Effect of treatment on the activity of PREP and Transglutaminase-IgA(Ttg-IgA) and other biomolecules in patients with celiac disease (CeD)

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Abstract

Celiac disease (CeD) is a long-term inflammatory disorder of the small intestine, triggered by the ingestion of gluten in genetically susceptible individuals. When gluten is consumed, it leads to damage to the intestinal lining, resulting in poor absorption of nutrients. Although nutrient malabsorption is a significant risk for various complications associated with CeD, genetic, immune, and environmental factors also play key roles. The clinical presentation of CeD can vary widely, ranging from no symptoms to severe symptoms, reflecting its impact on multiple organ systems. Currently, the only evidence-based treatment is a gluten-free diet (GFD), which can fully repair the intestinal damage and stop disease progression. If left undiagnosed, CeD can lead to serious complications in both children and adults. In the study, 40 patients with type 1 diabetes (DMT1) and 40 patients with celiac disease (CD) divided into two groups: treated and untreated patients were compared with 40 control patients. The results showed ROC curve of PERP the patients with treated gluten cut of value (3.46) higher than cut of value (2.62) of patients with untreated gluten. Anti-tTga ROC curve showed that the cut-off value for people with treated gluten was 9.15 be less than cut of value for people with untreated gluten was (14.87). As for patients with diabetes mellitus type 1 and patients with untreated gluten the results appear Ttg-IgA level higher with significantly p-value (0.02) compared with healthy people. The PERP level with higher significantly p-value≤0.0001 for patients with diabetes mellitus type1 and patients with untreated gluten compared with healthy people. This results clearly showed the role of PERP and Anti-tTga may be useful in identifying gluten intolerance as a type 1 diabetes risk factor. Furthermore, other variables were measured as part of the study, including glutamic pyruvic transaminase, hemoglobin A1c (HbA1c), and fasting blood sugar (FBS). Glutamic Pyruvic Transaminase (GPT), Complete Blood Count (CBC), Glutamic Oxaloacetic Transaminase (GOT), and Hemoglobin (HBG)

Keywords: Celiac disease, prolyl-endopeptidase, Transglutaminase-IgA(tTG-IgA), Fasting Blood Sugar (FBS), Hemoglobin A1c (HbA1c), Glutamic Pyruvic Transaminase (GPT), Glutamic Oxaloacetic Transaminase (GOT), Complete Blood Count (CBC).

Introduction:

Gluten: It's a protein. The ingestion of gluten proteins from wheat, rye, and barley The proteins gliadins and glutenins have a major role in the formation of the gluten network. Gluten can cause serious side effect in certin individuals by bring toxin, causing ones immune cells overrated attacked, these individual are sensitive to gluten. Gluten function has a major impact on baked goods quality. Gluten proteins are powerful food allergens due to their extraordinarily high proline and glutamine concentrations. (1)(2)

Gluten Sensitivity (GS) includes a range of conditions brought on by gluten consumption in people who are genetically vulnerable. Within this continuum, celiac disease (CD), or gluten-sensitive enteropathy, is the most well-characterized condition . In addition to gastrointestinal symptoms such bloating, stomach discomfort, diarrhea, and constipation, extraintestinal signs of GS are frequently the first to show. Not all GS patients also have CD. They might be positive for antibodies linked to gluten sensitivity serologically. (3)(4)

Having an immune disease could lead to other immune diseases like diabetes mellitus type1. More than 10% of people with T1DM also have celiac disease. **Type 1 Diabetes Mellitus (T1DM)**, also known as autoimmune diabetes, is a chronic illness that results in hyperglycemia from the loss of pancreatic beta cells, which prevents the body from producing enough insulin. While the majority of the time the symptoms start in childhood or adolescence, they can sometimes manifest much later in life. Although the exact cause of T1DM is unknown, T cell-mediated cellular death is believed to play a part in the disease's pathophysiology.(5)(6)
Diabetes mellitus is diagnosed and treated by the **glycosylated haemoglobin (HbA1c)** assay. Although within-subject variability in measured HbA1c impacts its interpretation and clinical value, no thorough systematic research has addressed this issue.(7)

The quickest and easiest test to diagnose diabetes is fasting blood glucose (FBG)This test measures the amount of glucose in the blood. It is best to have the test conducted in the morning

following a fast. If the subject's fasting blood glucose is greater than 126 mg/dl, they may have diabetes. (8)

In those with type 1 diabetes, celiac disease is highly prevalent. Additionally, a gluten-free diet affects how well type 1 diabetics maintain their blood sugar levels, which lowers their chance of developing diabetes complications and helps screen for celiac disease in those with type 1 diabetes mellitus. (9)

One extremely specific marker to identify subjects with latent celiac disease is **transglutaminase-IgA (tTG-IgA)**. In people with type 1 diabetes, it may test positive. In patients with CD, endomysial antibodies recognize it as the main autoantigen. (10)

The enzymes **aspartate transaminase (AST)** and **alanine transaminase (ALT)** are located in the liver and are also present in various bodily tissues at lesser concentrations. When liver injury and acute pancreatitis occur, their levels increase because they aid in the conversion of proteins into energy.(11)

The most often used laboratory test is the **Complete Blood Count (CBC)**. The evaluation furnishes information about the synthesis of all blood cells and establishes the patient's oxygen carrying capability by evaluating red blood cell (RBC) indices, hemoglobin, and hematocrit. Understanding the immune system can also be gained from differentiating the white blood cell (WBC) count. (12)(13)

The purpose of this study is to investigate the role that gluten sensitivity plays as a risk factor for type 1 diabetes (T1DM) via:

1. Investigate the prolyl endopeptidase (PREP) activity as a marker for gluten sensitivity (GS) in individuals with type 1 diabetes in all studied groups.

2. Assessing each group under study for their level of tTG-IgA, a marker for Type 1 Diabetes Mellitus (T1DM) and Gluten Sensitivity (GS) condition.

3. Checking the FBG, HbA1C, CBC, ALT, and AST activity.

4. Assign prolyl endopeptidase (PREP) to corresponding parameters with other parameters under investigation.

5. Determine how tTG-IgA correlates with the other parameters under investigation.

6. Research on ROC analysis using tTG-IgA, HbA1c, and prolyl-endopeptidase (PREP) as indicators of T1DM incidence in patients with gestational syndrome (GS).

Materials and Methods Patients

This research was carried out in Iraq assessed the correlation between Anti-Ttga and PERP levels in individuals with gluten sensitivity and Type 1 Diabetes. Eighty people in two groups, ages (8 - 40), from the medical city in Baghdad, Iraq, participated in the study between September 2023 and January 2023. We compared 40 healthy (equal in age and sex) controls to Celiac disease 40 patients which was divided into (treated, untreated patients) and with Type 1 Diabetes mellitus 40 patients (DMT1). Each research group's measurements included weight, height, and BMI.

Samples

Five milliliters of blood were drawn by venipuncture from each participant in this study, and to maintain the serum separate, two milliliters were placed within an EDTA tube and three milliliters inside a gel tube. A serum aliquot was isolated from the blood samples using a centrifuge, and kept at -20° C until testing.

Sample Analysis

To measure serum (PERP, IgA), provided an ELISA kit for PERP by BT lab and ELK Biotechnology kit for Anti-Tissue Transglutaminase (tTG-IgA). Additionally, using the Cobas Integra, D10, and Nihon, biochemical indicators such as FBS, AST, ALT, HbA1c, and CBC were assessed.

Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability. Estimate of correlation coefficient between variables i in this study.

Results and discussion

Table (1) shows the different between mean of Anti-tTga for Gluten treated (8.32) and Gluten untreated (29.62), mean of Gluten untreated patients higher than that mean of Gluten treated patients with P-value≤0.05, table (1) indicate that the mean of Anti-tTga for DMT1 patients also raised compared to healthy people this study supports the study he conducted Pérez L, et al.'s which is useful After starting a gluten-free diet (GFD), individuals with celiac disease (CD) have low levels of IgA. (14)

Table 1: Comparison between difference groups in Anti-tTGA and PREP

Sub Group	Mean ± SE	
	Anti-tTGA	PREP
Gluten treated	8.32 ±0.51 b	2.89 ±0.12 bc
Gluten untreated	29.62 ±6.07 a	1.88 ±0.09 c
DMT1	27.15 ±7.41 a	3.56 ±0.53 ab
Control	9.10 ±0.37 b	4.57 ±0.23 a

LSD value	8.063 *	1.180 **
P-value	0.0217	0.0001
Means having with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).		

As for PERP mean of Gluten treated and untreated patients (2.89, 1.88) respectively, where is the mean of untreated patients less than the mean of treated patients with P-value≤0.01, as stated in the study he conducted Noori E, et al.'s Additionally, studies have shown where in the GI system endopeptidases break down gluten peptides. (15)

Table (2) shows the mean of HbA1c for treated gluten patients (6.46) and untreated gluten patients (6.45) is close as the researchers Krupa et al.'s pointed out stated that a year of treated gluten had no effect on HbA1c. (16)

where it is higher compared with healthy people mean (4.85) and less than the mean for DMT1 patients (8.45) with highly significant p-value ≤0.01.

Table 2: Comparison between difference groups in HbA1c and FBS

Sub Group	Mean ± SE	
	HbA1c (%)	FBS (mg/dl)
Gluten treated	6.46 ±0.51 b	123.78 ±19.67 b
Gluten untreated	6.45 ±0.46 b	126.32 ±17.35 b
DMT1	8.45 ±0.30 a	183.20 ±17.76 a
Control	4.85 ±0.29 c	88.32 ±2.33 b
LSD value	0.912 **	44.01 **
P-value	0.0001	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).		

As for mean of FBS, they are close in treated and untreated gluten patients (123.78, 126.32) in a row, they mean less compared to DMT1 (183.20) and up from healthy people (88.32) with P-value≤0.01. This study supported Sukriti et al.'s revealed that, in comparison to patients receiving treatment for gluten, those with gluten had greater FBS and HbA1c. (17)

Table (3) explain the difference between treated and untreated gluten patients whose mean of WBC is up than untreated patients (8.01>6.68), as compared to healthy people mean which is (6.65).

Table 3: Comparison between difference groups in WBC, Lymphocyte and HGB

Sub Group	Mean ± SE		
	WBC (10^9/L)	Lymphocyte (%)	HGB (mg/dl)
Gluten treated	8.01 ±0.51 ab	2.65 ±0.24	14.38 ±1.41 ab

Gluten untreated	6.68 ±0.47 b	2.29 ±0.20	15.18 ±0.71 a
DMT1	8.33 ±0.59 a	2.63 ±0.14	13.47 ±0.43 bc
Control	6.65 ±0.24 b	2.54 ±0.12	13.18 ±0.23 c
LSD value	1.446 *	0.492 NS	1.206 *
P-value	0.0169	0.519	0.03529
Means having with the different letters in same column differed significantly. * (P≤0.05).			

As for the mean of HBG, patients with untreated gluten have mean higher comparison with treated gluten patients (15.18, 14.38) respectively with P-value≤0.05. Table (3) also shows the mean of Lymphocyte for treated, untreated gluten patients, DMT1 patients and healthy people (2.65, 2.29, 2.63, 2.54) their mean are almost identical. This table supports a study Atia A on the value of WBC and differs from me on the value of HGB which states:the research revealed that following therapy, the values of WBC and HBG were much greater than they had been previously.(18) It also supported my results by Zingone F et al.'s it has been suggested that celiac disaes may cause hyposplenism or functional asplenia Which in turn increases the HGB Because it prevent the breakdown of RBC. (19)

Results of GOT, and GPT were illustrated in table (4).

Table 4: Comparison between difference groups in GOT and GPT

Sub Group	Mean ± SE	
	GOT (U/L)	GPT (U/L)
Gluten treated	29.16 ±2.53 ab	26.05 ±2.07 ab

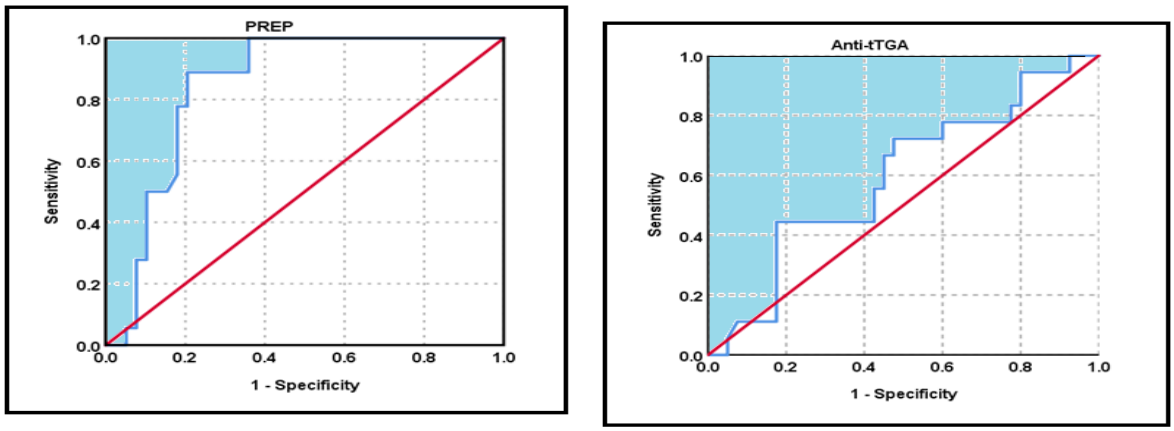
Gluten untreated	28.32 ±1.59 ab	31.63 ±1.89 a
DMT1	39.07 ±5.62 a	21.22 ±2.20 b
Control	26.62 ±0.94 b	20.32 ±1.01 b
LSD value	11.723 *	6.021 *
P-value	0.0498	0.0338
Means having with the different letters in same column differed significantly. * (P≤0.05).		

This table shows the mean value of GOT for treated (29.16), untreated (28.32) gluten patient and DMT1 (39.07) their mean are higher than healthy people (26.62). This table also explain the mean of GPT for treated (26.05) and untreated (31.63) where their mean are close compared to DMT1 (21.22) and healthy people (20.32) their mean are also close with Significant (P≤0.05). In CD, this study boosted by a study Saadah OI, et al.'s transaminase abnormalities are commonly observed, with increased AST being the most common anomaly. The most noticeable reaction to a GFD is a lower ALT level. (20)

Treated gluten

ROC analysis for biomarker in treated gluten sub group.

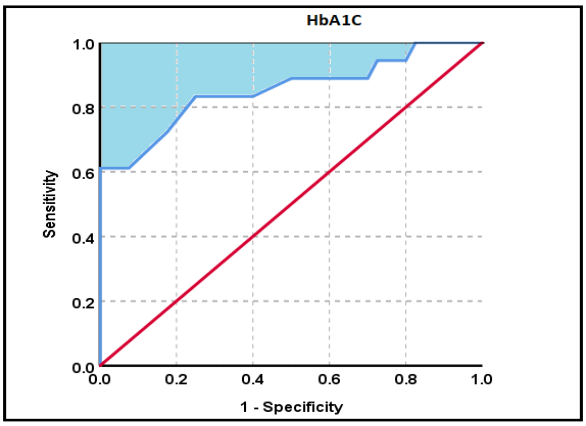
Variable	AUC	p-Value	Cut of value	Sensitivity	Specificity
tTgA	0.595	≤0.05	9.15	72	53
PERP	0.845	≤0.01	3.46	88.9	79
HbA1c	0.853	≤0.01	5.150	83.3	75
FBS	0.560	≤0.01	90.50	55.6	60

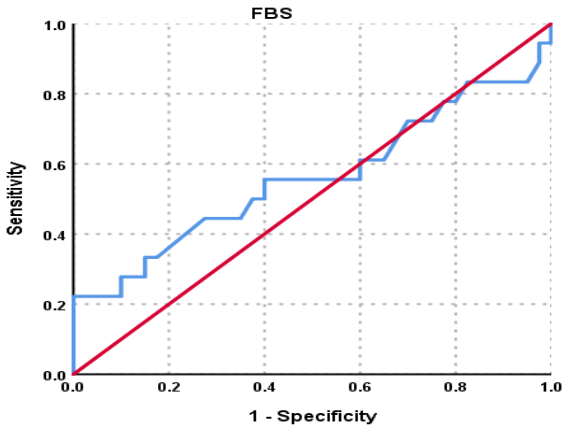


(A) (B)

Figure (A). The predictive value of PERP serum levels in treated gluten patient compared to healthy patients was investigated using a ROC curve analysis (Sensitivity88.9 , specificity 79 , criterion 3.46), p ≤0.01.

Figure (B). The predictive value of Anti-tTga serum levels in treated gluten patient compared to healthy patients was investigated using a ROC curve analysis (Sensitivity 72 , specificity 53 , criterion 9.15), p ≤0.05.





(C) **(D)**

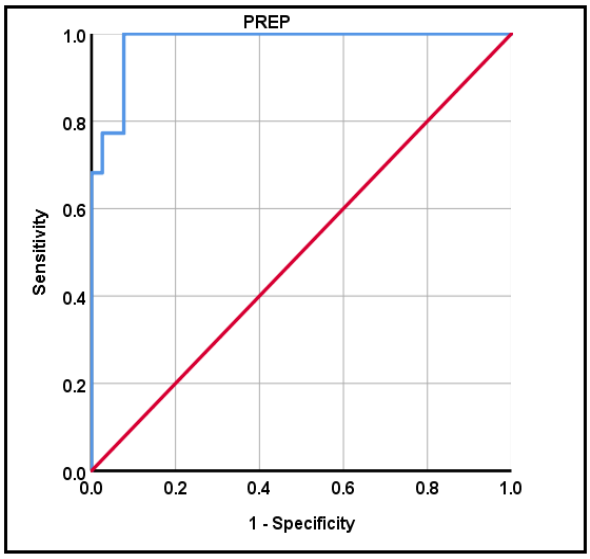
Figure (C). The predictive value of HbA1c serum levels in treated gluten patient compared to healthy patients was investigated using a ROC curve analysis (Sensitivity 83.3 , specificity 75 , criterion 5.150), p ≤0.01.

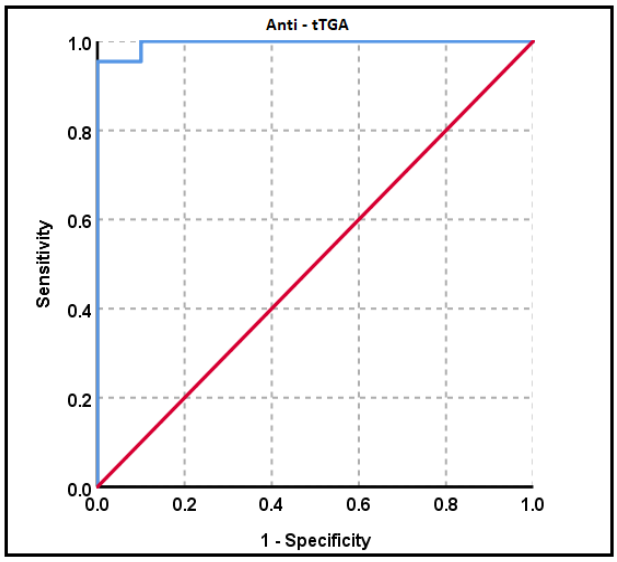
Figure (D). The predictive value of FBS serum levels in treated gluten patient compared to healthy patients was investigated using a ROC curve analysis (Sensitivity 55.6 , specificity 60, criterion 90.50), p ≤0.01.

Untreated gluten

ROC analysis for biomarker in untreated Celiac disease patients.

Variable	AUC	p-Value	Cut of value	Sensitivity	Specificity
tTgA	0.995	≤0.05	14.87	90.9	100
PERP	0.980	≤0.01	2.62	100	93
HbA1c	0.829	≤0.01	5.05	86	60
FBS	0.573	≤0.01	92.50	54.5	62

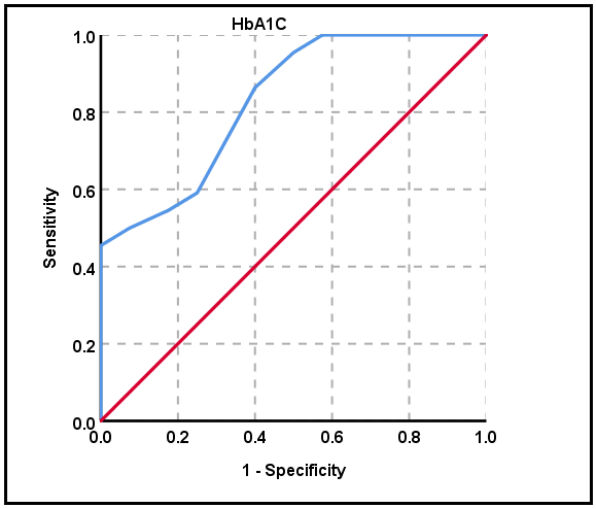


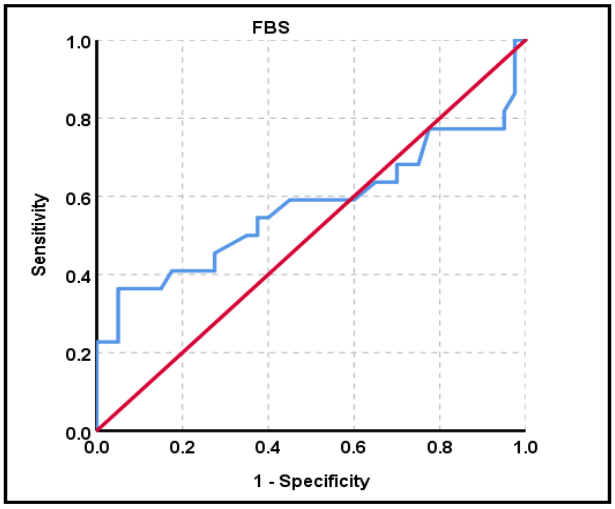


(A) **(B)**

Figure (A). The predictive value of PERP serum levels in untreated gluten patient compared to healthy patients was investigated using a ROC curve analysis (Sensitivity100, specificity 93 , criterion 2.62), p ≤0.01.

Figure (B). The predictive value of Anti-tTga serum levels in untreated gluten patient compared to healthy patients was investigated using a ROC curve analysis (Sensitivity 90.9 , specificity 100 , criterion 14.87), p ≤0.05.





(C) **(D)**

Figure (C). The predictive value of HbA1c serum levels in untreated gluten patient compared to healthy patients was investigated using a ROC curve analysis (Sensitivity 86 , specificity 60 , criterion 5.05), p ≤0.01.

Figure (D). The predictive value of FBS serum levels in untreated gluten patient compared to healthy patients was investigated using a ROC curve analysis (Sensitivity 54.5 , specificity 62, criterion 92.50), p ≤0.01.

Conclusion

The study's findings demonstrated that when treated CeD patients followed a gluten-free diet, their Ttg-IgA level decreased and their PREP activity increased. However, compared to the group of individuals with type 1 diabetes, the levels of HbA1c and FBG did not decrease significantly in the treated group. ROC analysis was used to determine the specificity of PREP, which yielded results of 93% for untreated CeD patients and 79% for treated patients. For anti-Ttg-IgA CeD patients, the results seem to be 100% for untreated CeD patients and 53% for treated patients.

Authors' declaration

- No conflict of interest.
- We so attest that each figure and table in the book is our own. Additionally, the authorization for reprinting the graphs and photos, which are not mine or ours, is linked to the manuscript.

- Ethical Clearance: The initiative has the approval of Baghdad University's local ethical commission.

Authors' Contribution Statement

P.H.SU. selected the research topic and supervised its writers, while F.F.M. collected samples, analyzed the results and wrote the research.

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