



ISSN: 0067-2904

Assessment of IL-8 and TNF- α Status as Inflammatory Mediators in Celiac and Non-Celiac Gluten-Sensitive Disease

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Received: 1/2/2025

Accepted: 4/5/2025

Published: xx

Abstract

Celiac disease (CeD) and non-celiac gluten sensitivity (NCGS) are disease conditions associated with exposure to a protein called gluten. That displays overlapping symptoms but distinct immune mechanisms. The study aimed to evaluate serum levels of Interleukin-8 (IL-8) and Tumour Necrosis Factor-alpha (TNF- α) in Iraqi patients with CeD and NCGS compared to healthy controls. This case-control study enrolled 80 patients diagnosed with coeliac disease, 20 gluten-sensitive patients, and 80 healthy individuals aged between 18-50 years; this study was conducted in Al-Najaf, Iraq, between August and December 2024. CeD patients exhibited significantly elevated IL-8 and TNF- α levels compared to NCGS and controls, with a consequent $p < 0.001$. Anti-tTG Ig was positive and correlated with IL-8 ($p=0.001$) but moderately with TNF- α ($p=0.012$). For NCGS, moderate positive correlations were found between anti tissue-transglutaminase-IgA and IL-8 ($p=0.020$) and tTG.IgG and IL-8 ($p=0.037$). The present study reveals that CeD exhibits chronic inflammation marked by IL-8 and TNF- α elevation. Furthermore, IL-8 can serve as a predictive biomarker for distinguishing between NCGS and CeD; however, additional study is needed for NCGS.

Keywords: Celiac Disease, Non-Celiac Gluten Sensitivity, IL-8, TNF-a, Anti-tissue transglutaminase Antibody.

تقييم حالة الأنترلوكين-8 وعامل نخر الورم-ألفا في مرضى حساسية الحنطة ومرض حساسية الغلوتين

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الخلاصة

حساسية الحنطة (مرض السيلياك) وحساسية الغلوتين غير المرتبطة بالسيلياك هما حالتان مرضيتان مرتبطتان بالتعرض لبروتين الغلوتين، وتتشابه أعراضهما مع اختلاف الآليات المناعية الكامنة. هدفت الدراسة إلى تقييم مستويات إنترلوكين-8 (IL-8) وعامل نخر الورم-ألفا (TNF- α) في مصل الدم لدى مرضى عراقيين مصابين بالداء البطني وحساسية الغلوتين غير المرتبطة بالسيلياك مقارنةً بالأفراد الأصحاء. شملت هذه الدراسة الحالات والشواهد 80 مريضاً مُشخصاً بالداء البطني، و20 مريضاً بحساسية الغلوتين، تتراوح أعمارهم بين ثمانية عشر إلى خمسين عاماً، مع 80 فرداً سليماً، وأُجريت في محافظة النجف، العراق، بين أغسطس

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وديسمبر 2024. أظهر مرضى حساسية الحنطة ارتفاعاً ملحوظاً في مستويات إنترلوكين-8 وعامل نخر الورم-ألفا مقارنةً بمرضى حساسية الغلوتين غير المرتبطة بالسيلياك والأفراد الأصحاء، مع قيمة احتمالية معنوية عالية ($p < 0.001$). ارتبطت الأجسام المضادة لأنسجة الترانسغلوتاميناز بشكل إيجابي قوي مع إنترلوكين-8 مع دلالة معنوية ($P=0.001$)، وارتباطاً متوسطاً مع عامل نخر الورم-ألفا مع دلالة معنوية ($p=0.001$). أما في مجموعة حساسية الغلوتين غير المرتبطة بالسيلياك، فقد وُجدت ارتباطات إيجابية متوسطة بين الأجسام المضادة من نوع IgA وإنترلوكين-8 مع فرق معنوي ($p=0.020$)، وكذلك بين الأجسام المضادة من نوع IgG وإنترلوكين-8 مع ظهور دلالة معنوية ($p=0.001$). تكشف الدراسة الحالية أن حساسية الحنطة تتميز بالتهاب مزمن يُعزى إلى ارتفاع مستويات إنترلوكين-8 (IL-8) وعامل نخر الورم-ألفا (TNF- α). علاوةً على ذلك، يُمكن لـ IL-8 أن يعمل كعلامة حيوية تنبؤية للتمييز بين حساسية الغلوتين غير المرتبطة بالسيلياك والداء البطني، إلا أن هناك حاجة إلى دراسات إضافية لفهم الآليات المناعية لحساسية الغلوتين غير المرتبطة بالسيلياك بشكلٍ أعمق.

1. Introduction

Celiac disease is a persistent autoimmune disorder that manifests in genetically predisposed subjects upon the ingestion of wheat [1,2]. This medical condition arises from an extensive collaboration of genetic-based and non-genetic-based factors and immune dysregulation, leading to an inappropriate T-cell reaction against gluten. These inefficient reactions create modifications within the surface covering of the small tract, especially identified through atrophied villi, giving multiple clinical signs [3]. The prevalence is well known according to the epidemiology; estimates for CeD vary between 0.7% and 2.9% globally, affecting both sexes, with more prevalence in females than males. Furthermore, those with autoimmune disorders have a higher incidence [4]. Nevertheless, CeD is predominantly underdiagnosed in developing nations and has more significant effects on paediatrics [5].

Gluten-related conditions include coeliac disorders, allergic to wheat, and non-celiac gluten sensitivity (NCGS). NCGS is a distinct clinical entity where patients develop digestive (e.g., bloated stomach, loose stools) as well as non-digestive manifestations (e.g., headaches, exhaustion, neurological abnormalities) after gluten consumption, despite lacking CeD-specific antibodies or wheat allergy [6,7]. NCGS is linked to rye or wheat consumption and may involve neurological symptoms like limb numbness and anxiety [8-10]. However, its diagnosis remains controversial due to reliance on self-reported symptoms, absence of histopathological markers, and overlap with other conditions [11,12]. Prevalence estimates for NCGS range widely (0.5%–15%), complicating epidemiological assessments [13].

Serological screening for autoantibodies targeting tissue transglutaminase (anti-tTG) and gliadin (AGA) is a non-invasive diagnostic approach for CeD [14]. The disease mechanism involves gluten peptides crossing the intestinal epithelium, where transglutaminase 2 (TG2) deamidates them. These peptides are presented by HLA-DQ2/DQ8 molecules, activating gluten-specific CD4+ T cells. This triggers B-cell production of TG2 autoantibodies and deamidated gliadin antibodies alongside cytotoxic intraepithelial lymphocyte (IEL) responses that damage enterocytes and cause villous atrophy [15]. Cytokines like IL-8 surge within hours of gluten exposure, reflecting rapid T-cell reactivation and symptom onset [16].

In CeD, inflammatory cytokines such as TNF- α are overexpressed in the gut, correlating with disease severity and villous atrophy. In contrast, NCGS involves transient TNF- α release, which may explain extra-intestinal symptoms (e.g., fatigue, neurological issues) but lacks the autoimmune-driven intestinal damage seen in CeD [17]. Consequently, this study investigates the functions of chemokines with cytokines in the progression of diagnostic methods to differentiate between NCGS conditions and CeD in patients from Iraq.

2. Material and method

The study dealt with 180 participants: 80 diagnosed with CeD through serological screening and clinical examination, 20 with NCGS identified after ruling out celiac disorders and wheat allergy, and 80 healthy controls matched for age, sex, and socioeconomic standing recruited from different hospitals. The study involves participants aged 18–50 with confirmed CeD or NCGS. The Patients were diagnosed by specialized GIT physicians according to ACG clinical guidelines, while NCGS is confirmed through the exclusion of other IBD symptoms with resolution after gluten elimination. Individuals with autoimmune disorders, chronic inflammatory bowel disease, kidney or liver dysfunction, cancer, pregnancy, or active infections are excluded from the study. All participants underwent serological antibody detection for anti-tissue transglutaminase (tTG-IgA/IgG) and anti-gliadin (AGA-IgA/IgG) antibodies, which were conducted using chemiluminescence immunoassays (CLIA) on the IDS-iSYS multidisciplinary system from the Immunodiagnostic Systems Limited, United Kingdom Company. Results are considered positive if they are 10 AU/mL or higher and negative if they are below 10 AU/mL based on the manufacturer's instructions. Cytokine levels of IL-8, and TNF- α in serum were quantified using a sandwich ELISA method. By strictly following the manufacturer's procedure, ELISA kits from Sun-long Biotech, a Chinese company, were used to detect the concentration of selective cytokines, IL-8, and TNF- α , in all participant samples.

Statistical analysis

The analysis of data was done using SPSS edition 23. Descriptive statistics were calculated for all variables, with categorical data described as percentages and frequency, whereas the continuous variables were displayed as the mean \pm standard deviation, as well as Pearson correlation. The analysis of variance (ANOVA) was conducted, followed by a post hoc test of least significant differences (LSD) to compare among parametric data. We employed the Kruskal-Wallis test to assess non-parametric data, whereas the categorical comparisons were studied using the Pearson Chi-square test (χ^2 -test). The level of statistical significance was established at $p < 0.05$.

3. RESULTS

The analysis revealed that the age distributions among the groups were comparable, with the majority of participants (42.5%) falling within the age range of 31 to 40 years, followed by 37.2% in the 21 to 30 years category, and 11.1% under the age of 21. It is noteworthy that, although the overall distribution of participant sex did not reveal statistically significant differences ($P = 0.440$), with females constituting 66.3% of the total group, a subgroup analysis indicated a significant disparity between patients with celiac disorder and the healthy control group ($P = 0.004$), as detailed in Table 1.

Table 1: Differences in age group and sex across study groups.

Variables			Groups (N=180)			Total	P-value
			Celiac Disease N=80	Gluten Sensitivity N=20	Healthy Control N=80		
Age groups (years)	<21	Count%	8 10%	4 20%	8 10%	20 11.1%	0.923
		P-value	0.689	0.164	0.689		
	21-30	Count%	30 37.5%	6 30%	31 38.8%	67 37.2%	
		P-value	0.920	0.484	0.689		
	31-40	Count%	34 42.5%	8 40%	33 41.3%	75 41.7%	
		P-value	0.841	0.841	0.920		
	>40	Count%	8 10%	2 10%	8 10%	18 10%	
		P-value	0.987	0.987	0.987		
Total	Count%	80 100%	20 100%	80 100%	180 100%		
P-value		0.783	0.262	0.637			
Sex	Male	Count%	27 33.7%	9 45%	34 42.5%	70 38.9%	0.440
	Female	Count%	53 66.3%	11 55%	46 57.5%	110 61.1%	
Total	Count%	80 100%	20 100%	80 100%	180 100%		
P-value		0.004	0.655	0.219			

The socio-demographic evaluation indicated that 81.3% of patients with CeD resided in urban areas, compared to 70% of individuals with NCGS and 80% of the control group (P = 0.531). A significant discrepancy was identified in family tendency, with 81.3% of CeD patients reporting a positive family history, in contrast to 35% of NCGS subjects and none of the controls (P < 0.001). Employment status (5% employed, 87.5% unemployed) and marital status (71.3% married) did not exhibit significant intergroup differences (P > 0.05), as detailed in Table 2.

Table 2: Variations in sociodemographic characteristics across study groups

Characteristics		Groups (N=180)			Total	P-value
		Celiac Disease N=80	Gluten Sensitivity N=20	Healthy Group N=80		
Residency	Urban	Count% 65 81.3%	14 70%	64 80%	143 79.5%	0.531
	Rural	Count% 15 18.8%	6 30%	16 20%	37 20.5%	
Count% Total		80 100%	20 100%	80 100%	180 100%	
P-value		0.302	0.076	0.285		
Occupation	Employed	Count% 4 5%	2 10%	8 10%	14 7.8%	
	Student	Count 6 7.5%	2 10%	6 7.5%	14 7.8%	
		%	70 87.5%	16 80%	66 82.5%	152 84.4%
	unemployed	Count 70 87.5%	16 80%	66 82.5%	152 84.4%	
Count% Total		80 100%	20 100%	80 100%	180 100%	
P-value		0.216	0.054	0.240		
Marital status	Single	Count% 23 28.8%	6 30%	28 35%	57 31.7%	0.687
	Married	Count% 57 71.3%	14 70%	52 65%	123 68.3%	
Count% Total		80 100%	20 100%	80 100%	180 100%	
P-value		0.192	0.076	0.173		
Family history	Yes	Count% 65 81.3%	7 35%	0 0.0%	72 40%	
	No	Count% 15 18.8%	13 65%	80 100%	108 60%	
Count% Total		80 100%	20 100%	80 100%	180 100%	
P-value		0.001	0.458	1.000		

#P-value describes the probability level statistically

In addition, serological profiling revealed significantly elevated levels in patients with CeD compared to those with NCGS and the control group. Specifically, anti-tTG-IgA levels were recorded at 79.411 ± 43.209 AU/mL for CeD, compared to 10.439 ± 3.889 AU/mL for NCGS and 2.3325 ± 1.975 AU/mL for controls, upon a large effect size ($\eta^2 = 0.639$) explaining 63.9% of the variance. While anti-tTG-IgG levels were 76.263 ± 51.508 AU/mL for CeD, 21.031 ± 9.710 AU/mL for NCGS, and 11.119 ± 2.318 AU/mL for controls ($P < 0.001$), with a moderate effect size ($\eta^2 = 0.301$), explaining 30.1% of variance. Similarly, AGA-IgA (68.318 ± 61.321 AU/mL for CeD vs. 5.2200 ± 2.142 AU/mL for NCGS vs. 17.111 ± 7.634 AU/mL for controls, upon small effect size ($\eta^2 = 0.217$), explaining 21.7% of variance) and AGA-IgG (61.133 ± 57.426 AU/mL for CeD vs. 4.4700 ± 2.081 AU/mL for NCGS vs. 11.850 ± 5.806 AU/mL for controls, alongside moderate effect size ($\eta^2 = 0.268$), explaining 26.8% of variance) demonstrated significant intergroup differences ($P < 0.001$), as illustrated in Table 3.

Table 3: Difference in serological parameters level among studied groups

Parameters (AU / mL)		Groups (N=180)			P-value	Effect Size (η^2)**
		Celiac Disease N=80	Gluten Sensitivity N=20	Healthy Individual N=80		
tTG.IgA	Mean \pm S.D	79.411 \pm 43.209	10.439 \pm 3.889	2.3325 \pm 1.975	0.001[#]	0.639
tTG.IgG	Mean \pm S.D	76.263 \pm 51.508	21.031 \pm 9.710	11.119 \pm 2.318	0.001[#]	0.301
AGA.IgA	Mean \pm S.D	68.318 \pm 61.321	5.2200 \pm 2.142	17.111 \pm 7.634	0.001[*]	0.217
AGA.IgG	Mean \pm S.D	61.133 \pm 57.426	4.4700 \pm 2.081	11.850 \pm 5.806	0.001[*]	0.268

(#) Analysis of variance (ANOVA); post hoc test of least significant differences (LSD); *The Kruskal-Wallis test was employed to compare non-parametric data of the gluten-sensitive groups; P-value describes the probability level statistically; ** η^2 describes Eta squared level.

Immunological analysis indicated elevated levels of pro-inflammatory cytokines in patients with CeD. Interleukin-8 (IL-8) concentrations were significantly higher in CeD patients (56.664 \pm 19.650 pg/mL) compared to those with NCGS (24.082 \pm 7.208 pg/mL; $P < 0.001$) and controls (28.551 \pm 16.554 pg/mL; $P < 0.001$); upon moderate effect size ($\eta^2 = 0.390$), explaining 39% of the variance. TNF- α levels exhibited a similar trend, with CeD patients showing markedly increased values (44.241 \pm 21.891 pg/mL) compared to NCGS (21.026 \pm 5.285 pg/mL; $P < 0.001$) as well as controls (15.583 \pm 12.451 pg/mL; $P < 0.001$); upon moderate effect size ($\eta^2 = 0.390$), explaining 39.5% of the variance, as detailed in Table 4.

Table 4: Difference in immunological markers level among studied groups

Parameters (pg /ml)		Groups (N=180)			P-value	Effect Size (η^2)**
		Celiac Disease N=80	Gluten Sensitivity N=20	Healthy Subjects N=80		
IL-8	Mean \pm S.D	56.664 \pm 19.650	24.082 \pm 7.208	28.551 \pm 16.554	0.001[*]	0.390
TNF Alpha	Mean \pm S.D	44.241 \pm 21.891	21.026 \pm 5.285	15.583 \pm 12.451	0.001[*]	0.395

P-value describes the probability level statistically; *The Kruskal-Wallis test was employed to compare non-parametric data of the gluten-sensitive groups; ** η^2 describes the Eta squared level.

Furthermore, the correlative analyses conducted among CeD demonstrated a strong positive association between anti-tTG-IgA and IL-8 ($r = 0.630$, $P < 0.001$), while anti-tTG-IgG exhibited a moderate correlation with IL-8 ($r = 0.328$, $P = 0.041$) and TNF- α ($r = 0.275$, $P = 0.012$). These findings are illustrated in Table 5.

Table 5: Association between immunological markers level among studied groups

Immunological Biomarker		Celiac Disease(N=80)	
		Pearson Correlation	P-value
tTG.IgA (AU/ml)	IL-8	0.630	0.001**
	TNF-Alpha	0.158	0.053
tTG.IgG (AU/ml)	IL-8	0.328	0.041[*]
	TNF-Alpha	0.275	0.012[*]
AGA.IgA (AU/ml)	IL-8	0.140	0.200
	TNF-Alpha	0.200	0.071
AGA.IgG (AU/ml)	IL-8	0.190	0.110
	TNF-Alpha	0.220	0.080

P-value describes the probability level statistically; **the correlation coefficients statistical significance levels are set at 0.001; * at the 0.05 level.

On the other hand, subjects with NCGS, both anti-tTG-IgA, and anti-tTG-IgG, demonstrated significant positive correlations with IL-8 ($r = 0.515$, $P = 0.020$; $r = 0.486$, $P = 0.037$, respectively), although no such associations were observed with TNF- α ($P > 0.05$), as described in Table 6.

Table 6: Correlation between selective markers in gluten sensitivity subjects

Immunological Biomarker		Non-Celiac Disease(N=20)	
		Pearson Correlation	P-value
tTG.IgA (AU/ml)	IL-8	0.515	0.020*
	TNF-Alpha	0.300	0.250
tTG.IgG (AU/ml)	IL-8	0.486	0.037*
	TNF-Alpha	-0.165	0.484

P-value describes the probability level statistically; *the correlation coefficients statistical significance levels are set at the 0.05 level.

4. Discussion

Celiac disorders, or gluten-sensitive enteropathy, are chronic inflammatory conditions that impact the small bowel's epithelial and lamina propria layers in individuals with a genetic predisposition [18]. These disorders can develop at any age, and individuals may initially test negative but later exhibit symptoms. For instance, someone who tests negative for gluten intolerance at 50 may still develop it by age 65 [19]. The research focused on case-control patients aged eighteen to fifty years with coeliac sprue, comparing them to age-matched non-celiac gluten-sensitive individuals and healthy controls without age restrictions. The study found no significant differences in age or sex among the entire groups, reducing confounding factors and enabling a more accurate assessment of the effects being studied. Despite that, the subgroup comparisons indicate a significant sex-based difference between patients with celiac disease and healthy controls. This finding is consistent with established epidemiological data, which suggest that celiac disease is more prevalent in females. The results from this study also match with that expressed by Abd-Alrazaaq, [20], which suggests that it was found to be non-statistically significant differences between the ages of patient groups, both celiac and non-celiac disease, and the control, particularly when comparing overall groupings. Similarly, these investigations found no significant differences in sex assistance studies between the CeD, NCGS group, and the healthy group, comparing entirely, aligning with previous research done by Abd and Hamad [3].

Concerning a comparison of subgroups, several local studies conducted by Eiden and Mubark ; Abdullah and Al-Badran [21,22] indicate that there are statistically significant differences based on sex in celiac disease patients. These studies reveal a higher prevalence of celiac disease among women in comparison to men, thereby underscoring that women are predominantly affected. It is suggested that sex hormones, especially estrogen, may affect the regulation of the immune response, thereby increasing the susceptibility to autoimmune illnesses like CeD in women, highlighting that women are predominantly affected, suggesting a gender disparity in CeD incidence.

Despite a gluten-free diet (GFD), serum (IL-8) remains high for a long time due to duodenal healing. This finding may help identify CeD in GFD-abiding individuals [23]. Patients with CeD had a higher number of cells that produce IL-8 compared to both controls and patients with Non-Celiac Self-Reported Wheat Sensitivity (NCSRWS). The research discovered that those with CeD have a more robust inflammatory response than individuals with NCSRWS, who do not produce IL-6 or IL-8 but express cytokines that cause inflammatory disorders [24]. Recently, postulates suggested that circulating cytokines could be useful as biomarkers for distinguishing between CeD and NCGS, and IL-8 was identified as an extremely effective cytokine for demonstrating prejudice between both medical conditions [6].

TNF- α is a key pro inflammatory cytokine that impacts immune cells, leading to cell death and the creation of additional cytokines that promote inflammation. Increased levels of TNF-alpha in serum may assist in detecting CeD, especially in instances with inconclusive biopsy results [25]. Following six hours of stimulation, TNF- α was detected over or equal to the estimation threshold in the medium used for stimulation among the majority of TCCs [26].

Similarly, individuals with sensitivity to gluten and refractory celiac disease (RCD) often have different cytokine profiles, particularly in relation to TNF- α levels.

TNF-alpha levels have dramatically risen in the rectal mucosa of individuals with CeD and non-celiac wheat sensitivity (NCWS), particularly in specific immune cells. This level rise could modify both intestinal and systemic symptoms and might be connected to TLR pathways, showing that gluten causes an innate immune response [27].

Present research on the attitude of antibodies (anti-tTG IgA) showed that the strong positive linkage of IL-8 is significant, whilst IL-8 has a non-significant linkage to AGA-immunoglobulin-A. Additionally, celiac disorder patients exhibited a positive correlation between tumor necrosis factor-alpha and IgG isotypes of anti-gliadin autoantibodies. The additional studies addressed the link involving levels of cytokines and human serum anti-TTG IgA concentrations. Excluding for INF-gamma, anti-TTG IgA levels showed strong positive associations with all other cytokines [3].

Furthermore, the current study indicates that various cytokines play a role in the humoral response in CeD. This role particularly promotes the differentiation of plasma cells that produce immunoglobulin-A (IgA) in the intestinal mucosal layer [20].

The immune reaction to high amounts of tissue transglutaminase immunoglobulin-A (tTG-IgA) in NCGS is not well known and may vary from that in CeD. This complexity in gluten-related diseases shows a need for further study into the immune reactions involved [28]. Negative association between gluten sensitivity and immunoglobulin G (IgG) types of anti-gliadin antibodies (AGA), with a moderate relationship between AGA IgG and NCGS. While IgG-AGA may assist in diagnosing NCGS, its low relevance and reliability hamper its practical usage [3,29]. Further, future studies are required to develop better gluten-sensitivity biomarkers and expand knowledge of the syndrome and its immunological features.

5. Conclusion

The current study demonstrates that CeD is characterized by chronic inflammation, as indicated by elevated levels of IL-8 and TNF- α . Moreover, IL-8 has the potential to function as a predictive biomarker for differentiating between NCGS and CeD; however, further research is required to investigate NCGS.

Ethical Certification

This research received approval from the Committee of Medical Ethics, reference number (MEC-74), Medical College, Kufa University, and full authorization from the Najaf Health Directorate.

Disclosure and conflict of interest

The authors declare that they have no conflicts of interest.

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