Diagnostic value of antigliadin antibodies in gluten sensitive enteropathy

Antigliadin antikorlarının gluten enteropatisi tanısındaki yeri

Hülya Çetinkaya M.D.[1], Buket Altıntaş M.D.[2], Murat Palabıyıkoğlu M.D.[1], Arzu Ensari M.D.[3], Kadir Bahar M.D.[1], Nazım ErgünM.D.[1], Kazım Soylu M.D., Yasemin Yavuz M.D., Yıldır Atakurt M.D.[4], Ali Özden M.D.[1], Nurten Girgin M.D.[2], Özden Uzunalimoğlu M.D.[1]

Departments of Gastroenterology[1], Pediatric Gastroenterology[2], Pathology[3] and Medical Statistics[4], Ankara University, Ankara,

ÖZET: *Bu çalışmada sağlıklı birey, aktif ve remisyonda Gluten Sensitif Enteropati (SE), İnflamatuar Barsak Hastalığı (IBD) ve Kronik İdiopatik Diare (CID)'li hastalardan oluşan 307 kişide serum IgA ve IgG antigliadin antikor tayini yapılarak, bu antikorların gluten enteropatisi tanısındaki yeri ortaya konmaya çalışıldı. Antigliadin antikorlar için en yüksek titreler aktif Gluten Sensitif Enteropati grubunda saptandı. IgA grubu antigliadin antikorlar özellikle GSE için sensitif bulundu. Spesifitesi hemen tüm gruplarda eşitti. IgG grubu antikorlar, sağlıklı grupta pozitiflik göstermesi, düşük sensitivite ve pozitif belirleyicilik değerleri nedeniyle, özellikle GSE olgularında değerli bulunmamıştır.*

Anahtar Kelimeler : **Gluten sensitif enteropati, IgA ve IgG antigliadin antikorlar**

SUMMARY: *The presence of IgA and IgG antigliadin antibodies were investiated in a group of 307 patients with active or quicent of gluten sensitive enteropathy (GSE), inflammatory bowel disease (IBD), chronic idiopathic diarrhoea (CID) and healthy volunteers. The aim was to explore the diagnostic value of IgA and IgG antigliadin antibodies in GSE. The highest antibody titers were found in active GSE. IgA antibody was a sensitive marker as well for the same disease while its specificity did not differ among the groups. On the other hand, IgG antibody was not found to be an oustanding diagnostic parameter because of its occurrence also in the healthy population with relatively low sensitivity and a low positive predictive value.*

Key words: **Gluten sensitive enteropathy, IgA and IgG antigliadin antibodies**

INTRODUCTION

Gluten sensitive enteropathy (GSE) occurs in individuals susceptible to gliadin with a permanent intolerance and is pathologically characterized by flattening of villi and crypt hypertrophy. As features of both humoral and cellular reactions had been determined in sera and intestinal mucosa of the patients, an aetiopathogenetic role of immune reaction against gliadin was suggested. High titer antibodies to gliadin were found for the first time in the late 1950's and at the beginning of the 1960's and supported the hyphothesis concerning pathogenetic events (1). However, it was later understood that antigliadin antibody was not specific for GSE patients and that it could be positive also in healthy individuals besides other gastrointestinal disorders. This fact prompted search aimed at different antibodies for screening and diagnostic procedures (2).

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In this study, both IgA and IgG antigliadin antibodies were detected in patients with GSE (with or without dietary gluten exclusion), IBD, CID and in healthy volunteers. They were compared and specificity, sensitivity, positive and negative predictive values were evaluated for each group in order to ascertain their diagnostic validity.

MATERIAL and METHOD

Study population

Group I:

The first group was composed of 12 GSE patients (9 women, 3 men; mean age: 37 ± 13.5 years; range: 25-52 years) without dietary gluten exclusion.

Group II:

This group consisted 27 GSE patients (17 women, 10 men; mean age: 32.8 ± 8.8 years; range: 16-52 years) with gluten-free diet. Patients had been consuming a diet without gluten for 25.8 (between 3-120) months on the average.

Group III:

a) This subgroup included patients with established IBD (5 with Crohn's disease and 41 with ulcerative colitis; 19 women, 27 men; mean age: 43.4 ± 15.2; range: 14-76 years). The diagnosis was confirmed using laboratory, endoscopical and histological techniques.

b) The second subgroup had 108 CID patients (36 women, 72 men; mean age: 36.1 ± 14.6; range: 16-80 years).

Control group: The group included 114 healthy individuals selected from medical staff and students and children with informed consent of their parents (mean age: 30.5; range: 5-74 years).

Material

Histopathological diagnosis of GSE was made using villous effacement and/or shortening, crypt hyperplasia/hypertrophia, a diffuse increase in the number of intra-epithelial lymphocytes both in surface and in crypt epithelium, a lymphoplasmocytic inflammatory cell infiltration of lamina propria and increase in eosinophils of lamina propria as histological criteria.

The diagnosis was also supported by clinical response to exclusion of gluten from diet. Venous blood samples were taken and then sera were stored at - 20 C until the evaluation of antigliadin antibody levels were completed.

IgA and IgG antibody quantities were calculated by commercially available ELISA (from Euroimmun). The "cut-off" values of this method (for individuals over 2 - years - old) are given below in relative-unit/ml(RU/ml):

Negative	Weak-positive	Positive
<25 RU/ml	25-50 RU/ml	>50 RU/ml

Statistical analysis

A paired t-test was used for mean and ± standard deviation. Significance value for statistical analysis was accepted as 0.01. Sensitivity, specificity, positive and negative predictive values were calculated according to the formulas given below:

$$\text{Sensitivity (\%)} : \frac{\text{Patients who have the disease in whom the test result is positive}}{\text{All patients with disease}} \times 100$$

$$\text{Specificity (\%)} : \frac{\text{Healthy subjects in whom the test result is negative}}{\text{All patients without disease}} \times 100$$

$$\text{Positive predictive value (PPV)} : \frac{\text{Patients who give positive results and have the disease}}{\text{All patients in whom test results is positive}} \times 100$$

$$\text{Negative predictive value (NPV)} : \frac{\text{Patients who give negative results and don't have the disease}}{\text{All patients in whom test results is positive}} \times 100$$

RESULTS

In the control group, IgA antigliadin antibody levels were estimated between 1-114 RU/ml (mean: 16.9 ± 9 RU/ml) and IgG values between 11-162 RU/ml (mean: 44.5 ± 31.2 RU/ml) (Table - I). According to the cut-off values, including weak positivity, IgA antigliadin antibody was positive in 20 and IgG antibody in 81 individuals. IgA and IgG antibodies were both positive in 16 individuals (Table-II).

Table 1. *IgA and IgG antigliadin antibody levels in control group.*

IgG (RU/ml)		AGA IgA (RU/ml)	AGA
AGE GR	n	Med±SD	Med±SD
05 - 14	15	15.2 ± 8.8	62.6 ± 35.0
15 - 24	37	15.0 ± 9.3	51.7 ± 38.6
25-34	18	15.9 ± 6.2	3.9 ± 20.0
35-44	19	18.1 ± 7.7	37.5 ± 22.3
45 - 54	14	21.6 ± 13.3	39.6 ± 25.8
55 - 74	11	19.6 ± 5.6	31.5 ± 15.8
TOTAL	114	16.9 ± 9.0	44.5 ± 31.2

In group - I of GSE patients (12 patients without gluten-free diet), IgA antigliadin antibody was positive in 11 (between 20-188 RU/ml; mean: 93.3 ± 48.8 RU/ml) and IgG antibody in all the patients (between 60-150 RU/ml; mean: 105.08 ± 29.2 RU/ml). 11 patients had positive values of both (Table-II).

In group-II of GSE patients (27 patients with gluten-free diet), IgA antigliadin antibody was positive in 16 (between 11-67 RU/ml; mean: 33.5 ± 16.7 RU/ml) and IgG antibody in 22 patients (between

Table 2. *IgA and IgG antigliadin antibody levels in GSE patients with and without gluten exclusion, IBD, CID and in control group.*

	AGA IgA (RU/ml				AGA IgG (RU/ml)			
Group	Positive (n)	Negative (n)	Range	Med ± SD	Positive (n)	Negative (n)	Range	Ort ± SD
Control	20	94	1-114	16.9 ± 9.0	81	33	11 - 116	44.5 ± 31.2
GSE (Non-GF)	11	1	20 - 118	92.3 ± 44.8	12	-	60 - 150	105.08 ± 29.2
GF - GSE	16	11	11 - 67	33.5 ± 16.7	22	5	12 - 128	54.8 ± 30.9
IBD	18	28	11 - 64	24.9 ± 14.3	38	8	13 - 121	45.1± 28.6
CID	50	58	9 - 188	56.2 ± 37	72	36	11 - 175	90 ± 13.8

12-128 RU/ml; mean: 54.8 ± 30.9 RU/ml). 14 patients had positive values of both (Table - II).

Patients with IBD (subgroup III-a) had IgA antigliadin antibody measures between 11-64 RU/ml (mean: 24.9 ± 14.3 RU/ml) with a positivity in 18 patients whereas IgG antibody measures in the same group occurred between 13-121 RU/ml (mean: 45.1 ± 28.6 RU/ml) with a positivity in 38 patients. IgA and G counts were both positive in 18 cases (Table-II).

In the other subgroup (III-b), patients with CID had IgA antibody counts between 9-188 RU/ml (mean: 56.2 ± 37 RU/ml) with 50 positive individuals. IgG measures were between 11-175 RU/ml (mean: 90 ± 13.8 RU/ml) with 72 positive patients. They were both positive in 41 cases.

IgA antigliadin antibody mean vaules of the control group was less than all the other groups (I, II, III-a and III-b) and the difference was significant ($p<0.001$).

Mean titers of IgG antibody of the control group showed significant difference with group-I (patients without gluten-free diet) ($p<0.001$), but the difference wasn't significant in comparison to other groups ($p>0.01$).

Table 3. *Calculations of sensitivity / specifity and predictive values in intestinal diseases.*

Group	AGA	Sensitivity	Specificity	PBD	NBD
GSE	IgA	% 91	% 94	% 79	% 98
(Non-GF)	IgG	% 25	% 1	% 1	% 85
GF-GSE	IgA	% 48	% 94	% 72	% 85
	IgG	% 8	% 7	% 50	% 79
IBD	IgA	% 19	% 96	% 69	% 72
	IgG	% 0	% 96	% 0	% 67
CID	IgA	% 27	% 96	% 88	% 55
	IgG	% 5	% 96	% 55	% 48

When mean IgA and IgG antigliadin antibody titers were compared within patient groups, the values in group-I (GSE patients without gluten-free diet) were higher than group-II and the difference was significant for both antibody type ($p<0.001$ and $p<0.01$ respectively).

Group III-b (with CID) mean values of both and IgG antibodies were significantly lower than group I ($p<0.01$), but were higher when compared with group II ($p<0.01$ and $p<0.001$ respectively).

IBD group had mean IgA and IgG antibody measures that showed no significant difference with group II ($p>0.01$). However, these values remained low when compared with group I and the difference was significant ($p<0.01$).

Comparison of two subgroups in group III revealed higher titers of both IgA and IgG antibodies in CID subgroup and the difference was significant ($p<0.01$).

For all patient groups, sensitivity, specificity, positive predictive and negative predictive values of IgA and IgG antigliadin antibodies were evaluated. Those were as follows (respectively): Group I: IgA: 91%, 94%, 79%, 98%, IgG: 25%, 1%, 1%, 85%; Group II: IgA: 48%, 94%, 72%, IgG: 8%, 7%, 50%, 79%; IBD group (III-a): IgA: 19%, 96%, 69%, 72%; IgG: 0%, 96%, 0%, 67%; CID group (III-b): IgA: 27%, 96%, 88%, 55%; IgG: 5%, 96%, 55%, 48% (Table III).

DISCUSSION

Antigliadin antibody titers, as most of the anti-nutrient antibodies, may be detected as weak-positive or positive in healthy individuals. It's known that some of these might be yet undiagnosed GSE cases (3). However, the cause of high values in healthy population is not fully understood. Trials conducted to ascertain the association

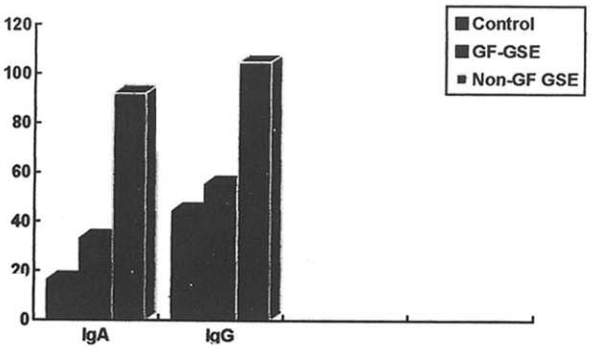

Figure 1. *IgA and IgG antigliadin levels in GSE patients with and without gluten exclusion and in control group*

GF GSE: Gluten - free gluten sensitive enteropathy
Non - GF GSE: Non-gluten free sensitive enteropathy

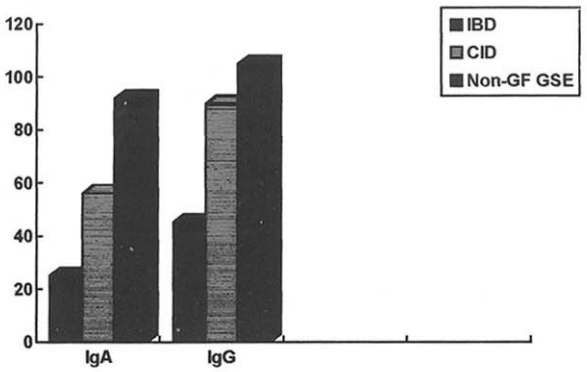

Figure 2. *IgA and IgG antigliadin antibody levels in patients with CID, IBD and in Non-GF GSE.*

IBD: Inflammatory bowel disease.
CID: Chronic idiopathic diarrhea.
Non-GF GSE: Gluten sensitive enteropathy witout gluten exclusion.

of HLA types in GSE with healthy volunteers having high antigliadin antibody levels revealed no clues for such a relationship (4). In our trial, mean IgA antibody measures of healthy controls were lower than "cut-off" levels while IgG values were positive showing no significant difference with other patient groups except GSE group without gluten-free diet (Graph I). This findings is in parallel with the current information pointing out that IgA antibody is more specific for GSE (2).

Recent studies suggest that serum antigliadin antibodies detected by ELISA method could be a diagnostic factor in evaluating GSE (5-7). GSE patients without gluten-free diet have high counts of IgA and IgG antigliadin antibodies. In patients with gluten-free diet (who achieve remission), IgA values decrease to similar measures with the controls while IgG values remain steadily high between levels of GSE patients without gluten exclusion and the controls (8,9). In this study, the measures of both IgA and IgG antibodies were higher in GSE patients without gluten exclusion than GSE patients with exclusion ($p<0.001$ and $p<0.01$ respectively). Mean IgG antibody titers of GSE patients with gluten exclusion did not differ from those of the controls and this was in harmony with the current literature. It's known that antigliadin antibody levels may be increased in various gastrointestinal disorders other than GSE (such as post-infectious malabsorbtion, Crohn's disease, cow-milk protein sensitivity, etc.) (2). In these cases, it's generally informed that IgA antibody values are higher than IgG class demonstrating a stronger correlation with small intestine mucosal damage (6). However, IBD group as well as CID group of our trial revealed higher IgG measures. Mean IgA and IgG counts were significantly increased in CID group when compared with the IBD group (Graph 2). The high occurence rate of ulcerative colitis patients with intact small intestine in IBD group might be a satisfactory explanation for this significance. This result supports the notion that antigliadin antibody titers exhibit direct relationship with small intestine mucosal damage. On the other hand, similar antibody values both in IBD and GSE patients with remission also add more to support this notion (Graph 2).

GSE group without gluten exclusion had the highest mean IgA and IgG antibody values among all and the difference was significant. This coincides with recent knowledge that active GSE exhibits very high antigliadin antibody calculations (10). After antibody estimations had been taken place in clinical practice, their sensitivity and specificity were detected and sensitivity was determined as higher. When questioned separately, IgA class antibody was more sensitive (82 - 100% (2,11,14).

IgA sensitivity was the highest in GSE group without gluten exclusion (91 %). Parallel with the recent data, IgG class antibody had the lowest sensitivity in all of the trial groups. The specificity of IgA was above 90 % in all groups, also being in harmony with the literature, especially for GSE group. Apart from GSE group, specificity of IgG antibody was 96 % and was quite low in GSE patient group both with or without gluten exclusion. That result suggested IgG class antibody had no specifity for GSE cases.

IgA antibody had higher positive and negative predictive values than IgG when all patient groups were concerned. The highest positive predictive value (PPV) for IgA was determined in CID group

(88 %) and the highest negative predictive value (NPV) was observed in GSE group (98 %) without gluten exclusion. In active GSE (group I), PPV for IgA antibody was higher than accepted while NPV was in similar range. PPV for IgG antibody was quite low whereas NPV showed harmony with current literature (14).

We conclude that antigliadin antibodies could have high titers in diseases thought to have small intestine mucosal damage. However, the highest values are detected in active GSE (patients without dietary gluten exclusion). In this study, IgA antigliadin antibody has been determined sensitive particularly for GSE cases. Its specificity is nearly the same in all groups. Because of its occurence also in healthy population with relatively low sensitivity and PPV, IgG antigliadin antibody is not accepted to be a valuable diagnostic parameter especially for GSE. In our opinion, patients who have positive antigliadin antibodies should be detected also for antiendomysial antibody, thought to be more sensitive and specific (12-14), to support the diagnosis and besides should undergo jejunal biopsy to highlight possible pathologic conditions.

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