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Predictive Value of the Intestinal Free Fatty Acid Binding Protein in Celiac Disease

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ABSTRACT

Purpose: Non-invasive tests used in the screening and follow-up of celiac disease (CD) are currently below expectations because they show false negatives/positives and are not always correlated with duodenal histology. We aimed to investigate the predictive value of intestinal free fatty acid binding protein (FABP-I) which can easily be released into the circulation in the presence of enterocyte damage in a short time in CD.

Methods: This study included 59 patients with CD who were not on gluten free diet (GFD)(n=24) and who were on GFD(n=35) and 52 healthy controls. Demographic variables, complete blood count, ferritin, vitamin D, calcium, phosphorus, vitamin B12, prothrombin time, INR, and serum FABP-I levels were recorded for all groups.

Results: There was no difference between the groups in terms of complete blood count, ferritin, calcium, phosphorus, vitamin B12, prothrombin time and INR (all p>0.05). Mean serum FABP-I was determined as 499.2±33.3 ng/L for patients with CD who were not on GFD and as 487.7±48.0 ng/L for who were on GFD, and these values were significantly higher when compared to the healthy controls 432.2±63.8 ng/L(p<0.001). The sensitivity, specificity, positive predictive value, and negative predictive value of FABP-I for a cut-off value of 456.8 ng/L were 84.7%, 69.2%, 10.2% and 61.5%, respectively(AUC=0.785).

Conclusion: This study has shown that FABP-I can serve as a non-invasive predictive marker for CD diagnosis. Overlooked diagnosis in serology-negative patients and false serology positivity for reasons other than CD will be prevented with its use in clinical practice since FABP-I directly reflects intestinal damage.

Keywords: Celiac disease, enterocyte damage, FABP2, FABP-I, gluten-free diet, intestinal free fatty acid binding protein

İntestinal Serbest Yağ Aside Bağlayan Proteinin Çölyak Hastalığındaki Prediktif Değeri

ÖZET

Amaç: Çölyak hastalığının tarama ve takibinde kullanılan non invaziv testler, yalancı negatiflik/ pozitiflik göstermeleri ve duodenal histoloji ile her zaman korele olmamaları nedeniyle günümüzde beklentinin altında kalmaktadır. Son çalışmalar, çölyak hastalığındaki bu eksikliği tamamlamak için barsak epitelyal hasarını gösteren direk belirteçler üzerine yoğunlaşmaktadır. İntestinal serbest yağ asidi bağlayan protein (FABP-I); suda eriyen küçük bir protein olduğu için enterosit hasarını takiben kısa süre içinde dolaşıma salınır. Çalışmamızda FABP-I'nın çölyak hastalığındaki prediktif değerini araştırmayı amaçladık.

Yöntem: Çalışmaya glutensiz diyete (GDF) uyum göstermeyen (n=24) ve GFD'ye uyum gösteren (n=35) 59 çölyak hastası ve 52 sağlıklı kontrol dahil edildi. Tüm gruplarda demografik veriler ve tam kan sayımı, ferritin, vitamin D, kalsiyum, fosfor, vitamin B12, protrombin zamanı, INR ve serum FABP-I düzeyleri kaydedildi.

Bulgular: Tam kan sayımı, ferritin, kalsiyum, fosfor, vitamin B12, protrombin zamanı, INR düzeyleri açısından gruplar arasında farklılık görülmedi (tüm p>0.05). Ortalama serum FABP-I düzeyi GFD uyumsuz çölyak hastalarında 499.2±33.3 ng/L, GFD uyumlu çölyak hastalarında 487.7±48.0 ng/L saptandı ve bu sonuçlar sağlıklı kontrole göre (432.2±63.8 ng/L) anlamlı derecede yüksek bulundu (p<0.001). Serum FABP-I için cut-off değeri 456.8 ng/L alındığında, FABP-I'nin çölyak hastalığı tanısında duyarlılığı, özgüllüğü, pozitif ve negatif prediktif değeri sırasıyla %84.7, %69.2, %10.2 ve %61.5 bulundu (AUC=0.785).

Sonuç: Serum FABP-I, çölyak hastalığı tanısında direkt barsak hasarını gösteren non invaziv prediktif bir belirteçtir. FABP-I'nın klinik kullanıma girmesiyle seronegatif hastalarda tanının gözden kaçması ve çölyak hastalığı dışındaki nedenlere bağlı serolojinin yanlış pozitif olduğu durumlarda tanıdaki zorluklar önlenmiş olacaktır.

Anahtar Kelimeler: Çölyak hastalığı, enterosit hasarı, FABP2, FABP-I, glutensiz diyet, intestinal serbest yağ asidi bağlayan protein

Celiac disease (CD) is an auto-immune, familial disease that develops in genetically predisposed individuals due to intolerance to gluten found in grains and grain products, which typically progresses with malabsorption, creates characteristic lesions in the small intestine and shows clinical improvement when a gluten-free diet (GFD) is adopted. In CD, the primary mechanism responsible for the loss of villous architecture, which is tasked with absorption, is excess enterocyte apoptosis; and the degree of apoptosis is directly related to the extent of villous atrophy. Apart from markers of apoptosis, researchers have discussed searching for different parameters that could indicate intestinal epithelial damage.

Fatty acid binding proteins are 14-15 kDa cytoplasmic proteins that are abundantly expressed in almost all mammalian cells. FABPs regulate the lipid response in cells and are associated with metabolic and inflammatory pathways. Tissues with a rapid rate of fat metabolism, such as intestine, liver, adipose, and muscle tissues, demonstrate high FABP levels that parallel fatty acid intake and use. According to the most recent studies, intestinal free fatty acid binding protein (FABP-I) also known as FABP2 is a sensitive marker of enterocyte damage in CD and its serum levels are correlated with the severity of histological lesions (1,2). This study aims to investigate the predictive value of the FABP-I in CD.

MATERIALS AND METHODS

Study Population and Laboratory Assessments

This study included patients with CD who were on follow-up in the gastroenterology outpatient clinic between October 2017 and January 2018. Group 1 was composed of patients with CD who were not on GFD (n:24) (antibody-positive), Group 2 was composed of patients with CD who were on GFD (antibody-negative) (n:35), and Group 3 was composed of healthy controls working in the hospital (n:52). Patients over the age of 18 who were diagnosed with CD were included in the study. Exclusion criteria were age below 18, positive personal or family history concerning inflammatory bowel disease, pregnancy and/or breastfeeding, liver or kidney disease, use of supplementation with vitamin D and/or calcium salts.

The diagnosis of CD based on the presence of positive–specific autoantibodies (anti- tissue transglutaminase (tTG) IgA) and concomitant diagnostic intestinal biopsies (at least six biopsies) according to Oberhuber/Marsh criteria when Marsh ≥2 (Marsh 0: normal mucosa; Marsh 1: increased number of intraepithelial lymphocytes, exceeding 40 per 100 enterocytes; Marsh 2: the proliferation of the crypts of liberkuhn; Marsh 3: variable villous atrophy; Marsh 3A: partial villous atrophy, Marsh 3B: subtotal villous atrophy, Marsh 3C: total villous atrophy) (3). anti-tTG and endomysial antibodies (anti-EMA) were presented as negative, weakly positive, positive and strong positive. A negative HLA test excluded CD when negative serology and histologic findings were observed. In these cases with conflicting results, serology is repeated after at least six weeks after on gluten containing diet and determining positive autoantibodies confirmed the diagnosis (4). Age, body mass index (BMI), gender, complete blood count parameters, calcium, phosphate, vitamin D, vitamin B12, ferritin, prothrombin time (PT), INR, and FABP-I levels (Biont, YLA1817HU Elisa Kit, China, Shanghai) were recorded for all groups. Age at diagnosis, immunoglobulin A (IgA) levels, anti-tTG IgA, anti-EMA IgA and Oberhuber/Marsh classification of duodenal biopsy specimens at the time of diagnosis were also recorded for patients.

Statistical Analysis

Statistical data were analyzed using SPSS v.23.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as mean ± standard derivation (SD) for continuous variables. Variables were tested for normality using the Kolmogorov-Smirnov test. The Mann-Whitney U or Kruskal- Wallis H were used to compare non-normally distributed variables. Categorical data was analyzed using a chi-square test or Fisher's exact test. The capacity of serum FABP-I values in predicting the presence of CD was analyzed using ROC (Receiver Operating Characteristics) curve analysis. When a significant cut-off value was observed, the sensitivity, specificity, positive and negative predictive values presented. Statistical significance was considered at $p \leq 0.05$.

RESULTS

This study included 59 patients with CD and 52 healthy controls. The mean age of patients with CD was 39.3 ± 11.9 years and 38 (64.4%) of them were female. There were no differences between the groups in terms of age, gender, and BMI. Demographic data of patients and healthy controls have been presented in Table-1. There were no differences between the groups in terms of calcium, phosphate, ferritin, vitamin B12, PT, INR, and complete blood count parameters, except vitamin D and FABP-I (Table-2). Vitamin D level was 21.1±15.0 ng/ml in CD patients who were not on GFD and significantly lower than CD patients who were on GFD (29.2±18.9 ng/ml) and healthy controls (33.7±11.5 ng/ml) **(p= 0.004)**.

Table-1. Demographic and Follow-up Characteristics of the Study Population				
	Celiac Ab (+) Patients	**Celiac Ab (-) Patients**	**Healthy Controls**	**P value**
Number of patients, n (%)	24 (21.6)	35 (31.5)	52 (46.8)	
Sex, female, n (%)	16 (66.3)	22 (62.9)	32 (61.5)	0.911
Age (year) ± SD	38.5 ± 10.2	39.9 ± 13.0	43.9 ± 15.5	0.214
BMI (kg/m^2) ± SD	25.6 ± 2.8	25.6 ± 3.4	26.6 ± 2.7	0.226
IgA (mg/dl) ± SD	241.4 ± 119.6	190.9 ± 121.0	-	0.119
Time since diagnosis mth± SD	46.8 ± 31.5	28.6 ± 26.2	-	0.019
anti-EMA IgA, n(%)				
negative	0(0)	35 (59.3)	-	-
weakly positive	5 (8.5)			
positive	5 (8.5)			
strong positive	14 (23.7)			
anti-tTG IgA, n(%)				
negative	0(0)	35 (59.3)	-	-
weakly positive	9 (15.3)			
positive	2 (3.4)			
strong positive	13 (22.0)			
Marsh Grade, n (%)				
0	0(0)	8 (22.9)		
1	0(0)	3 (8.6)		
2	0(0)	4 (11.4)		
3a	2 (8)	14 (40)		
3b	11 (46)	4 (11.4)		
3c	11 (46)	2 (5.7)		

anti-EMA, anti-endomysium antibody; anti- tTG: anti –tissue transglutaminase; BMI, body mass index; IgA, immunoglobulin A; SD: Standard deviation

Table-2. Laboratory Characteristics of the Study Populations				
	Celiac Ab (+) Patients	**Celiac Ab (-) Patients**	**Healthy Controls**	**P value**
FABP-I(ng/L) ± SD	499.2±33.3	487.7±48.0	432.2±63.7	0.001[a]
Vit D (ng/mL) ± SD	21.1±15.0	29.2±18.9	33.7±11.5	0.004[b]
Ca (mg/dl) ± SD	9.1±0.9	9.4±0.8	9.36±0.4	0.163
P (mg/dl) ± SD	3.1±0.7	3.4±0.7	3.2±0.6	0.074
Ferritin (mg/L) ± SD	34.7±29.1	58.8±78.7	68.5±95.2	0.236
Vit B12 (pg/Ml) ± SD	482.8±394.7	331.8±102.6	407.3±305.0	0.133
PT (s) ± SD	13.0±4.8	12.3±3.4	11.9±1.0	0.315
INR ± SD	1.1±0.4	1.1±0.3	1.03±0.1	0.340
Hb (g/dl) ± SD	13.9±1.4	13.8±1.8	13.9±2.3	0.965
Htc (%) ± SD	41.0±3.5	41.3±4.2	41.8±5.6	0.789

Ca, Calcium; FABP-I, Intestinal fatty acid binding protein; Hb, Hemoglobin; Htc, Hematocrit; INR,International Normalized Ratio; P, Phosphate; PT, prothrombin time; SD, Standard deviation; vit B12, vitamin B12; vit D, Vitamin D
[a]Difference of Celiac Ab (+) and Celiac Ab (-) patients from the healthy controls
[b]Difference of Celiac Ab (+) patients from Celiac Ab (-) patients and healthy controls

Mean FABP-I was determined as 499.2±33.3 ng/L for antibody-positive celiac patients and as 487.7±48.0 ng/L for antibody-negative celiac patients, and these values were significantly higher when compared to the healthy controls as it was 432.2 ± 63.7 ng/L (p<0.001). The distribution of FABP-I levels by groups has been shown in Figure-1. The cut-off value of FABP-I was computed as 456.8 ng/L. The sensitivity, specificity, positive and negative predictive values of FABP-I at this level were found as 84.7%, 69.2%, 10.2%, 61.5%, respectively (Figure-2).

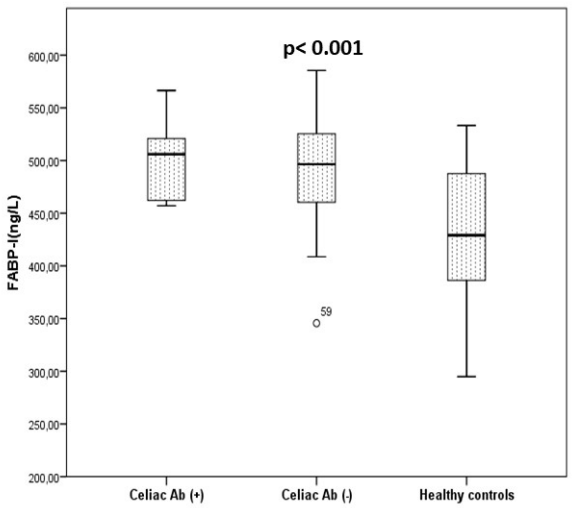

Figure-1. Distribution of FABP-I levels by groups

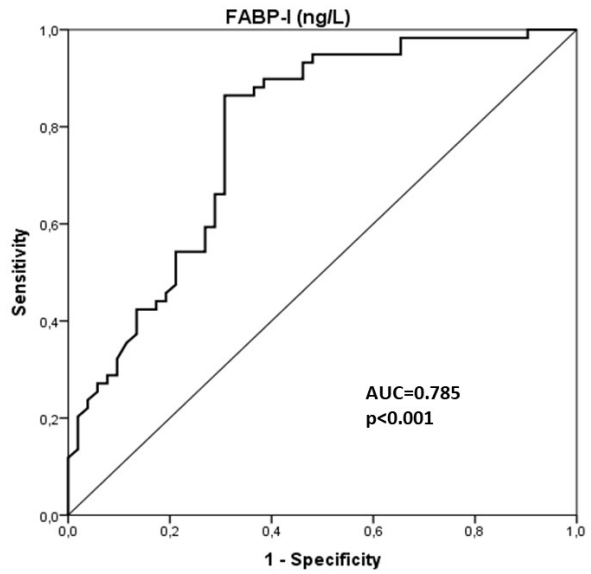

Figure-2. ROC curves of the FABP-I for predicting celiac patients

DISCUSSION

FABP-I can easily be released into the circulation in the presence of enterocyte damage in a short time as they are small and water-soluble proteins. In the present study, we have shown that patients with CD (both compatible and incompatible with GFD) had higher serum FABP-I levels when compared to healthy controls. The sensitivity of FABP-I to predict CD was 84.7% which was lower than anti- EMA IgA (93.7%) and anti-tTG IgA (96.8%) (5). We think its relative lower sensitivity can be improved while a cut-off value can be determined for FABP-I by further wide populations studies. It should also be emphasized that conventional serologic tests also have some restrictions. First, anti-tTG IgA may be negative at a rate of 5-16%, even in cases of villous atrophy confirmed by biopsy (6). Second, the biopsy is not recommended for pediatric patients who also have lower anti-tTG-IgA (7,8). Third, serum samples for antibody measurement for CD diagnosis should be done after at least six weeks of gluten-containing diet intake. Otherwise, false negative results will be seen. In a study that investigated the relatives of patients with CD who were genetically positive for HLA DQ2 and DQ8 and had antibodies in the intestinal tissue despite they had negative serology (9). Consequently, negative serology does not eliminate a diagnosis of CD. On the other hand, anti-tTG IgA is not associated with duodenal mucosal damage and may also cause false positivity besides false negativity (10). Thus, when children with Down syndrome, autoimmune diseases and chronic liver disease are screened for CD, anti-tTG IgA levels are detected even if the mucosal structure is normal (11-13). As a marker that directly reflects intestinal damage, it seems plausible that FABP-I will be able to eliminate this screening confusion.

In the present study, one half of patients with CD who were not on GFD had strong anti-tTG IgA positivity, did not demonstrate any Marsh grades lower than grade III and had the highest FABP-I levels. A study conducted on pediatric celiac patients showed higher serum levels of FABP-I in patients with Marsh grade IIIC changes who show severe villous atrophy than in patients with Marsh grade IIIA changes and mild atrophy. It has been proposed that the relatively lower FABP-I levels seen in some patients who are histologically determined to have villous atrophy are linked to mild mucosal damage that is limited to the proximal small intestine (1). Similarly, it has been reported that the antibody positivity increases in parallel with FABP-I levels (14). However, a correlation between Marsh grade, antibody strongness degree and FABP-I

levels was not observed in the present study may be due to low patient number.

GFD is the only treatment modality for CD. After conversion to a GFD, apoptosis decreases and approaches normal levels (2). GFD compliance of patients can be evaluated, either by negative antibody levels or by demonstration of mucosal healing on biopsy which is an invasive method. Despite anti-tTG IgA is a commonly used test to identify patients with CD adhering to a GFD, its absence in the circulation does not indicate healing of the intestinal epithelium (5,15). In addition, it may take more than a year for celiac antibodies to become negative despite GFD (16). Certain studies done on pediatric patients have shown that FABP-I levels decrease sooner than serum auto-antibodies on a GFD (7). Supporting this, the current United European Gastroenterology (UEG) guideline emphasized that serum FABP-I may be a new marker in evaluating dietary compliance (17). In the present study, we determined higher serum FABP-I levels in CD patients who were on GFD than those who were not, despite the difference was not significant, statistically. In a study that evaluated patients with high pre-treatment FABP-I levels after 12 months of adherence to a GFD, FABP-I levels were found to decrease but did not reach levels comparable to those of healthy controls, indicating persisting mucosal damage in celiac patients even under treatment. Supporting this, refractory CD patients had decreased anti-EMA and anti-tTG IgA levels but increased FABP-I levels with long-term GFD (2). So, FABP-I can be a potential follow-up marker for patients with refractory CD (8).

This study had some limitations. First, it was a cross sectional study. Therefore, we could not compare FABP-I levels at diagnosis and while on a GFD and in histological remission. Second, the antibody levels and Marsh grading could not be included in the statistical analysis due to the low number of cases.

In conclusion, patients with CD have higher serum FABP-I levels when compared to healthy controls. Serum FABP-I level which is an independent marker of enterocyte damage can be detected in the serum before serum antibodies in the early period as a predictive marker. Overlooked diagnosis in serology-negative patients and false serology positivity for reasons other than CD will be prevented with its use in clinical practice.

DECLARATIONS

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Authors Contributions: Conceptualization: PG, EC, KO; Resources: HOD, PG, EC, HO, HLD; Investigation: GA, HOD, PG, HO, KO; Formal Analysis: GA, OO, PG, HO, HLD; Writing – original draft: OO, PG, EC, KO; Writing – review & editing: All authors; Supervision: GA, HOD, OO, PG, HLD

Ethics Committee Approval: The study was approved by the local ethics committees of Sivas Cumhuriyet University with the number 2017-09/06.

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