısı kondu. Bu iki hastada anti-gliadin immunglobulin A titresi 70 ünitenin üzerindeydi.* ***Sonuç:*** *Doku transglutaminaz ve endomisyal antikor immunoglobulin A pozitifliği olmadığında, izole anti-gliadin immunglobulin A pozitifliği olması durumunda, çölyak hastalığı tanısı ihtimali düşünülmelidir. Bu latent çölyak hastalığı veya gluten hipersenstivitesi gibi durumlarda olabilecek non-spesifik bir durum olabilir. Bu çocuklarda uzun süreli klinik takip, tanının kesinleştirilmesi için önerilmektedir.*

Anahtar kelimeler: İzole anti-gliadin immunglobulin A antikoru, negatif doku transglutaminaz ve endomisyal antikorları, çölyak hastalığı; Marsh sınıflaması; gastrointestinal semptomlar, gluten hipersenstivitesi

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INTRODUCTION

Celiac disease (CD) may present with both gastrointestinal and non-gastrointestinal manifestations, including symptoms such as chronic abdominal pain, constipation, vomiting, short stature, or unexplained anemia (1). The diagnosis of CD requires evaluation of clinical symptoms, serologic studies, and intestinal biopsies (1). One of the challenges in the diagnosis of CD is utilizing the serologic studies available as a noninvasive screening method to determine which patients require confirmatory testing with small bowel biopsy as a gold standard investigation. The use of anti-gliadin antibodies immunoglobulin G and A (AGA IgG and IgA) for CD screening has decreased due to the higher specificity and sensitivity of tissue transglutaminase and endomysial antibodies (TTG and EMA IgA) (1). Our aim was to determine if AGA IgA has any clinical importance in diagnosing CD in children who have chronic gastrointestinal symptoms.

MATERIALS AND METHODS

Children with a chronic history of vomiting, diarrhea, abdominal pain, and other gastrointestinal symptoms were evaluated for CD in the outpatient clinic of West Virginia University Hospitals. TTG IgA and AGA IgG and IgA in serum were determined by ELISA test, and EMA IgA was determined by indirect immunofluorescence. Some children with isolated positive AGA IgA were further evaluated by proximal gastrointestinal biopsies. The number of small bowel biopsies was a minimum of four specimens from both the proximal and distal duodenum and/or jejunum. The classic pathology changes of CD in the small bowel are categorized by the Marsh classification (1) as Marsh type 0, or pre-infiltrative stage with normal mucosa; type 1, or increased number of intraepithelial lymphocytes, usually exceeding 20 per 100 enterocytes; type 2, or proliferation of the crypts of Lieberkühn; type 3, or partial (3a), subtotal (3b), or complete/total villous atrophy (3c); and type 4, or hypoplasia of the small bowel architecture (total villous atrophy with crypt hyperplasia). Although HLA DQ 2 and 8 testing is not a routine test, it is performed to evaluate a tendency harboring CD in suspected CD cases (1).

Outpatient records of these children with a chronic history of gastrointestinal symptoms were reviewed after internal review boards at West Virginia University School of Medicine approved the study.

RESULTS

Sixteen children (8 males, 8 females; mean age: 9.7 years) had positive AGA IgA but negative TTG and EMA IgA antibodies. Vomiting, chronic intermittent diarrhea, recurrent abdominal pain, and chronic constipation were presenting symptoms in 6, 8, 13, and 4 of our patients, respectively. None had a history of steatorrhea or fat malabsorption. None had IgA deficiency. Only Patient 5 had a family history of CD. Thirteen underwent an upper endoscopy with multiple small bowel biopsies. Two patients had villous atrophy (Marsh 3a) consistent with a diagnosis of CD. Serum AGA IgA titers were significantly higher in patients who had Marsh histologic type 3a (Patients 1 and 2) as compared to those with type 0 (Patients 3-16) with the cut-off above 70 Units. Mean AGA IgA titers in type 3a vs. type 0 were 94.5 Units (70.0–119.0) vs. 31.5 Units (22.0–60.0) (Mann-Whitney test = 0.00, p=0.017). HLA DQ 2 and 8 testing was performed in only 6 patients due to insurance limitation, and 3 were positive (Table 1).

Table 1. Demographic data of patients with isolated positive AGA IGA

Characteristics	n=16
Age (yrs), median (range)	11.3 (3.0-17.0)
Gender, male/female	8/8
Marsh histologic subtype	Type 0-14
	Type 3a-2
Symptom frequency	
Vomiting (%)	37.5
Diarrhea (%)	50.0
Abdominal pain (%)	81.3
Constipation (%)	25.0
Serum AGA IgA level (U/L), median (range)	34.5 (22.0-119.0)
Serum AGA IgG level (U/L), median (range)	27.5 (2.0-97.0)

AGA: Anti-gliadin antibody. IgA: Immunoglobulin A. IgG: Immunoglobulin G.

Clinical data in all patients are summarized in Table 2. The relationship between AGA IgG, IgA antibodies and Marsh classification of intestinal biopsies are depicted in Figures 1 and 2. Patient 1 had Down syndrome and a history of duodenal atresia repair, bacterial overgrowth, and osteoporosis. Although the patient had a clinical history of CD, his HLA DQ 2 and 8 testing was negative. The parents decided not to place him on a gluten-free diet since he responded well to antibiotic therapy. All gastrointestinal symptoms of Patient 2 resolved and he was able to tolerate lactose after a gluten-free diet. The parents decided not to have a repeat endoscopy to confirm normal small intestinal pathology.

DISCUSSION

Awareness of the CD diagnosis has increased along with the incidence of the disease since the development of CD serology. The accuracy of the serologic testing improves the screening and management outcome. Currently, most of the guidelines for the diagnosis of CD, including recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition, advise primary care physicians and gastroenterologists to start the screening with TTG IgA ELISA kits and to a lesser extent EMA IgA antibody (1). Even with newer TTG ELISA assays, the sensitivity and specificity are the low to mid 90% range.

Table 2. Clinical manifestations of patients with isolated positive AGA IgA and/or IgG

Patient	Age (years), gender	Vomiting, diarrhea, abdominal pain, constipation	AGA IgG/IgA (Units)	Mucosal histology (Marsh)	HLA DQ2/8	Esophageal, gastric histology	Small intestinal histology	Final diagnoses
1	12, M	+, +, +, -	97,119	3a	-, -	Normal	Partial villous atrophy, slight increase in IEL	Bacterial overgrowth, probable CD, Down syndrome, hypothyroidism
2	3, M	+, +, +,-	73,70	3a	+, -	Mild reflux	Partial villous atrophy, slight increase in IEL	Lactase deficiency, probable CD
3	3, M	+, -, -,-	91,30	0	Not done	Lymphocytic esophagitis	Increased lymphocytes and plasma cells in duodenum, increased lymphoid aggregate	Food allergy, congenital adrenal hyperplasia
4	3, M	+, -, +,-	82,33	0	-, +	Normal	Lymphoid aggregate in duodenum and rectum	GERD, asthma, congenital glaucoma
5	15.5, M	-, +, +,-	54,47	0	Not done	Chronic non-atrophic gastritis	Normal	IBS
6	7, F	-, +, +,-	34,36	0	Not done	Chronic active gastritis with *H. pylori*	Normal	*H. pylori* gastritis
7	10.5, F	-, -, +,-	45,24	0	-, -	Eosinophils in antrum, gastritis, gastric erosion	Eosinophils in duodenum	Gastroparesis, hiatal hernia
8	3.5, F	+, +, +,-	3,50	0	Not done	Not done	Not done	GERD
9	13, F	+, +, +,-	2	0	+, -	Not done	Not done	Cystic fibrosis, cirrhosis, GERD
10	16, F	-, -, +,-	58	0	Not done	Chronic inactive gastritis	Lymphoid aggregate in ascending, transverse, descending colon and rectum	Gastritis, hypercholesterolemia, somatoform disorder
11	17, F	-, -, +,+	3	0	-, -	Not done	Not done	Chronic constipation
12	8, M	-, -, +,+	3	0	Not done	Normal	Normal	Chronic constipation, encopresis
13	12, M	-, -, -,+	21	0	Not done	Chronic active gastritis	Normal	Chronic constipation encopresis, gastritis
14	6, F	-, -, +,+	2	0	Not done	Normal	Normal	Chronic constipation, encopresis
15	13, F	-, +, -,-	2	0	Not done	Normal	Normal	GERD, IBS
16	13.5, M	-, +, +,-	2	0	Not done	Normal	Normal	IBS

M: Male. F: Female. +: Present. -: Absent. GERD: Gastroesophageal reflux disease. IBS: Irritable bowel syndrome. IEL: Intraepithelial lymphocyte.

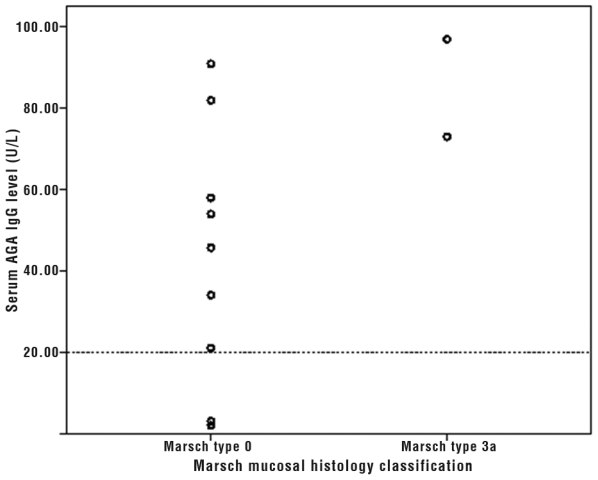

Figure 1. Relationship between AGA IgG antibodies and Marsch classification of intestinal biopsies.

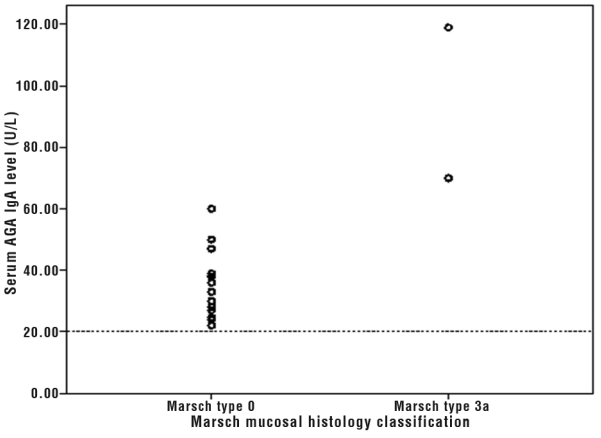

Figure 2. Relationship between AGA IgA antibodies and Marsch classification of intestinal biopsies.

Negative TTG and EMA IgA results have been reported in young celiac patients (unpublished data from Tonutti et al. presented at the 5th International Congress on Autoimmunity-Sorrento, 2006). Serum AGA IgA titers were significantly higher in our patients who had Marsh histologic type 3a (Patients 1 and 2) as compared to those with type 0 (Patients 3-16). Although these two patients could have CD, the confidence level for the diagnosis was not high since EMA IgA is expected to be positive given their intestinal histology (Marsh 3a). The lack of a positive HLA DQ 2 and 8 result in Patient 1 makes the likelihood of a CD diagnosis extremely low; small bowel bacterial overgrowth could have caused the villous atrophy, increased intraepithelial lymphocytes, and mucosal inflammation in the small intestine (1). Serum AGA IgG titers were significant in both groups with Marsh histologic type 0 and 3a. This phenomenon has been reported in the era prior to the use of the more specific and sensitive antibody testing (EMA and TTG IgA), but the statistical power is still very low in this study (2). Juto et al. (2) reported that elevated serum level of AGA IgA was strongly correlated with villous atrophy and was seen in infants younger than 3 years of age. The sole appearance of conventional AGA IgA in most children with gastrointestinal symptoms may be interpreted as a non-specific immunomodulation phenomenon (3).

Despite the higher sensitivity and specificity of TTG and EMA IgA and the NASPGHAN recommendation to use TTG IgA as the sole CD screening test, AGA IgA testing has been included in a part of CD serology as a screening panel by primary pediatricians and gastroenterologists (1). The anti-gliadin IgA and IgG tests are based on a sandwich enzyme immunoassay (ELISA) utilizing a horseradish peroxidase conjugated detection antibody. The sensitivity and specificity of anti-gliadin immunoglobulin testing has been shown to be inferior to that of TTG and EMA testing except for being slightly higher when using in treated celiac patients (1). Higher titers of AGA IgA may have more clinical significance in children with high index of suspicion for CD (4). In a study by Bonamico et al. (5), it was suggested that even low titers of AGA IgA in non-celiac patients may be an indication of increased intestinal permeability to macromolecules. In those patients with probable or proven CD, higher levels of AGA IgA of more than 70 Units/ml exhibit more severe mucosal damage in the small intestine (3). AGA IgA appeared to be a good indicator of the immune reaction in the small intestine triggered by gluten (2,6,7). The extent of elevated AGA IgA in combination with a clinical history may be valuable in discerning which patients should undergo upper endoscopy for biopsies. Therefore, it is reasonable to infer that its levels correlate to some extent with mucosal injury. In this study, we cannot conclude that having positive and/or high titers of AGA IgA is sufficient for the diagnosis of CD.

Recently, the identification of specific B-cell epitopes on the gliadin molecule and the use of specific, synthetic, and conformationally intact B cells epitopes increasingly improved the development of the new test, Deamidated Gliadin Peptide (DGP) (8). The selective deamidation of gliadin peptide is

a process in which amino acid glutamine is converted to glutamic acid in the small intestine after gluten ingestion by the enzyme TTG (8), and it immensely increases the gliadin peptide antigenicity and the sensitivity of DGP antibody assays or DP-AGA IgA and IgG (9). Kaukinen (4) found that all antibody levels declined in line with mucosal recovery with a gluten-free diet. The DP-AGA antibody test interestingly was positive in six of the nine cases with small bowel mucosal damage persisting on a gluten-free diet, whereas positive TTG IgA was detected in only two cases and positive EMA IgA in none. The sensitivity and specificity are 91% and 98%, respectively (4). Unfortunately, none of our patients had residual serum stored in the laboratory to confirm the diagnosis with this test, but the patients were informed to follow up with their primary physicians for further evaluation if clinical symptoms of CD manifested later, including repeating all CD serology tests and upper endoscopy with small bowel biopsies.

In conclusion, higher levels of AGA IgA may have more clinical significance in children with a high index of suspicion for CD. The authors believe that some of these patients who have gastrointestinal symptoms with isolated AGA IgA should be followed, since the negative results of more specific antibodies like TTG IgA or EMA IgA could be falsely negative. Further immunopathogenic study from the small bowel biopsy to detect an early phase of CD might elucidate this phenomenon in this group of patients. The use of DP-AGA IgA in conjunction with TTG and EMA IgA could provide a more sensitive screening test for further evaluation for the diagnosis of CD.

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