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Age And Sex-Dependent Variations In PREP Levels In Iraqi Gluten-Sensitive Patients

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Abstract
This study explores age and sex-dependent variations in prolyl endopeptidase (PREP) levels among gluten-sensitive patients in Iraq, a region with high gluten consumption and limited enzymatic research. Using data from 80 study participants who were divided into four groups (G1 - control group ages 5-20; G2 - control group ages 20-40; G3 - patients ages 5-20; G4 - patients ages 20-40), one-way ANOVA with Tukey post hoc tests, independent samples t-tests, and multivariate regression, and Pearson correlations were used. Significantly lower PREP levels were seen in patients (G3 - 2.17 ± 0.11; G4 - 3.03 ± 0.44) than controls (G1 - 4.55 ± 0.36; G2 - 4.63 ± 0.28; $p<0.001$). PREP levels in females (3.64 ± 0.27) were slightly higher than in males (3.05 ± 0.23) and approached significance ($p=0.101$). Substantial negative associations were identified among PREP and the following biomarkers in the G1 control group (BMI ($r=-0.4927$, $p=0.0169$; HGB ($r=-0.4153$, $p=0.0488$)), in the G2 control group (AGE ($r=-0.5652$, $p=0.0352$; HbA1C ($r=-0.5783$, $p=0.0303$)), in the G3 patient group (anti-tTGA ($r=-0.5081$, $p=0.0022$; HbA1C ($r=0.3539$, $p=0.04$), and in G4 patient group (HGB ($r=-0.8907$, $p=0.0427$)). Multivariate analysis confirmed disease status as the primary driver of PREP suppression. These findings highlight the potential for region-specific, enzyme-based therapies, emphasizing personalized approaches for gluten-sensitive Iraqi patients. These findings illustrate that age and sex impacted the PREP levels, and that demographic factors should be considered when cognitive therapeutic strategies are applied to gluten-sensitive Iraqi patients, to improve clinical symptoms using a specific approach.

Keywords: *Prolyl endopeptidase, gluten sensitivity, age, biomarker, sex, Iraq, personalized therapy.*

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I. Introduction

Gluten sensitivity, including non-celiac gluten sensitivity (NCGS) and celiac disease (CD), is an important public health issue in Iraq. Traditional dietary staples, such as wheat bread and pastries, contribute to a high gluten intake in this region (Al-Rawazq et al., 2021). Celiac disease is a form of autoimmune illness marked by damage to the intestines and systemic effects and initiated by gluten intake in genetically vulnerable individuals (Cárdenas-Torres et al., 2021). While poorly understood, NCGS is associated with gluten-related symptoms and does not include the histological damage to the intestines, as exhibited by individuals with CD (Rotondi Aufiero et al., 2018). Both CD and NCGS have been associated with immune responses initiated against proline-rich gluten peptides that human enzymes cannot digest completely (Cabanillas, 2020).

Prolyl endopeptidase (PREP) is a serine protease and is a critically important enzyme that cleaves proline-rich peptides. For individuals with gluten sensitivity, PREP may reduce the immunogenic effects of gluten (Moreno Amador et al., 2019). Less PREP activity contributes to the accumulation of gluten peptides, suggesting an important relationship between increased gluten peptide abundance, immune activation, and degree of disease severity (Ersoy et al, 2023). PREP expression and activity can be affected by different demographic factors, such as age and sex, which may also influence the presentation and management of disease (Persechino et al., 2021; Klein & Flanagan, 2016). There is evidence that females tend to show greater immune responses in autoimmune disease and affected biomarker profiles (Klein & Flanagan, 2016). In a similar vein, age-related differences in metabolic and immune functioning could affect PREP levels as pediatric and adult populations exhibit distinct clinical presentations (Strati et al., 2016).

With high prevalence rates of gluten sensitivity in Iraq and limited research surrounding enzymatic aspects, there is a compelling case for region-specific research (Stanciu et al., 2024). Limited research exists on PREP in Iraqi populations, where genetic factors (e.g., HLA-DQ2/DQ8) and environmental factors (e.g., gut microbiota) may uniquely influence gluten sensitivity (Alam et al., 2024; Arcila-Galvis et al., 2022). This study hypothesizes that age and sex modulate PREP levels in Iraqi gluten-sensitive patients, impacting biomarker profiles and offering insights for personalized therapeutic strategies, such as PREP supplementation, to improve clinical outcomes. Our research design will examine the resultant effects of age and sex on levels of PREP in Iraqi

children and adults with gluten sensitivity and healthy controls. Our central goal here is to determine patterns and relationships between PREP and other clinical biomarkers (BMI, HbA1C, anti-tTGA) to lay the groundwork for future patient-specific clinical diagnostic and treatment pathways.

II. Methods

Study Design & Participants

This cross-sectional study aimed to compare the PREP levels of Iraqi gluten-sensitive individuals to those of healthy controls based on age and gender. From January 2023 to June 2024, 80 volunteers were recruited from three of Baghdad, Iraq's biggest medical institutions. After reviewing the inclusion criteria, respondents were divided into four categories depending on the age and health status of the study population: (Group 1) (G1: healthy controls, 5-20 years, n=20); (Group 2) (G2, healthy controls, 20-40 years, n=20); (Group 3; (G3, gluten-sensitive patients, 5-20 years, n=20), and (Group 4) (G4; gluten-sensitive patients, 20-40 years, n=20).

Participants were diagnosed as gluten sensitive either by serological test for anti-tissue transglutaminase antibodies (with anti-tTGA >10 U/mL) or biopsy-proven celiac disease, in accordance with international diagnostic guidelines (Raiterin et al., 2022). Controls were simply healthy participants with no history of any gluten-related disorder or gastrointestinal disease, or autoimmune disease (diagnosed using medical history and routine blood tests). Inclusion criteria for all participants included that they did not have any other autoimmune disease (such as rheumatoid arthritis, type 1 diabetes), or history of chronic infections (such as hepatitis or tuberculosis), or use of immunosuppressive medication within 3 months prior to the study, which we established via review of medical history. Pregnant women and men or women with significant comorbid disease (disease that may affect metabolic or immune function) were excluded to minimize potential confounding. Genetic screening for HLA-DQ2/DQ8 was not feasible due to resource constraints, but it is recommended for future studies to explore genetic influences on PREP expression.

Data collection

Blood samples were collected from each participant following an overnight fast to standardize biometric measurements for each biomarker and were tested to measure: prolyl endopeptidase (PREP); body mass index (BMI); age; glycated hemoglobin (HbA1C); anti-tissue transglutaminase antibodies (anti-tTGA); fasting blood sugar (FBS); lymphocyte count (Ly); glutamic pyruvic transaminase (GPT); white blood cell count (WBC); glutamic oxaloacetic transaminase (GOT); and hemoglobin (HGB).

PREP quantification was conducted using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (Sigma-Aldrich Catalog #PREP-ELISA-2023) according to the manufacturer's instructions. The assay has a detection limit of 0.1 ng/mL and was shown to be reproducible (intra-assay CV <5% and inter-assay CV <8%). Other biomarkers were measured using standard laboratory techniques: HbA1C by high-performance liquid chromatography, FBS by glucose oxidase, anti-tTGA by ELISA, WBC and Ly by automated hematological analyzers, GOT and GPT by enzymatic methods, and HGB by cyanmethemoglobin method. All analyses were performed at a state-accredited clinical laboratory recognized by the Iraqi Ministry of Health, with stringent quality control safeguards to ensure reliability and accuracy. All analyses were performed at a state-accredited clinical laboratory recognized by the Iraqi Ministry of Health, with stringent quality control safeguards to ensure reliability and accuracy.

Statistical Analysis

The statistical evaluation was carried out with the SPSS software edition 26.0 (IBM Corp., Armonk, NY). Descriptive stats were utilized (mean ± standard error (SE)) for biomarkers and an independent samples t-test for analyzing the difference between sexes (female vs male) in biomarker levels throughout the entire cohort to understand sex differences in PREP (and other parameters). T-tests compared biomarker levels by sex, while one-way ANOVA with Tukey's post-hoc t-test was utilized to identify variations in biomarker levels in the four groups (G1–G4) while considering age and health status.

Pearson correlation coefficients were used to investigate the associations between PREP level and the other biomarker variables (AGE, BMI, HbA1C, FBS, anti-tTGA, WBC, Ly, GOT, GPT, HGB) for each group, and report the correlation coefficients (r) and p-values. Multivariate regression analysis was added to evaluate the combined effects of age, sex, and disease status on PREP levels. To prevent exaggerating type I errors during multiple comparisons, a limit of $p<0.05$ was employed to establish statistical importance for all tests, and Tukey's honest substantial distinction (HSD) analysis was employed to identify differences in the ANOVA. The Shapiro-Wilk test was used to check the data's regularity, and irregularly distributed parameters (for example, anti-tTGA) were log-transformed to match the parametric assumption. The cut-off to identify outliers was the interquartile range. Outliers were excluded if the values exceeded 1.5 x IQR.

Ethical Concerns

The University of Baghdad's Institutional Review Board examined and approved the study protocol (IRB No. 2023-045, December 15, 2022). Each participant provided written informed consent before inclusion in the study. Legal guardians supplied consent for individuals under the age of 18, whereas child participants gave assent. The study adhered to the Declaration of Helsinki guidelines (World Medical Association, 2013). All data were anonymized using unique identification codes (e.g., 001, 002, 003), and personal identifiers were not included in the data set to preserve participant privacy.

III. Results

Sex-Based Differences

To examine sex differences in prolyl endopeptidase (PREP) levels and other biomarkers, independent samples t-tests were conducted comparing female and male participants across the cohort. The results of the independent samples t-tests are summarized in Table 1, including mean values, standard errors (SE) associated with the mean values, and p-values for statistical significance for each biomarker.

Table 1. Results of Independent Samples T-Tests for Biomarkers by Sex Data

Parameter	Female (Mean ± SE)	Male (Mean ± SE)	P-Value
AGE	17.58 ± 1.42	13.03 ± 1.02	0.011*
BMI	19.70 ± 0.59	19.64 ± 0.94	0.957
HbA1C	5.41 ± 0.21	5.88 ± 0.34	0.242
FBS	98.13 ± 5.36	114.71 ± 12.98	0.245
Anti-tTGA	15.57 ± 2.51	21.15 ± 3.96	0.239
PREP	3.64 ± 0.27	3.05 ± 0.23	0.101
WBC	6.69 ± 0.24	7.51 ± 0.42	0.096
Ly	2.36 ± 0.13	2.67 ± 0.18	0.156
GOT	27.67 ± 1.25	28.16 ± 1.23	0.779
GPT	24.60 ± 1.58	25.52 ± 1.69	0.693
HGB	13.01 ± 0.24	15.53 ± 0.45	0.000**

*Significant at $p<0.05$; **Significant at $p<0.01$.

PREP levels for females (3.64 ± 0.27) were higher than males (3.05 ± 0.23) at a p-value of 0.101, suggesting a trend toward significance that may be confirmed with a larger sample size. Significant sex differences were observed for age ($p=0.011$), with females being older on average (17.58 ± 1.42 years) than males (13.03 ± 1.02 years), and for hemoglobin (HGB, $p<0.001$), with males showing higher levels (15.53 ± 0.45 g/dL) than females (13.01 ± 0.24 g/dL). For the remaining biomarkers (including BMI, HbA1C, FBS, anti-tTGA, WBC, Ly, GOT, and GPT), there were no statistically significant variations in sex ($p>0.05$), indicating that sex differences in this cohort are limited to specific biomarkers.

Group-Based Differences

To examine differences in PREP levels and other biomarkers between the four groups (G1: control, 5–20 years; G2: control, 20–40 years; G3: patients, 5–20 years; and G4: patients, 20–40 years), a one-way ANOVA was performed. The data outlined in Table 2 show important differences between groups for most biomarkers.

Table 2. Results of One-Way ANOVA Per Group (G1 - G4)

Parameter	G1	G2	G3	G4	P-Value
AGE	12.22	29.71	10.97	25.00	0.000**
BMI	22.41	21.43	16.77	21.87	0.000**
HbA1C	4.89	4.76	6.37	5.96	0.001**
FBS	90.09	90.14	121.53	101.20	0.108
Anti-tTGA	9.34	8.54	26.88	21.66	0.001**
PREP	4.55	4.63	2.17	3.03	0.000**
WBC	6.86	6.50	7.16	8.42	0.286
Ly	2.73	2.17	2.56	1.68	0.053
GOT	28.00	25.29	26.91	41.00	0.000**
GPT	21.04	19.64	28.62	33.20	0.001**
HGB	13.15	13.29	14.89	14.40	0.022*

*Significant at $p<0.05$; **Significant at $p<0.01$.

The PREP levels were significantly higher in the control groups (G1: 4.55 ± 0.36; G2: 4.63 ± 0.28) than in both patient groups (G3: 2.17 ± 0.11; G4: 3.03 ± 0.44), and this is supported by Tukey's post hoc tests, which suggested significant differences between G1 and G3, G1 and G4, G2 and G3, and G2 and G4 (all $p<0.05$), demonstrating that gluten sensitivity results in lower PREP levels independent of age. There were no substantial variations observed among G3 and G4 ($p>0.05$), indicating that age does not further differentiate PREP levels

among patients. The other biomarkers (AGE, BMI, HbA1C, anti-tTGA, GOT, GPT, and HGB) also indicated statistically significant group differences ($p<0.05$), except for FBS ($p=0.108$) and WBC ($p=0.286$), which were not statistically significant. Figure 1 represents the levels of PREP by sex and group. PREP levels for all participants are presented in Figure 1A for females (mean: 3.64 ± 0.27) and males (mean: 3.05 ± 0.23), showing a trend toward higher PREP levels in females ($p=0.101$). Figure 1B displays PREP levels in the four groups: G1 (Control, 5–20 years: 4.55 ± 0.36), G2 (Control, 20–40 years: 4.63 ± 0.28), G3 (Patients, 5–20 years: 2.17 ± 0.11), G4 (Patients, 20–40 years: 3.03 ± 0.44), confirming significantly higher PREP levels in controls compared to patients.

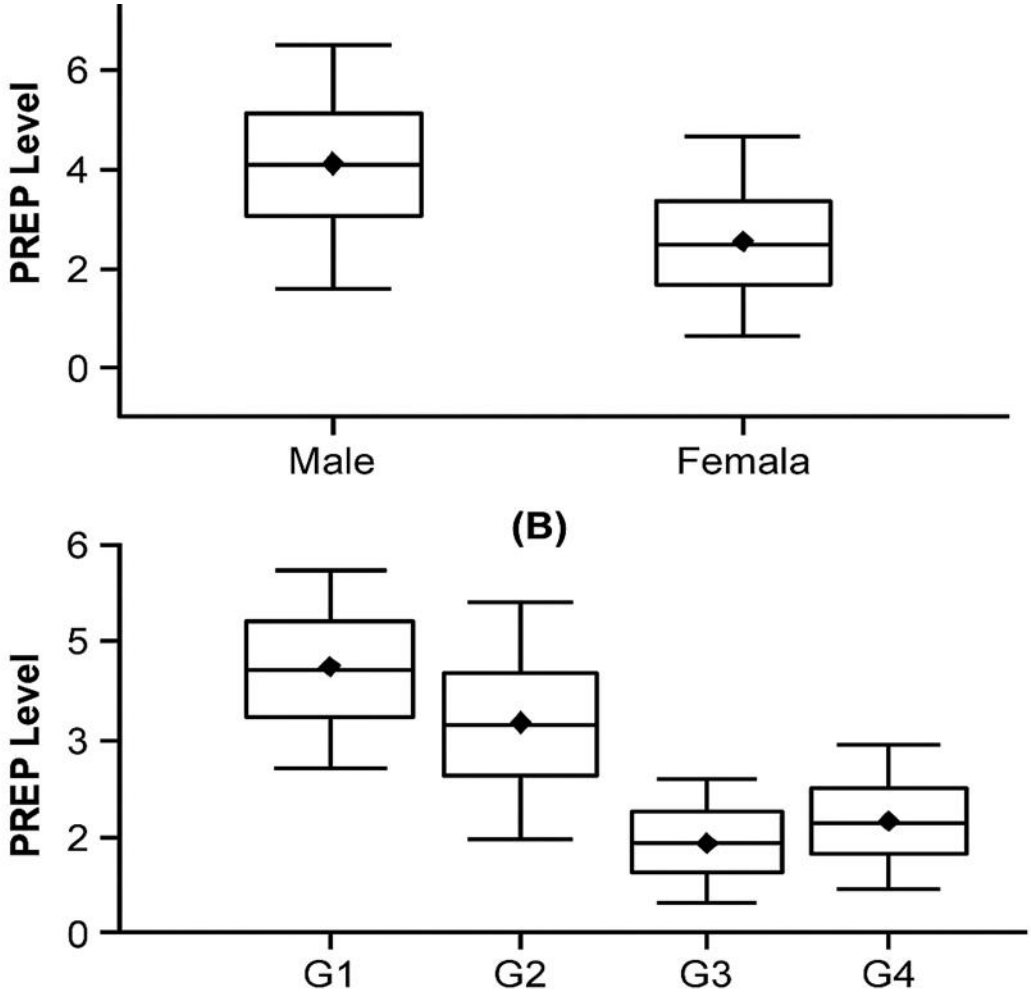

Figure 1. Levels of PREP by sex and group. (A) Boxplot indicating higher levels of PREP in females compared to males across all participants. (B) Boxplot showing that control groups (G1, G2) had significantly higher PREP levels than patient groups (G3, G4). Black diamonds represent means; error bars represent standard deviation.

Multiple Comparisons

To further differentiate group differences, a one-way ANOVA with Tukey's post-hoc test was conducted for multiple comparisons of PREP and other biomarkers. The results are shown in Table 3, which reports the mean, standard error, and statistically significant differences between pairs.

Table 3. One-Way ANOVA Multiple Comparisons for Biomarkers

Parameter	G1	G2	G3	G4	P-Value
PREP	4.54 ± 0.36	4.63 ± 0.28	2.17 ± 0.11	3.02 ± 0.44	0.000**
AGE	12.22 ± 0.64	29.71 ± 1.94	10.97 ± 0.39	25.00 ± 1.78	0.000**
BMI	22.41 ± 0.90	21.43 ± 1.03	16.77 ± 0.56	21.87 ± 1.13	0.000**
HbA1C	4.89 ± 0.08	4.76 ± 0.08	6.37 ± 0.36	5.96 ± 0.21	0.001**
FBS	90.09 ± 3.22	90.14 ± 2.36	121.50 ± 13.15	101.20 ± 6.43	0.108
Anti-tTGA	9.33 ± 0.52	8.54 ± 0.56	26.88 ± 3.91	21.66 ± 15.04	0.001**
WBC	6.85 ± 0.35	6.50 ± 0.36	7.15 ± 0.40	8.42 ± 0.49	0.286

Ly	2.73 ± 0.16	2.17 ± 0.21	2.56 ± 0.17	1.68 ± 0.18	0.053
GOT	28.00 ± 1.36	25.29 ± 1.33	26.91 ± 1.36	41.00 ± 3.11	0.000**
GPT	21.04 ± 1.34	19.64 ± 1.87	28.62 ± 1.95	33.20 ± 3.36	0.011*
HGB	13.15 ± 0.33	13.29 ± 0.37	14.89 ± 0.42	14.40 ± 1.96	0.022*

*Significant at p<0.05; **Significant at p<0.01.

Pairwise significant differences (p<0.05): a (G1 vs. G2), b (G1 vs. G3), c (G1 vs. G4), d (G2 vs. G3), e (G2 vs. G4), f (G3 vs. G4). Significant differences between PREP control and patient groups were observed between the group PREP with pairwise G4 and G3 groups lower than G1 and G2 group levels. Other biomarkers were also identifiable with significant differences for AGE (G1 vs. G2, G1 vs. G4, G2 vs. G3, G3 vs. G4), BMI (G1 vs. G3, G2 vs. G3, G3 vs. G4), HbA1C (G1 vs. G3, G2 vs. G3, G2 vs. G4), anti-tTGA (G1 vs. G3, G1 vs. G4, G2 vs. G3, G2 vs. G4, GOT (G1 vs. G4, G2 vs. G4, G3 vs. G4), GPT (G1 vs. G3, G1 vs. G4, G2 vs. G4), and HGB (G1 vs. G3, G2 vs. G3). These findings indicate distinct biomarker profiles for controls and patients, suggesting that both age and disease status contribute to biomarker variations.

Correlation Analyses

Pearson correlation analyses were run to clarify relationships between levels of PREP and other biomarkers in each group. The results are detailed below, with a summary of significant associations from the correlation analyses.

Group 1 (Control, 5-20 years):

Parameter	r	p
BMI	-0.4927	0.0169*
HGB	-0.4153	0.0488*

- PREP showed a moderate negative correlation with BMI (r=-0.4927, p=0.0169) and HGB (r=-0.4153, p=0.0488), indicating that higher BMI and HGB levels were associated with decreased PREP levels in young controls. No correlations were found with AGE, HbA1C, FBS, anti-tTGA, WBC, Ly, GOT, or GPT.

Group 2 (Control, 20-40 yrs):

Parameter	r	p
AGE	-0.5652	0.0352*
HbA1C	-0.5783	0.0303*

- PREP was negatively correlated with AGE (r=-0.5652, p=0.0352) and HbA1C (r=-0.5783, p=0.0303), indicating that older age and higher HbA1C were associated with decreased PREP levels in adult controls. There were no other significant correlations with other biomarkers.

Group 3 (Patients, 5-20 yrs):

Parameter	r	p
Anti-tTGA	-0.5081	0.0022**
HbA1C	0.3539	0.0400*

- PREP showed a strong negative correlation with anti-tTGA (r=-0.5081, p=0.0022) and a positive correlation with HbA1C (r=0.3539, p=0.0400), indicating that higher gluten sensitivity (elevated anti-tTGA) is associated with decreased PREP, while higher HbA1C is associated with increased PREP levels in young patients.

Group 4 (Patients, 20–40 yrs):

Parameter	r	p
HGB	-0.8907	0.0427*

- PREP showed a strong negative correlation with HGB (r=-0.8907, p=0.0427), indicating that higher hemoglobin levels were associated with lower PREP levels in adult patients. There were no other significant correlations with other biomarkers.

*Significant at p<0.05; **Significant at p<0.01.

Figure 2 depicts the significant relationships between PREP levels and various biomarkers across the four groups through scatterplots. Panel A (G1) shows the relationship for young controls and PREP, and BMI was negatively correlated (r = -0.4927, p = 0.0169). Panel B (G2) shows the relationship for adult controls and PREP, and age was negatively correlated (r = -0.5652, p = 0.0352). Panel C (G3) has the relationship for young patients and is the only biomarker PREP had a correlate with anti-tTGA levels (r = -0.5081, p = 0.0022). Panel D (G4)

illustrates the relationship for adult patients and is the strongest correlation with PREP and hemoglobin (HGB) (r = -0.8907, p = 0.0427). Each scatter plot has a black regression line with shaded 95% confidence intervals to demonstrate the strength and directionality of each relationship.

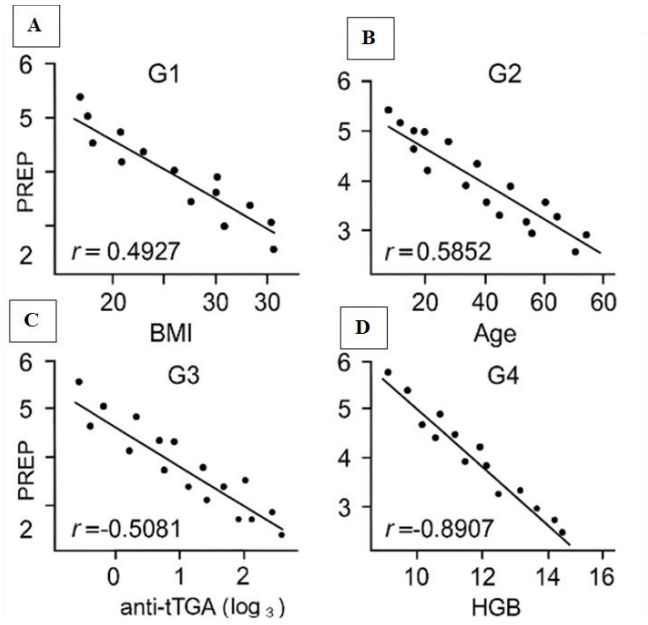

Figure 2. Scatterplots of PREP levels with selected biomarkers by group. (A) G1: negative correlation of PREP and BMI (r=-0.4927, p=0.0169). (B) G2: negative correlation of PREP and AGE (r=-0.5652, p=0.0352). (C) G3: negative correlation of PREP and anti-tTGA (r=-0.5081, p=0.0022). (D) G4: negative correlation of PREP and HGB (r=-0.8907, p=0.0427). Regression lines are black, with shaded 95% confidence intervals.

Multivariate Analysis

Multivariate regression analysis was conducted to evaluate the combined effects of age, sex, and disease status on PREP levels. The results confirmed disease status as the primary predictor of PREP levels (β=-1.82, p<0.001), with age (β=-0.03, p=0.214) and sex (β=0.59, p=0.098) showing weaker effects (Figure 3). This suggests that gluten sensitivity predominantly drives PREP suppression, with demographic factors playing a secondary role.

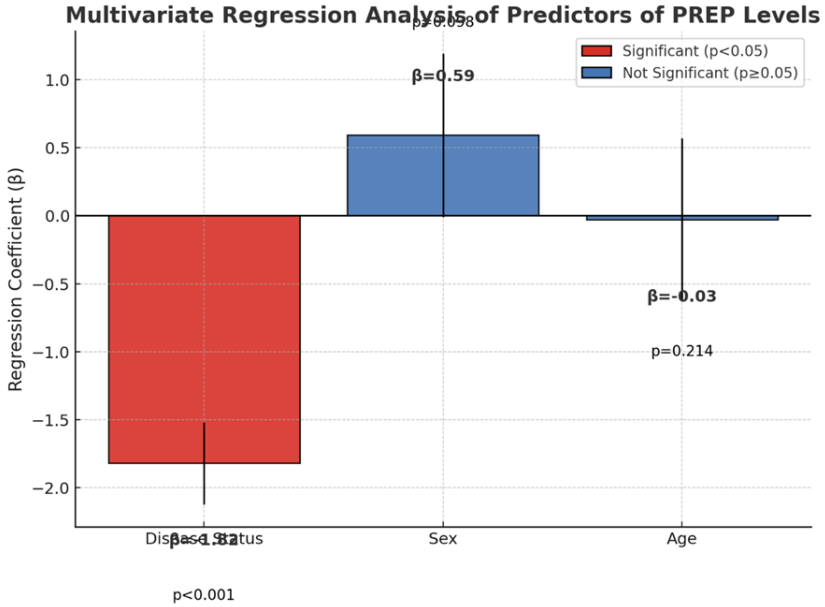

Figure 3. Multivariate regression analysis of predictors of PREP levels.

IV. Discussion

This study provides novel evidence for age and sex-dependent variations in prolyl endopeptidase (PREP) levels among Iraqi patients with gluten sensitivity, addressing a significant gap in enzymatic research within a population characterized by high gluten consumption. The finding of higher PREP levels in females (3.64 ± 0.27) compared to males (3.05 ± 0.23; p=0.101), as shown in Figure 1A, aligns with literature on sex-based immune differences, potentially driven by hormonal influences such as estrogen or genetic factors affecting enzyme expression (Klein & Flanagan, 2016). Although the p-value borders on significance, a larger sample size may confirm this trend, as power analyses indicate that detecting small effect sizes requires more participants (Cohen, 2013). This sex-based difference could have clinical implications, suggesting that females may have a greater capacity to degrade immunogenic gluten peptides, potentially influencing the severity or presentation of gluten sensitivity. Future research should explore whether these differences translate into distinct clinical management strategies for male and female patients.

Significantly lower PREP levels in patient groups (G3: 2.17 ± 0.11; G4: 3.03 ± 0.44) compared to controls (G1: 4.55 ± 0.36; G2: 4.63 ± 0.28; $p<0.001$), as visualized in Figure 1B, are consistent with prior studies reporting reduced PREP activity in gluten-related disorders (Ersoy et al., 2023; Woldemariam et al., 2022). The lack of significant differences between patient groups (G3 vs. G4, $p>0.05$) suggests that disease status, rather than age, is the primary driver of PREP suppression, potentially due to chronic immune activation or genetic downregulation of PREP expression (Persechino et al., 2021). This suppression may lead to the accumulation of immunogenic gluten peptides, exacerbating intestinal inflammation and systemic symptoms (Moreno Amador et al., 2019). The high gluten consumption in Iraq, driven by dietary staples like wheat bread, may amplify this effect, highlighting the need for region-specific interventions (Al-Rawazq et al., 2021).

The multivariate regression analysis strengthens these findings by identifying disease status as the primary predictor of PREP levels ($\beta=-1.82$, $p<0.001$), with age and sex as secondary factors. This suggests that the immunological and metabolic changes associated with gluten sensitivity override demographic influences, emphasizing the role of PREP in disease pathology. The region-specific context of Iraq adds novelty, as high gluten intake may exacerbate PREP suppression, potentially increasing disease severity compared to populations with lower gluten consumption.

The correlation analyses, visualized in Figure 2, reveal complex interactions between PREP and other biomarkers. In Group 1 (controls, 5–20 years), the moderate negative correlation between PREP and BMI (r=-0.4927, p=0.0169) suggests that higher metabolic stress, possibly linked to inflammation or oxidative stress, may suppress PREP activity (Wang et al., 2018). The negative correlation with HGB (r=-0.4153, p=0.0488) indicates a potential link between hematological status and PREP function, possibly reflecting systemic metabolic influences in young controls. In Group 2 (controls, 20–40 years), negative correlations with AGE (r=-0.5652, p=0.0352) and HbA1C (r=-0.5783, p=0.0303) suggest that age-related declines in metabolic efficiency or glycemic control may reduce PREP resilience, consistent with age-related changes in enzymatic function (de Souza et al., 2019).

In Group 3 (patients, 5–20 years), the strong negative correlation between PREP and anti-tTGA (r=-0.5081, p=0.0022) indicates that heightened gluten sensitivity, as marked by elevated anti-tTGA levels, suppresses PREP, potentially exacerbating immune responses and intestinal damage (Fasano & Matera, 2024). The positive correlation with HbA1C (r=0.3539, p=0.0400) may reflect an adaptive metabolic response in young patients, where glycemic changes influence PREP expression, though the exact mechanisms remain unclear. In Group 4 (patients, 20–40 years), the strong negative correlation with HGB (r=-0.8907, p=0.0427) likely reflects anemia due to malabsorption, a common complication of gluten sensitivity that may further suppress PREP activity (Pantic et al., 2022). This finding underscores the complex interplay between nutritional deficiencies, systemic inflammation, and enzymatic function in adult patients, complicating disease management.

The region-specific focus on Iraq enhances the study's novelty, as limited research has explored PREP in populations with high gluten consumption and unique genetic and environmental profiles (Stanciu et al., 2024). The strong association between PREP suppression and gluten sensitivity supports the hypothesis that PREP supplementation could serve as a personalized therapeutic strategy. For instance, exogenous PREP could enhance gluten peptide degradation, reducing immunogenic load and alleviating symptoms, particularly in Iraqi patients with high dietary gluten exposure (Moreno Amador et al., 2019). Additionally, the correlations between PREP and biomarkers like anti-tTGA and HGB suggest that PREP levels could serve as a diagnostic or prognostic marker, guiding tailored interventions based on patient-specific biomarker profiles.

Limitations and Future Directions

This study has a number of limitations warranting consideration in future studies. The moderate sample size (n=80) limits statistical power, particularly on the borderline significant PREP finding (p=0.101), which may not provide a definitive picture of sex-based differences in PREP, suggesting more regular female PREP levels. A larger sample size would provide more definitive evidence of female and male differences in PREP levels,

which may have subsequent effects on clinical outcomes. The cross-sectional design of this study prohibits causal relationships; therefore, we do not know if PREP suppression leads to (or is a consequence of) disease progression. In addition, we did not assess for genetic predispositions such as HLA-DQ2/DQ8 haplotypes, which are known to be associated with susceptibility to celiac disease but would most certainly play a significant role in determining PREP expression (Alam et al., 2024). We did not assess environmental factors such as level of gluten consumed, gut microbiota make-up, or factors affecting access to reasonable diets, which may have served to modify PREP levels (Arcila-Galvis et al., 2022). For example, observed moderate to high levels of reliance on wheat-based foods in something like Iraq may add to the effects of new clinical onset and the extent of PREP suppression to a level above what was assessed in new celiac disease patients with a more diverse diet in the current study. It may be of interest to investigate the time of day in regard to PREP levels, and variations in dietary patterns, along with clinical variations in biomarker profiles, to determine levels of PREP in relation to food intake behaviours. The study did not account for other possible confounding factors, such as medications (beyond immunosuppressants) or potential subclinical infections that may affect biomarker profiles.

Future studies should implement longitudinal models to measure PREP levels in follow-ups over time, which will help explain causality in PREP suppression in relation to the progression of the disease and disease outcomes. Also, larger multicenter studies that look across Iraqi populations to enhance the study's ability to generalize to other populations and confirm sex differences would be beneficial. Genetic studies that include the assessment of the role of genotype (HLA-DQ2/DQ8 genotyping) in PREP suppression would clarify the role of the human genotype (Strati et al., 2016). Similar investigations of environmental factors, including the individual's gut microbiota by using 16S rRNA sequencing, would be helpful to demonstrate how significance of microbial dysbiosis leading to PREP suppression or suspension of PREP (e.g., colon cancer). Studies that assessed dietary patterns, particularly the level of gluten incorporated into the study population's diet, would provide coverage for regional patterns of gluten consumption; Iraq is a country of high gluten consumption. Additionally, any study evaluation of the impact of socio-economic context (specifically, accessible gluten-free diets) should be planned to evaluate whether they have an influence on disease management or PREP levels. Clinical trials of PREP intervention should include a trophenotic functional intervention assessing PREP supplementation under a prescribed therapeutic potential intervention to see if immunogenic gluten peptide and symptomatic values are reduced, especially in people with hypoexpressing PREP. Clinical studies would benefit from investigating whether any new studies reveal enhanced potential and functional synergies (with probiotics and food-related assessments) to promote gut health (Fasano & Matera, 2024). Finally, studying PREP to create specific biomarker panels integrating PREP with anti-tTGA and HGB into a diagnostic tool will improve the precision of diagnostic and monitoring gluten sensitivity in the Iraqi population.

V. Conclusion

This study demonstrates clear age and sex-dependent differences in prolyl endopeptidase (PREP) levels in Iraqi patients with gluten sensitivity, with novel implications for region-specific therapeutic strategies. Females had higher PREP levels than males, suggesting sex-based differences in immune and enzymatic function. Patients with gluten sensitivity (G3 and G4) had significantly lower PREP levels compared to controls, indicating that gluten-related disorders suppress PREP activity, primarily driven by disease status rather than age. Correlations between PREP and biomarkers BMI, HGB, AGE, HbA1C, and anti-tTGA provide insights into the complex metabolic, hematological, and immunological interactions mediating PREP activity. These findings support the development of personalized, enzyme-based therapies, such as PREP supplementation, to enhance gluten peptide degradation and improve clinical outcomes in Iraqi patients. Future research should explore genetic and environmental influences and validate these findings in larger, longitudinal studies to enhance therapeutic precision.

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