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Comparison of serum antigliadin and antiendomysium antibodies in patients with Familial Mediterranean Fever

Ailesel Akdeniz Ateşi hastalarında serum antigliadin ve antiendomisiyum antikorlarının karşılaştırılması

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SUMMARY

This study was conducted to investigate the prevalence and clinical relevance of antiendomysium (EmA) and antigliadin (AGA) antibodies at the onset of Familial Mediterranean Fever (FMF) patients by serological methods. The high levels of AGA and EmA have been reported in patients with severe abdominal pain. Although it has been pointed out that coeliac disease (CD) has been shown to be associated with many different diseases, the relationship between the CD and FMF, in which main sign is abdominal pain, has not been investigated so far. A total of 25 children (ages between 5 to 17 years) were included in this study. Fifteen of them were FMF patients having abdominal cramps and discomfort, ten were healthy controls. AGA IgA and IgG antibodies were detected by a commercial ELISA (Euroimmun), EmA IgA and AGA IgA antibodies were determined by indirect immunofluorescence test (IFA, Euroimmun). Six (40%) FMF patients were found positive for AGA IgG, two (13.3%) were found positive for AGA IgA by ELISA, four (26.6%) were positive for EmA IgA and two (13.3%) were positive for AGA IgA antibodies by IFA. Statistical analysis revealed that there was statistical difference (p= 0.05, Fisher's chi-square test) for AGA IgG antibodies by ELISA between FMF patients and controls. There was no statistical difference for AGA IgA by ELISA, AGA IgA and EmA IgA by IFA,. Those found to be AGA IgG and IgA positive and/or EmA IgA positive by both methods could not be further investigated with the intestinal biopsy. In order to determine the accuracy of AGA and EmA antibodies for the diagnosis of CD in FMF children with digestive symptoms, it is necessary to search for these antibodies among larger FMF patient group.

Keywords : *Familial Mediterranean fever, coeliac disease, diagnosis, anti-gliadin antibodies, anti-endomysium antibodies, indirect immunofluorescence, enzyme-linked immunosorbent assay.*

ÖZET

Bu çalışma ailesel akdeniz ateşi (FMF) hastalarında antiendomisyum (EmA) ve antigliadin (AGA) antikorlarının prevalansının ve klinikle ilişkisinin serolojik yöntemlerle araştırılması amacıyla gerçekleştirilmiştir. Karın ağrısı olan hastalarda AGA ve EmA 'nın düzeylerinin yüksek olduğu bildirilmiştir. Çölyak hastalığının (CD) pek çok farklı hastalıkla ilişkili olduğuna dikkat çekilmiş olmakla birlikte en önemli belirtinin karın ağrısı olduğu CD ve FMF arasındaki ilişki yeterince araştırılmamıştır. Bu çalışma kapsamına 25 çocuk (% - 17 yaş arası) alınmıştır. Bunlardan 15 ' i FMF hastası olup karın krampları ve discomfort şikayetleri vardır; 10 çocuk ise sağlıklı kontrol grubunu oluşturmuştur. AGA İgA ve İgG antikorları ELISA (Euroimmun) , EmA İgA ve AGA İgA antikorları indirekt immunfloresan testi (IFA , Euroimmun) uyarınca saptanmıştır. ELISA yöntemiyle FMF hastalarından altısı (% 40) AGA İgG , ikisi (% 13.3) AGA İgA açısından pozitif bulunmuştur ; dört hasta (% 26.6) EmA İgA ve iki hasta ((% 13.3) AGA İgA antikorları için IFA yöntemiyle pozitif olarak belirlenmiştir. FMF hastaları ve kontrol grup arasında ELISA yöntemiyle saptanan AGA İgG antikorları açısından saptanan fark istatistiksel olarak anlamlı (p= 0.05 , Fischer ki-kare testi) bulunmuştur.ELISA yöntemiyle belirlenen AGA İgA , IFA yöntemiyle AGA İgA ve EmA İgA anasındaki fark anlamlı bulunmamıştır. Her iki yöntemle de AGA İgG ve İgA pozitif ve/veya EmA İgA pozitif bulunanlar intestinal biyopsi ile daha ileri incelemeye tabi tutulamamışlardır. Sindirim sistemi semptomları bulunan çocuklarda CD ve FMF ' tanısı için AGA ve EmA antikorlarının öneminin belirlenmesi amacıyla bu antikorların daha çok sayıda FMF hastalarında araştırılması gereklidir.

INTRODUCTION

Antigliadin antibodies (AGA) mark coeliac disease (CD), but AGA are also encountered in dermatitis herpetiformis, diabetes mellitus, selective IgA deficiency, psoriasis, sickled cell anemia, hepatic disorders, juvenile rheumatoid arthritis, ulcerative colitis (1,2). Intolerance to gluten leads to damage to the mucous membranes of the small intestine and immune system, plays a major role in the development of CD (1,3). The determination of IgG and IgA serum antigliadin (AGA) and antiendomysium antibodies (EmA) remains one of the most widely used screening tests for CD (4,5). Most authors agree that AGA IgG antibody determinations are sensitive, but not pathognomonic and AGA IgA antibodies are more specific, but less sensitive (6). In many studies, IgA EmA have been shown to be a more reliable marker than AGA for the diagnosis of CD, because the sensitivity and specificity of the test are much higher except in children younger than two years of age (7,8).

Familial Mediterranean Fever (FMF) is an autosomal recessive recurrent episodic inflammatory disorder. The cause of FMF is unknown. Fever and inflammation are such prominent signs that frequent attempts have been made to implicate infectious agents and/or their products (1).

Although AGA and EmA are major antibodies present in CD and ulcerative colitis (UC) which progress with inflammation in the intestinal mucosa, we aimed whether AGA and EmA responses play a role or not, in the serosal surface inflammation of intestine in FMF.

MATERIAL AND METHODS

Eight male and seven female children diagnosed of FMF, aged between five to 17 years with the mean age (10.3 ± Standard Deviation SD), were included in this study. Ten children with no complains were healthy controls. FMF children on the colchicine treatment were all in remission. All patients fit the "Tel Hashomer" (9) criteria for definitive diagnosis of FMF. Blood was obtained by venipuncture for determination of IgG and IgA AGA and EmA.

Serum EmA were tested with indirect immunofluorescence (IFA) using tissue sections from intestine of monkey (Euroimmun, Germany) as substrate for IgA and AGA IgA by IFA test on a gliadin-coated surface, with a titer of 1:10 or greater taken as positive. Total serum IgA was measured in all patients to exclude deficiency as a cause of false-negative EmA. AGA IgG and IgA were also tested using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun, Germany). ELISA microplate wells were coated with gliadin purified from wheat gluten as an antigen for AGA IgG and IgA, with a positive result taken as greater than 50 -100 relative units (RU) per millilitre.

Patients with positive EmA test results were advised to undergo small intestinal biopsy for definitive diagnosis. Among this group of children, antinuclear antibody (ANA) were also determined by IFA (Zeus Scientific, Inc., U.S.A.) using HEp-2 cell line as an antigen substrate. Above mentioned ELISA and IFA assays for AGA IgG, IgA and EmA IgA determinations were run according to the standard procedures.

C reactive protein (CRP), C3/C4 levels and total immunoglobulins (Behring. Germany) were also determined at the serology-immunology laboratory of Microbiology and Clinical Microbiology Department.

Statistical analysis: Fisher's chi-square test was used for the serological results of AGA IgG, IgA by ELISA; EmA IgA and AGA IgA by IFA between FMF patients and controls.

RESULTS

A total of 25 patients (ages between 5 to 17 years) were included in this study and AGA IgG and IgA antibodies were investigated in their sera by ELISA and IFA methods. Fifteen of them were FMF patients having abdominal

cramps and discomfort and ten were healthy controls. AGA IgA and IgG antibodies were detected by a commercial ELISA test, EmA IgA and AGA IgA antibodies were determined by IFA test. Six (40%) FMF patients were found positive for AGA IgG, two (13.3%) were found positive for AGA IgA by ELISA, four (26.6%) were positive for EmA IgA and two (13.3%) were positive for AGA IgA antibodies by IFA, respectively (Table 1).

Table1. Distribution of AGA IgG, IgA ELISA and AGA IgA, EmA IgA IFA results among FMF and control group

	ELISA AGA IgG				ELISA AGA IgA				IFA AGA IgA				IFA EmA IgA			
	Positive		Negative		Positive		Negative		Positive		Negative		Positive		Negative	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
FMF n= 15	6	40	9	60	2	13.3	13	86.7	2	13.3	13	86.7	4	26.6	11	73.4
Control n= 10	0	0	10	100	0	0	10	100	0	0	10	100	0	0	10	100

We found AGA IgG, IgA (ELISA), EmA IgA and AGA IgA (IFA) positive in only one FMF patient (Table 2). Our results also showed great correlation between IFA AGA IgA and ELISA AGA IgA in all cases. Three out of four EmA IgA positive patients were not found positive by IFA AGA IgA and ELISA AGA IgA (Table 3).

Statistical analysis revealed that there was statistical difference (p=0.05) of the AGA IgG antibodies by ELISA between FMF patients and controls. There was no statistical difference for AGA IgA by ELISA, AGA IgA and EmA IgA by IFA, respectively.

ANA was also found positive in only one FMF children with centromeric pattern. CRP were negative, C3/C4 levels and total immunoglobulins were normal in FMF and control group. Because AGA and EmA positive FMF patients refused to have intestinal biopsy, histopathological investigation could not be performed.

Table 2. Results of serologic tests for FMF patients

Patient No:	ELISA AGA IgA	ELISA AGA IgG	IFAT EmA IgA	IFAT AGA IgA
1	Negative	Positive	Positive	Negative
2	Negative	Negative	Negative	Negative
3	Negative	Negative	Negative	Negative
4	Negative	Positive	Negative	Negative
5	Negative	Positive	Negative	Negative
6	Positive	Negative	Negative	Negative
7	Negative	Negative	Negative	Positive
8	Negative	Negative	Negative	Negative
9	Negative	Negative	Negative	Negative
10	Negative	Negative	Negative	Negative
11	Negative	Negative	Negative	Negative
12	Positive	Positive	Positive	Positive
13	Negative	Positive	Positive	Negative
14	Negative	Positive	Positive	Negative
15	Negative	Negative	Negative	Negative

Table 3. Comparison of AGA IgA, IgG ELISA and EmA IgA, AGA IgA IFA results of FMF group

		IFAT EmA IgA		IFAT AGA IgA	
		Positive	Negative	Positive	Negative
ELISA AGA IgA n=15	Positive (n=2)	1	1	2	0
	Negative (n=13)	3	10	0	13
ELISA AGA IgG n=15	Positive (n=6)	4	2	1	5
	Negative (n=9)	0	9	1	8

DISCUSSION

Familial Mediterranean fever is an inherited disorder of unknown aetiology, which usually begins in childhood and occurs primarily in certain populations in the Mediterranean area. It is characterised by short, self-limited, febrile episodes that may occur alone or with inflammation of serosal surfaces(1). Although it has been pointed out that CD has been shown to be associated with many different diseases (1,10), the

relationship between the CD and FMF has not been investigated so far. Antigliadin, antiendomysial, and antireticulin antibodies have been widely used in the diagnosis of CD(1,5). Measurement of AGA is considered a highly sensitive test for CD in children. Specificity, however, appears to vary due to the presence of AGA in other diseases (11,12). Antibodies against endomysium and gliadin are rarely detected in healthy individuals and in patients with other intestinal diseases (13,14). In many cases, the determination of antibodies against endomysium and gliadin can take the place of endoscopy and the analysis of biopsy material (15-17). The aim of our study was to examine the importance of AGA and EmA antibodies and whether their responses play a role or not in FMF patients.

In our study, AGA IgA and IgG antibodies were detected by a commercial ELISA; EmA IgA and AGA IgA antibodies were determined by IFA technique. According to statistical analysis it can be said that there is difference of the AGA IgG by ELISA between the FMF patient group and controls. However, the reason why there is no significant corelation determined with other important parameters like AGA IgA by ELISA, AGA IgA and EmA IgA by IFA may be explained that the patients were in clinically inactive period due to the colchicine treatment. It can be questioned that whether elevated AGA IgG responses is a consequence of the process of FMF or whether inflamation process due to gluten intolerance may cause FMF. The aim and the results of this study is far from giving a definite answer to this question. To clarify this, the whole cases should be classified according to their genotype, phenotype and clinical status including amyloidosis and also serum samples should be taken regularly before, during and after the activation of the disease. In our study, we were not able to do classification according to genotype nor we could determine amyloidosis. In addition to this, the presence of amyloidosis could not be shown by histopathologically. Consequently, we could get different results in amyloidosis cases because CD may have a progression with amyloidosis, too.

Kull et al. searched for the frequency of AGA and EmA in the sera of patients with UC. They found 17 of the 50 patients with UC (34%) were positive for IgA-or/and IgG type AGA. There was no correlation between the presence of AGA and the duration or extent of the disease, or disease activity. But they found 5 patients with both IgA and IgG types of AGA had extensive colitis. EmA were not detected in any of them (18). Reifen et al. investigated the relationship of prolactin levels between CD and FMF. Both groups were chosen because of their inflammatory nature. They found significant correlation between serum prolactin concentration and activity of serum EmA (19). The results from different laboratories are not always comparable, on account of changes in the technique and the different ways of expressing the results. In our unpublished data, we found that prevalence of AGA IgG, IgA by ELISA and EmA by IFA were positive 70%, 40% and 30%, respectively in a group of patient clinically suspected CD. Those found AGA IgG and IgA positive and/or EmA positive by both methods could not be further investigated with the intestinal biopsy.

Combined determinations of AGA, EmA, and antireticulin antibodies offer optimal sensitivity and specificity for the detection of CD. Tests based on the measurement of AGA and EmA antibodies have gained success as non-invasive screening tests; however, the ultimate diagnosis still is based on the finding of a severe histologic lesion of the jejunum (5,10).

So herein we have demonstrated that AGA IgA by ELISA is positive in a significant population of FMF patients even under the colchicine treatment. Although the number of cases included in this study is small for a strict conclusion, the results point out to a common mechanism between CD and FMF which are both inflammatory

lesions of the intestinal tract.

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