Original Article

Assessing the Utility of FABP-I in Serum and Biopsy Samples for Diagnosis and Monitoring of Celiac Disease

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Abstract

Background: Celiac disease is immune-mediated disease characterized by small intestine inflammation as a consequence of gluten ingestion in susceptible patients (HLA-DQ2/DQ8). FABP-I small protein comprised of 1%–2% of total cytosolic protein inside enterocytes that play an important role in enterocytes damage and serve as biomarker. **Objectives:** Evidence supports the point regarding celiac disease diagnosis by serological tests. This study aimed to reveal whether the presence of FABP-I in serum or biopsy suggests a new criterion for disease diagnosis and treatment. **Materials and Methods:** A total of 100 patients of different genders and ages were positively diagnosed with CeD by serological tests, (100) healthy individuals were enrolled. For the same patients, 45 biopsies were taken by specialists from suspected individuals, all oriented well, of proper size and good quality for analysis, processed by histopathologist for further use. **Results:** This study included a total of 200 individuals in which 100 clinical samples positively diagnosed including (40) males and (60) females, age range (3–45 years), 100 sample serves as control group from the beginning of February to August 2024 at Imam Al-Hussain Teaching Hospital in Karbala Governorate, indicating a significant difference between patients and controls. Serum FABP-I levels significantly higher in patients rather than in controls, while in biopsy the protein was not detected in either group. **Conclusion:** Study indicated that FABP-I shows a higher concentration in the serum of CeD patients in comparison to the control group. While, it is absent in biopsy samples of both patients and controls.

Keywords: Celiac disease, FABP-I, serological diagnosis

Introduction

The disease (CeD) can be defined as a chronic autoimmune condition occur as a result of protein termed gluten, characterized by enteropathy of small intestine in persons with genetic susceptibility (HLA-DQ2/DQ8). CeD is diagnosed depending on certain tests (serologic) which include IgG-DGP, IgA-TG2, or IgA-EmA, confirmation of these tests accomplished with histological changes such as enteropathy that consist of increased intraepithelial lymphocytes present in the biopsies of duodenum, crypt hyperplasia, and villous atrophy.[1]

FABP-I a protein of low molecular weight, specialized to epithelial cells of the small intestine. Because FABP-I expressed in the cytoplasm in high level, the I-FABP circulating level it can be considered as a hallmark for controlling enterocytes damage, also it has been suggested as a potential biomarker of disease progression in CeD.[2]

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FABP-I considered attractive biomarker for tissue damage in the upper gastrointestinal tract because of its ability to be measured in available non-invasive samples (such as urine) beside the moderately invasive (serum) as well as of FABP-I tissue specificity together suggested its importance as a disease biomarker.[3]

FABP-I has been aiding in diagnosis when used as a non-invasive diagnostic tool for CeD in combination with TGA levels.[4] Circulating FABP-I is a proxy biomarker for the injury intensity of intestinal epithelial cell. This

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protein considered helpful biomarker in estimation the damage of epithelial cells in intestine in different disease such as necrotizing enterocolitis, intestinal ischemia, as well as mesenteric infarction.[5] Elevated circulating levels of FABP-I in active CeD patients introduced evidences of enterocytes damage.[6,7]

The best procedure for the assessment of intestinal damage in CeD still endoscopy accompanied with biopsy, however, the routine assessment of disease progress depends on patient self-reported symptoms because endoscopy considered an invasive procedure, so together with blood and fecal inflammatory biomarkers which often do not reflect histological damage or following therapy recovery.[8,9]

In the present study, ELISA was performed to assess FABP-I levels in the serum of CeD patients as well as immunofluorescence technique was also used to detect the presence of FABP-I in the biopsy samples of the same patients to recommend a new approach of diagnosis depending on the non-invasive procedures.

Materials and Methods

Subjects

A total of 250 individuals attended Karbala Center for Gastroenterology and Hepatology, only 100 patients enrolled in this study, as well as 100 healthy controls, in Imam AL Hussein Medical City in Karbala Province. Only the participant whom matches the criteria were enrolled in this study (patients 3–45 years old, attended gastrointestinal tract unit whom diagnosed to have CeD by specialists), while any age bellow 3 years or if there any suspicion in the family history for CeD, if the person was on GFD or if there is any autoimmune disease rather than CeD were excluded from the study. The patient subject and controls matched as possible as could by age and gender. Diagnosis was done based on the clinical features, serological markers, and histopathological pictures of CeD in duodenal mucosa all together according to the current international guidelines.[10]

Ethical approval

The study agrees with the ethics of the Training and Developing Center in Karbala province, in which hospitals samples were taken and all participants provided the needed approvals for the study be proceeded at date January 13, 2023) number (015).

ELISA assay for determination of FABP-I

The ELISA assay was performed according to the manufacturer's instructions. Hunan FABP-I (Intestinal Fatty Acid Binding Protein) ELISA kit was supplied by Elabscience Biotechnology/ USA, catalog No:E- EL-H0159.

Immunofluorescence staining kit (Anti-Rabbit IgG- Elab Fluor®594)

In this procedure, the target protein can be detected if there is a suitable antibody, fluorescence can be visualized by immunofluorescence staining kit, for immunofluorescence detection of cells and tissue sections. Immuno Fluorescence Staining Kit (Anti-Rabbit IgG-Elab Fluor®594) composed of Goat Anti-Rabbit IgG (H + L) (Elab Fluor®594conjugated) (because it is able to recognize epitopes on human antigens that are not immunogenic in rodents and their collective ability to either engage different epitopes of human antigens or to engage these epitopes differently in comparison with murine and human antibodies), named the secondary antibody that can detect the primary antibody from rabbit with bright red fluorescence. The kit is provided with anti-fluorescence quenching sealing solution that makes the fluorescence more lasting.

Preparation immunofluorescence staining

The first step in the staining procedure was preparation of fixation solution, in which 4% paraformaldehyde was recommended as fixative (E-IR-R113), triton X-100 has been used as a permeable solution (E-IR-R122). TBST is the washing buffer recommended to use, while primary antibody dilution buffer recommended to use is Elabscience ® Antibody Dilution Buffer (E-IR-R106), dilution of primary antibody according to manual instructions as well as dilution of secondary antibody by antibody dilution buffer (E-IR-R106) PBS as secondary antibody dilution Buffer.

Immunofluorescence staining for tissue slice

The first step in tissue slice staining of paraffin sections was de-waxing, hydration, and antigen repair, incubation with fixative at room temperature for 15–30 min or more. TBST working buffer washed the slice about 3–5 min, 3 times, and the liquid is discarded. 100 µL of Normal Goat Blocking Buffer (Ready-to-Use) (E-IR-R110) was added, incubate for 30 min. Discarded the blocking buffer, then add 100 µL of diluted primary antibody and incubate for 60 min at 37°C in a wet box (Or incubated at 4°C overnight). Discard the primary antibody, and the glass with TBST working buffer for 3~5 min, 3~5 times. 100 µL of diluted secondary antibody was added and then incubated for 60 min at 37°C in wet box. Wash the glass with TBST working buffer solution for 3–5 min, 3 to 5 times, during washing notice to avoid light. The nuclear staining DAPI reagent (E-IR-R103) was added and incubate for 5 min in wet box, then all glasses washed with TBST working buffer for 5 min, for 4 times to remove the redundant DAPI Reagent. On a glass slide one drop of Anti-Fluorescence Quenching Agent (E-IR-R119) was added; avoid air bubbles by covering the glass with cells. Finally, the result was observed using a fluorescence microscope.

Statistical analysis

Study participant's data, CeD patients and the healthy group, enrolled in the study and analyzed by the Statistical Package for the Social Sciences (SPSS) version 25 software for Windows, IBM, US, 2017. Before the analysis process, all parameters were analyzed for the presence of errors or inconsistency. The mean levels of these parameters analyzed using F test (The one way ANOVA). *P* value (level of significance) of 0.05 or less is considered significant.

Results

Assessment of FABP-I level in serum and biopsy in CeD patients and control

The present study has revealed that there is a significant difference in the level of FABP-I in the serum of patients with CeD and healthy controls, with patient group showing a higher level rather than in healthy controls, who did not show any elevation in cytokine concentration (*P* value =<0.0001), as shown in Table 1.

The current study also indicated the level of serum FABP-I in CeD patients according to different age group which was high in the age group of (23–32) with mean range of (65.25), and in the age group (43–52) with a mean range of (61.26), with less FABP-I concentration in the age group of (13–22) at P value <0.00 as shown in Table 2.

There were significant differences revealed in this study about the concentration of serum FABP-I among males and females, in which it was expressed at a high level in males with a mean range of (62.73) while in females, the concentration was less than in males, with a mean range of (42.35), as shown in Table 3 and Figure 1 which reveal the standard curve of FABP-I concentration by ELISA.

While, immunofluorescent technique used for biopsy examination with a fluorescent microscope demonstrated that there were no significant differences in FABP-I presence in biopsy of CeD patients compared to the control groups (H. pylori biopsy). Figure 2 shows the positive and negative immunofluorescence of FABP-I in the biopsy.

Table 1 Mean concentration of FABP-I among patients and control

FABP-I serum	Mean ± SEM	P value
Pateints	5.023 ± 0.253	<0.0001**
Control	0.383 ± 0.026	

** high significant at $P \leq 0.05$

Discussion

Studies regarding FABP-I localization demonstrated that it has been localized in the entire small intestine mucosa and specially the jejunum. Notice that FABP-I expressed in large amounts on the tops of the villi, in which villous atrophy of the small intestine is the hallmark of celiac disease.[11]

There are many studies revealed that patients with CeD (both compatible and incompatible with GFD) had higher serum FABP-I levels when compared to healthy controls,[2] while other investigations disagree with the results of this study which recommended that a duodenal biopsy is required. Indeed, they established that the biopsy of duodenum is the gold-standard method for appropriate diagnosis of this defect, because there is no currently available analysis in the serum provides enough sensitivity and specificity, although biochemical analyses are highly sensitive.[12]

Depending on the previous studies on enterocyte programmed cell death, it has been indicated that there is a relationship between villous atrophy and serum FABP-I levels in untreated CeD patients, depending on the fact that

Table 2 Serum FABP-I concentration according to age group

Variable	Age group	No.	Mean rank	Chi-square	Df.	P value
FABP-I serum	3–12	44	41.55	14.287	4	0.006**
	13–22	5	30.80			
	33–42	12	49.92			
	43–52	19	61.26			
	Total	100				

** high significant at $P \leq 0.05$

Table 3 Serum FABP-I according to sex

Variable	Sex	No.	Mean Rank	Sum of Ranks	Mann Whitney U	Wilcoxon W	Z	Asymp. Sig. (2- tailed)
FABP-I serum	Male	40	62.73	2509.00	711.000	2541.000	−3.441	0.001 **
	Female	60	42.35	2541.00				
	Total	100						

** high significant at $P \leq 0.05$

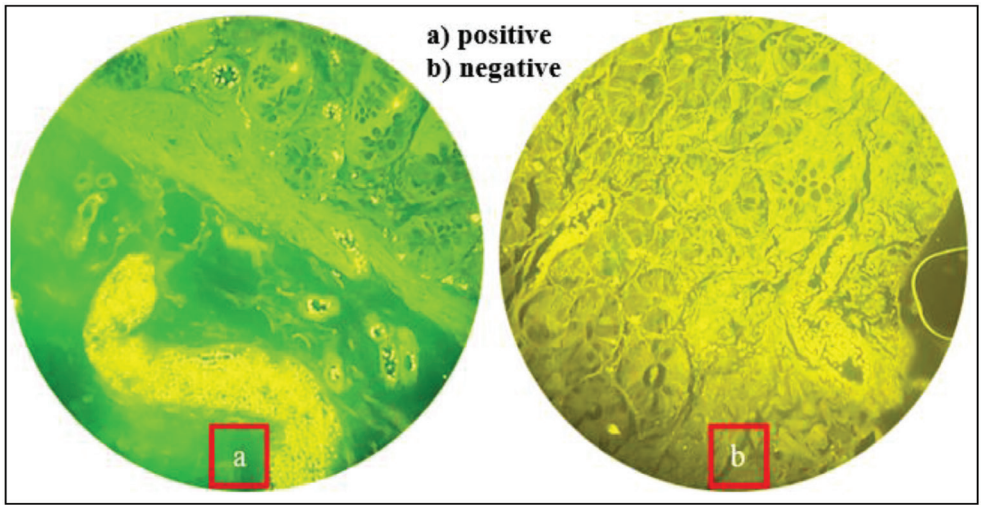

Figure 1: The standard curve for FABP-I concentration. (a) Positive test; (b) Negative test

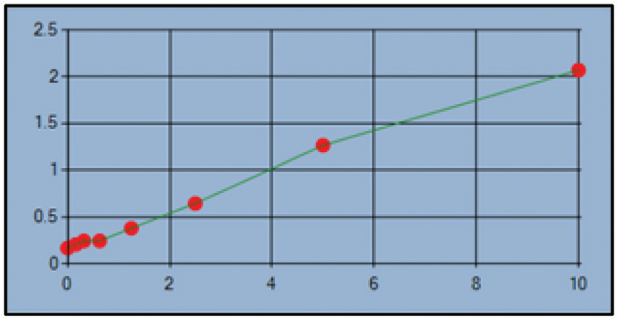

Figure 2: CeD biopsy under fluorescent microscope

enterocytes damage causes villous atrophy and considered a result of the damage, which is highly localized at the tips of the jejunal villi, which are released immediately during cell damage into the circulation.[13-15]

Studies have shown that elevated levels of FABP- I presented in patients with untreated CeD, and normalize after the initiation of a gluten-free diet.[2] Therefore, other research revealed that FABP-I has been introduced as a marker for a no-biopsy approach in patients not qualifying for 10-fold tTG rise.[16-18]

Conclusion

In the serum of CeD patients, the concentration of FABP-I presented at a higher level than the control group (negative for CeD) with a sensitivity of 0.88% and specificity of 0.79% making it of diagnostic validity. While, in biopsy the FABP-I absent in both patients and controls which explains the limited advantage of its use in the diagnosis of CeD.

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Conflicts of interest

There are no conflicts of interest.

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