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Article

Serum IL-15 and IL-21 as Diagnostic and Severity Biomarkers in Treatment-Naïve Celiac Disease: A Case-Control Study

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Abstract: Celiac disease (CD) is an enteropathy characterized by chronic immune-mediated intestinal damage due to dietary gluten. The consequence of celiac disease is a gradual decline of the small intestinal mucosal layer, dysregulated cytokine signaling, and a deranged balance between the two branches of the immune system (innate and adaptive). The pro-inflammatory cytokines IL-15 and IL-21 are involved as mediators for both innate and adaptive immunity in the development of celiac disease; however, very limited research has been conducted on the levels of IL-15 and IL-21 in patients with celiac disease prior to treatment (i.e., "treatment-naive") due to insufficient data that indicates their diagnostic usefulness and ability to reflect the severity of the disease in the population living in the Arab states of the Middle East (ME). In view of this gap in the existing literature, we aim to evaluate the concentrations of IL-15 and IL-21 in serum of treatment-naive patients with celiac disease to establish their utility as non-invasive markers for diagnosing celiac disease and assessing disease severity. Design: This was a prospective case-control study of 40 celiac disease patients who had never been treated and 40 matched healthy controls from the being seen in the Gastroenterology Unit at Al Diwaniyah Teaching Hospital, Iraq between October 2025 - April 2026. Serum concentrations of IL-15 and IL-21 were determined by using a sandwich enzyme-linked immunosorbent assay (sandwich ELISA). The Marsh-Oberhuber histopathological classification was used to evaluate disease severity. Receiver Operating Characteristic (ROC) curve analysis was then used to evaluate diagnostic accuracy. Pearson's Correlation Analysis was used examine the relationship between cytokine concentrations and disease severity criteria. The results of our study indicate that there were significantly higher levels of serum IL-15 in patients with celiac disease as compared to healthy control subjects (148.6 ± 28.4 pg/mL vs. 42.3 ± 11.7 pg/mL; P = 0.001). Thus, patients had approximately 3.5 times the level of IL-15 than did the healthy controls. In addition, IL21 serum levels were found to be significantly higher among celiac disease patients than among healthy control subjects (312.4 ± 54.7 pg/mL vs. 87.6 ± 21.3 pg/mL; P < 0.001), with approximately 3.6 times the level of serum IL-21 in celiac patients compared to healthy controls. The results of receiver operating characteristic (ROC) curve analysis also showed high sensitivity and

specificity for both IL-15 (> 85.5pg/ml cutoff; 92.5% and 95%, respectively) and IL-21 (>178.3 pg/ml cutoff; 95% and 97.5%, respectively). The area under the ROC curves (AUC) were il15 [AUC 0.981 (95%CI 0.956-1.000)] and il21 [AUC 0.994 (95%CI 0.985-1.000)] therefore indicating that these two measures would be useful as diagnostic tools for the diagnosis of celiac disease. IL-15 and il-21 serum levels were positively related to Marsh/Oberhuber histological staging ($r = 0.782$, $P < 0.001$ and $r = 0.841$, $P < 0.001$) as well as IL-15 being positively related to IL-21 ($r = 0.614$; $P < 0.001$). Concluding Remarks: Elevated serum levels of interleukin-15 and interleukin-21 from untreated patients with celiac disease demonstrate systemic elevation of both the innate and adaptive pro-inflammatory immune pathways. The diagnostic accuracy of both cytokines exceeded 0.98 AUC, suggesting they may serve as non-invasive biomarkers for the diagnosis of celiac disease, while also demonstrating correlation with the severity of histopathological mucosal damage based upon the Marsh-Oberhuber grading system. Therefore, the serum levels of interleukin-15 and interleukin-21 represent promising non-invasive biomarkers for diagnosis and classification of the severity of celiac disease, with potential for use as monitoring or assessment of clinical response to therapy.

Keywords: Celiac disease, Interleukin-15, Interleukin-21, Inflammatory cytokines.

Introduction

Celiac disease (CD) is an autoimmune disorder caused by the ingestion of gluten in genetically predisposed subjects, and it involves small intestine damage, which manifests itself as a consequence of villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis [1]. The prevalence of the disease in the general population is estimated to be around 1.4%, with a rising number of cases reported in the Middle East due to changes in diet and better diagnostic capabilities [2]. Despite being widespread, celiac disease still lacks diagnosis in many patients due to its wide array of symptoms and possible clinical manifestations [3]. The pathophysiology of CD includes a combination of genetic (HLA-DQ2/DQ8) and environmental (gluten) factors together with altered immune response in the gut (4). Central to this process is an imbalance in cytokine signaling — particularly an excess of pro-inflammatory mediators over regulatory counterparts — which drives and perpetuates intestinal tissue damage[4].

IL-15, a pro-inflammatory cytokine similar to IL-2, is mainly secreted by intestinal epithelial cells and innate immune cells due to gluten-mediated epithelial cell damage [5]. It is critical for the activation and proliferation of IELs using NKG2D receptor-mediated signaling pathways, conferring upon them the ability to cause cytotoxic damage to enterocytes [6]. In addition to its cytotoxic properties, IL-15 causes disruption of the tolerance-promoting activities of Treg cells, leading to an increased adaptive immune response towards gliadin peptides, and thus perpetuating inflammation [7]. In clinical settings, elevated levels of intestinal IL-15 expression have been shown to correlate positively with histologically graded intestinal damage according to the Marsh-Oberhuber classification system [8].

IL-21, mainly produced by activated CD4+ T helper and T follicular helper cells, plays a role in modulating several immune responses through the JAK1/STAT3 signaling pathway [9]. IL-21 is described as a key modulator of the adaptive immune cascade in celiac disease, stimulating differentiation of B cells into plasma cells generating disease-specific autoantibodies anti-tissue transglutaminase (anti-tTG) and anti-deamidated gliadin peptide (anti-DGP), alongside with

promoting Th17 proliferation and inhibiting the action of Tregs [10] Increased levels of IL-21 have been demonstrated in the duodenal mucosa of celiac patients prior to starting gluten-free therapy, proving its importance in causing the autoimmune reaction triggered by gluten consumption [11]. Significantly, IL-21 cooperates with IL-15 for activation of IELs, as well as potentiating the cytotoxic effect mediated by NKG2D, creating an immunological axis where innate responses mediated by IL-15 combine with adaptive responses modulated by IL-21, resulting in the complete pathogenesis of celiac disease [12].

Despite a detailed understanding of the involvement of IL-15 and IL-21 within mucosal tissues, the levels of these two molecules in circulation have not been fully elucidated nor their usefulness as diagnostic markers that do not require invasive sampling techniques. The scientific community has predominantly studied these two cytokines in tissues rather than blood, yielding little information on the systemic levels of IL-15 and IL-21 in untreated individuals [13], [14]. Alternatively, the immunologic spectrum of celiac disease among Middle Eastern populations is not well-defined. The HLA haplotypes, dietary intake, environmental factors, and intestinal microbial composition that are prevalent in this geographic area can result in unique cytokine expression levels when compared with individuals from Western and East Asian cultures [15]. Considering the rising prevalence of celiac disease in Iraq and other surrounding nations, there is an urgent need to investigate the immune response spectrum in this geographic region [16].

It is hypothesized that celiac patients who remain untreated will have significantly higher levels of serum IL-15 and IL-21 when compared to healthy controls, and that these will be proportionate to the level of intestinal damage in terms of histopathology. Therefore, the objectives of the current research will be to: (1) estimate and compare the serum levels of IL-15 and IL-21 in untreated patients of celiac disease with healthy controls; (2) evaluate the ability of these cytokines to diagnose celiac disease as biomarkers using ROC curve analysis; (3) determine the relationship between serum levels of IL-15 and IL-21 as mediators of a common pro-inflammatory pathway; and (4) explore the relationship between serum levels of these cytokines and the level of histopathological severity as determined according to Marsh-Oberhuber classification. The results of this investigation will be expected to provide new information on the systemic immunopathology of celiac disease among Iraqis, as well as the practical significance of IL-15 and IL-21 as biomarkers.

Materials and Methods

2.1 Study Design and Participants

The current study adopted a prospective case-control design that was carried out at the Gastroenterology Unit of Al-Diwaniyah Teaching Hospital, Al-Diwaniyah, Iraq. The duration of the experiment lasted from October 1, 2025, to April 1, 2026. The objective of this research was to determine the serum levels of interleukin-15 (IL-15) and interleukin-21 (IL-21) in treatment-naïve patients suffering from celiac disease relative to control subjects. The number of the samples was 80 individuals, 40 patients with celiac disease and 40 control subjects. The sample size of the patients with celiac disease was entirely composed of treatment-naïve individuals without a gluten-free diet, steroids, immunosuppressants, and biologics before participation in the research.

2.2 Inclusion and Exclusion Criteria

As regards participants with CD, the inclusion criteria included: (1) CD as confirmed through positive serology (anti-tissue transglutaminase IgA [anti-tTG IgA] and/or anti-deamidated gliadin peptide IgG [anti-DGP IgG]) together with characteristic Marsh-Oberhuber findings on duodenal biopsy; (2) ages between 18 to 60 years old; (3) treatment-naïve patients that had not yet gone on a gluten-free diet as well as being unexposed to any forms of corticosteroids, immunosuppressants, and biologics; and (4) possession of comprehensive clinical, serologic, and histopathologic information. The exclusion criteria include: (1) comorbidity conditions involving autoimmune disease such as rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes mellitus, or autoimmune thyroid disease; (2) existence of any chronic infection like hepatitis B, hepatitis C, tuberculosis, or HIV; (3) malignancy or having malignancy within the last five years; (4) pregnancy and/or lactation; (5) coexisting diseases of other types of gastrointestinal ailments such as inflammatory bowel disease, irritable bowel syndrome, or microscopic colitis; and (6) refusal to participate or insufficient clinical information.

Healthy subjects were identified among those who visited the clinic for general health screening or came along with patients to the hospital. The selection criteria for healthy subjects included the following: (1) age ranging from 18 to 60 years; (2) absence of history of celiac disease, gastrointestinal problems, and other inflammatory or autoimmune disorders; (3) negative serologic test for celiac disease at the screening stage; and (4) voluntary participation with written informed consent. The age (±5 years) and sex were matched between celiac disease and healthy subjects.

Table (2-1): Inclusion and Exclusion Criteria for Celiac Disease Patients.

Inclusion Criteria	Exclusion Criteria
Confirmed celiac disease based on positive anti-tTG IgA and/or anti-DGP IgG serology and confirmatory duodenal biopsy	Other autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes mellitus, or autoimmune thyroid disease)
Age between 18 and 60 years	Chronic infections (hepatitis B, hepatitis C, tuberculosis, or HIV)
Treatment-naïve status: no prior gluten-free diet, corticosteroids, immunosuppressants , or biological agents	Malignancy or history of malignancy within the past five years
Complete clinical, serological , and histopathological data	Pregnancy or lactation
Willingness to provide written informed consent	Other gastrointestinal diseases (IBD, irritable bowel syndrome, or microscopic colitis)
—	Refusal to participate or incomplete clinical data

2.3 Evaluation of the Level of Severity of Disease

Celiac disease patients' severity of disease was assessed using the internationally recognized and validated histopathological system for measuring the level of damage of the intestinal mucosa caused by celiac disease using the Marsh-Oberhuber Classification (Marsh-Oberhuber 1989). The classification is based on three histopathological criteria: intraepithelial lymphocyte density (IEL) number per 100 enterocytes, degree of crypt hyperplasia, and degree of villous atrophy. The Marsh-Oberhuber Classification is a system of grading the histology of celiac disease and is classified as follows: Marsh 1 has normal villous architecture, IEL count greater than 25 per 100 enterocytes, and no crypt hyperplasia; Marsh 2 has normal villi and presence of IEL and crypt hyperplasia; Marsh 3a has partial atrophy of villi; Marsh 3b has subtotal atrophy of villi; and Marsh 3c has total atrophy of villi and has a flat mucosal surface. For the purposes of this study, patients were classified into three levels of severity; mild (Marsh 1-2), moderate (Marsh 3a-3b), and severe (Marsh 3c). Gastroenterologists with experience in performing upper gastrointestinal endoscopy obtained biopsy specimens of the duodenum, which were assessed by pathologists who were blinded to the clinical information of the patients.

Table (2-2): Marsh-Oberhuber Classification for Histopathological Grading of Celiac Disease.

Grade	Villous Architecture	IEL Count (/100 enterocytes)	Crypt Hyperplasia	Severity
Marsh 1	Normal	> 25	Absent	Mild
Marsh 2	Normal	> 25	Present	Mild

Marsh 3a	Partial villous atrophy	> 25	Present	Moderate
Marsh 3b	Subtotal villous atrophy	> 25	Present	Moderate
Marsh 3c	Total villous atrophy	> 25	Present	Severe

2.4 Blood sample collection and processing

The peripheral venous blood (5 mL) was collected under sterile conditions from each subject using standard phlebotomy technique. Blood was drawn into plain tubes (no anticoagulant added) to permit subsequent separation of serum. Blood was allowed to clot (30 min at room temperature) and then centrifuged at 3000 x g for 15 min at 4°C. The serum that was resulting from this process was aliquoted into sterile microcentrifuge tubes and frozen at -80°C until analysis. Samples were processed no longer than 2 hours after collection to ensure stability of cytokines and to minimize repeated freezing and thawing.

2.5 Quantification of Serum IL-15 and IL-21 Levels

To measure the level of interleukin-15 (IL-15) and interleukin-21 (IL-21) in serum samples we used a sandwich ELISA (enzyme-linked immunosorbent assay). All assays were performed according to the manufacturer's protocol. The basic methodology involved utilizing 96-well plates coated with specific capture antibodies against IL-15 or IL-21, adding serum samples in duplicate and incubating for 90 minutes at 37 °C. After this incubation, wells were washed four times with wash buffer in order to remove unbound materials. Biotinylated detection antibodies were then added and the wells were incubated for an additional 60 minutes at 37 °C; after the incubation the wells were washed four times. Streptavidin-HRP (horseradish peroxidase) was then added and incubated at 37 °C for 30 minutes; after the final wash, tetramethylbenzidine (TMB) substrate solution was added to each well and incubated in the dark at room temperature for 15 minutes to develop colour. The reaction was terminated with addition of stop solution, plates were read at 450 nm using a microplate reader (BioTek Instruments, USA). All concentrations were expressed as pg/mL. The intra-assay and inter-assay coefficients of variation were ≤10%.

2.6 Statistical Analysis

The statistical analysis of our subjects' demographics and clinical characteristics was conducted using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were carried out on both the demographic and clinical characteristics of the participants. Continuous data was expressed as mean ± standard deviation (SD) when normally distributed, or median and interquartile range (IQR) when not normally distributed. Categorical data were expressed as frequencies and percentages. To determine if the data were normally distributed, a Kolmogorov-Smirnov test was conducted on all variables.

Independent sample T-tests were used for normally distributed data and Mann-Whitney U-test was used for non-normally distributed data to compare the levels of IL-15 and IL-21 between patients with celiac disease and healthy controls. To compare the levels of IL-15 and IL-21 between the three groups of patients with celiac disease classified according to the severity of their condition, normally distributed data were analyzed using one-way ANOVA and Tukey's post-hoc test and non-normally distributed data were analyzed using Kruskal-Wallis test and Dunn's post-hoc test. Receiver operating characteristic (ROC) curves were also created to determine diagnostic performance of IL-15 and IL-21, with area under the curve (AUC), sensitivity, specificity, positive and negative predictive values (PPV and NPV respectively) calculated based on the optimal cut-off values determined via the Youden index. To examine the relationship between IL-15 and IL-21 and the relationship between IL-15 and IL-21 and each Marsh-Oberhuber grade of severity, Pearson's correlation coefficients were computed. A two-tailed P-value less than 0.05 was considered statistically significant.

2.7 Ethical Considerations

The study complied with the ethical principles outlined in the Declaration of Helsinki. All participants were informed about the objectives, procedures and possible risks and benefits of the study prior to participating. Participation was completely voluntary after written informed consent was

obtained from all participants. Participant data was kept confidential during the study, and all participants had the right to withdraw from the study at any time, without repercussions.

Results

3.1 Demographic and Clinical Characteristics of Study Participants

Eighty participants were recruited to participate in the current investigation, including 40 treatment-naïve patients with CD and 40 age- and gender-matched healthy controls. Demographic and clinical features of both groups are given in Table 3-1. The mean age of CD patients was 34.2 ± 9.8 years, while that of healthy controls was 33.7 ± 9.4 years (P = 0.794). Gender was not a distinguishing factor since there were 18 men (45.0%) and 22 women (55.0%) within the group of CD patients and 19 men (47.5%) and 21 women (52.5%) in the group of healthy controls (P = 0.823). The mean BMI was significantly lower in patients than in healthy individuals (22.1 ± 3.2 vs. 23.8 ± 3.5 kg/m², P = 0.017) due to the malabsorption feature associated with CD. The mean time since diagnosis was 18.4 ± 11.6 months. According to histological examination, CD patients were divided into three categories: mild disease (Marsh 1-2) – 13 (32.5%), moderate disease (Marsh 3a-3b) – 18 (45.0%), severe disease (Marsh 3c) – 9 (22.5%). Positive family history of celiac disease was observed in 14 (35.0%) patients.

Table (3-1): Demographic and Clinical Characteristics of CD Patients and Healthy Controlsj.

Characteristics	CD Patients (n = 40)	Healthy Controls (n = 40)	Test	P-value
Age (years) – Mean ± SD	34.2 ± 9.8	33.7 ± 9.4	t = 0.26	0.794
Gender – Male, n (%)	18 (45.0%)	19 (47.5%)	$\chi^2 = 0.05$	0.823
Gender – Female, n (%)	22 (55.0%)	21 (52.5%)	—	—
BMI (kg/m²) – Mean ± SD	22.1 ± 3.2	23.8 ± 3.5	t = 2.42	0.017*
Disease duration (months) – Mean ± SD	18.4 ± 11.6	—	—	—
Positive family history, n (%)	14 (35.0%)	—	—	—
Marsh 1–2 (Mild) , n (%)	13 (32.5%)	—	—	—
Marsh 3a–3b (Moderate) , n (%)	18 (45.0%)	—	—	—
Marsh 3c (Severe) , n (%)	9 (22.5%)	—	—	—

n: number; SD: standard deviation BMI: body mass index; *: significant at P < 0.05; **: significant at P < 0.001; χ^2: Chi-square test; t: independent samples t-test.

3.2 Serum Interleukin-15 (IL-15) Levels

3.2.1 IL-15 Levels in CD Patients and Healthy Controls

Levels of serum IL-15 were measured in patients with Celiac Disease (CD) as well as healthy control subjects. The results have been provided in Table 3-2. Mean serum IL-15 levels were found to be significantly increased in patients suffering from CD compared with control subjects (148.6 ± 28.4 pg/mL vs 42.3 ± 11.7 pg/mL; P = 0.001), corresponding to about 3.5 times elevation. Serum IL-15 levels in patients with CD (94.2-211.7 pg/mL) were greatly elevated compared with those of healthy control individuals (21.8-68.4 pg/mL).

Table (3-2): Serum IL-15 Levels in CD Patientsj and Healthy Controlsj.

Groups	IL-15 Level (pg/mL)	Value

CD Patients (n = 40)	**Mean ± SD**	148.6 ± 28.4
	Range	94.2 – 211.7
Healthy Controls (n = 40)	**Mean ± SD**	42.3 ± 11.7
	Range	21.8 – 68.4
P-value	0.001** †	

n: number; SD: standard deviation; †: Independent samples t-test; **: significant at P < 0.001.

3.2.2 Diagnostic Accuracy of Serum IL-15

The ROC analysis was used to evaluate the diagnostic significance of serum IL-15 in distinguishing CD patients from healthy individuals, with the findings shown in Table 3-3. Using a cut-off level of > 85.5 pg/mL, the serum IL-15 demonstrated a sensitivity of 92.5%, a specificity of 95.0%, a positive predictive value (PPV) of 94.9%, and a negative predictive value (NPV) of 92.7%. The AUC was 0.981 (95% confidence interval [CI]: 0.956–1.000).

Table (3-3): Diagnostic Accuracy of Serum IL-15 in Celiac Diseasej (Cutoff > 85.5 pg/mL).

IL-15 Parameter	CD Patients (n = 40)	Healthy Controls (n = 40)
Cutoff value	> 85.5 pg/mL	
Sensitivity %	92.5%	
Specificity %	95.0%	
PPV %	94.9%	
NPV %	92.7%	
AUC (95% CI)	0.981 (0.956 – 1.000)	

AUC: area under the curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value**j**.

3.2.3 IL-15 Levels Across Clinical Attributes

Interleukin-15 serum concentration was further analyzed for its association with various clinical parameters, such as histopathological grade of the disease in terms of Marsh-Oberhuber grading system, duration of disease, and family history of the disease. As shown in Table 3-4, serum IL-15 level showed significant increase with the increase in histopathological severity of the disease, with a mean value in mild grade patients being 101.4 ± 19.3 pg/mL, moderate grade being 152.8 ± 22.6 pg/mL, and severe grade being 198.5 ± 24.1 pg/mL (P = 0.001). In contrast, there was no significant difference in IL-15 serum levels among patients with disease duration less than 12 months and more than 12 months (139.2 ± 26.8 vs. 155.7 ± 29.1 pg/mL; P = 0.072). Patients having a positive family history of celiac disease had significantly higher IL-15 levels than those having a negative family history of celiac disease (161.3 ± 27.4 vs. 141.8 ± 27.1 pg/mL; P = 0.038).

Table (3-4): Serum IL-15 Levels Across Clinical Attributes in CD Patients.

Characteristics	n	Mean ± SD	Range	P-value
Severity of Disease				

Mild (Marsh 1–2)	13	101.4 ± 19.3	72.1 – 138.5	
Moderate (Marsh 3a–3b)	18	152.8 ± 22.6	112.3 – 192.4	0.001**
Severe (Marsh 3c)	9	198.5 ± 24.1	158.7 – 211.7	
Disease Duration				
< 12 months	17	139.2 ± 26.8	94.2 – 192.0	
≥ 12 months	23	155.7 ± 29.1	102.4 – 211.7	0.072
Family History				
Positive	14	161.3 ± 27.4	118.6 – 211.7	
Negative	26	141.8 ± 27.1	94.2 – 200.3	0.038*

n: number; SD: standard deviation **: significant at P < 0.001; *: significant at P < 0.05. P-values for severity calculated by one-way ANOVA with Tukey's post-hoc test; P-values for duration and family history by independent samples t-test.

3.3 Serum Interleukin-21 (IL-21) Levels

3.3.1 IL-21 Levels in CD Patients and Healthy Controls

Levels of serum IL-21 were significantly increased in patients with CD compared to those in healthy subjects (312.4 ± 54.7 pg/mL vs. 87.6 ± 21.3 pg/mL; P = 0.001), indicating a 3.6-fold elevation (Tables 3-5). There was a larger spread in levels of IL-21 in CD patients (198.6-432.1 pg/mL) compared to those in healthy individuals (48.2-134.7 pg/mL), further confirming a marked increase in the level of this cytokine in patients

Table (3-5): Serum IL-21 Levels in CD Patients and Healthy Controls.

Groups	IL-21 Level (pg/mL)	Value
CD Patients (n = 40)	**Mean ± SD**	312.4 ± 54.7
	Range	198.6 – 432.1
Healthy Controls (n = 40)	**Mean ± SD**	87.6 ± 21.3
	Range	48.2 – 134.7
P-value	0.001** †	

n: number; SD: standard deviation †: Independent samples t-test; **: significant at P < 0.001.

3.3.2 Diagnostic Accuracy of Serum IL-21

The ROC curve analysis of IL-21 serum level resulted in an area under the curve (AUC) of 0.994 (95% CI: 0.985 – 1.000), showing excellent diagnostic accuracy for celiac disease (Tables 3-6). With the cutoff point being greater than 178.3 pg/mL, IL-21 exhibited high sensitivity (95.0%), specificity (97.5%), PPV (97.4%), and NPV (95.1%) to outperform IL-15's diagnostic

Table (3-6): Diagnostic Accuracy of Serum IL-21 in Celiac Disease (Cutoff > 178.3 pg/mL).

IL-21 Parameter	CD Patients (n = 40)	Healthy Controls (n = 40)
Cutoff value	> 178.3 pg/mL	

Sensitivity %	95.0%
Specificity %	97.5%
PPV %	97.4%
NPV %	95.1%
AUC (95% CI)	0.994 (0.985 – 1.000)

AUC: area under the curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value.

3.3.3 IL-21 Levels Across Clinical Attributes

Serum IL-21 concentration analysis showed an almost identical trend, compared to IL-15. There was an increase in IL-21 levels in relation to the increase in Marsh score: mild (Marsh 1-2) – 218.3 ± 41.6 pg/mL, moderate (Marsh 3a-3b) – 321.7 ± 48.2 pg/mL, and severe (Marsh 3c) – 419.8 ± 52.4 pg/mL (P = 0.001). The duration of the disease did not show any correlation with IL-21 concentration level (<12 months – 298.4 ± 52.1 pg/mL, ≥12 months – 323.1 ± 56.8 pg/mL; P = 0.124). A family history of the disease had a significantly positive correlation with IL-21 levels (338.6 ± 58.3 vs. 298.2 ± 51.4 pg/mL; P = 0.027).

Table (3-7): Serum IL-21 Levels Across Clinical Attributes in CD Patients.

Characteristics	n	Mean ± SD	Range	P-value
Severity of Disease				
Mild (Marsh 1–2)	13	218.3 ± 41.6	158.4 – 294.7	
Moderate (Marsh 3a–3b)	18	321.7 ± 48.2	238.5 – 401.3	0.001**
Severe (Marsh 3c)	9	419.8 ± 52.4	348.6 – 432.1	
Disease Duration				
< 12 months	17	298.4 ± 52.1	198.6 – 398.2	
≥ 12 months	23	323.1 ± 56.8	212.4 – 432.1	0.124
Family History				
Positive	14	338.6 ± 58.3	241.7 – 432.1	
Negative	26	298.2 ± 51.4	198.6 – 410.3	0.027*

n: number; SD: standard deviation; **: significant at P < 0.001; *: significant at P < 0.05.

3.4 Correlation Between IL-15, IL-21, and Marsh-Oberhuber Score

A Pearson correlation analysis was performed to determine the associations between IL-15 and IL-21 concentration in the blood sera, as well as between each cytokine and Marsh-Oberhuber histopathological grade. Table 3-8 illustrates the results of these analyses. It has been observed that serum IL-15 and IL-21 concentration is moderately associated in the case of celiac patients ($r = 0.614$, $r^2 = 0.377$, $P = 0.001$). This demonstrates that these cytokines are acting as co-mediators in a pro-inflammatory axis in celiac disease. Also, both the cytokines have high positive correlations with the degree of Marsh grading (IL-15: $r = 0.782$, $r^2 = 0.612$, $P = 0.001$; IL-21: $r = 0.841$, $r^2 = 0.707$, $P = 0.001$). It can be seen that IL-21 is more positively correlated than IL-15 with the histopathology score of the disease.

Table (3-8): Pearson's Correlation Between IL-15, IL-21, and Marsh-Oberhuber Score

Variable	r	r^2	P-value
IL-15 vs. IL-21	0.614	0.377	0.001**
IL-15 vs. Marsh Score	0.782	0.612	0.001**
IL-21 vs. Marsh Score	0.841	0.707	0.001**

r: Pearson's correlation coefficient r^2: coefficient of determination **: significant at $P < 0.001$.

Discussion

Celiac disease is an immune disorder characterized by damage to the small intestine, which is becoming progressively significant worldwide. The prevalence rate in the general population is about 1.4%, and its occurrence is on the rise, particularly in Middle Eastern countries like Iraq, where changing food habits and improved diagnosis have helped identify more cases of celiac disease [17], [18]. In the current study, we aimed to examine and compare the levels of serum IL-15 and IL-21 in 40 patients with celiac disease without any previous treatment and 40 normal subjects, who were selected from the Gastroenterology Unit of Al-Diwaniyah Teaching Hospital, Iraq. The average age of patients was 34.2 ± 9.8 years, whereas the average age of controls was 33.7 ± 9.4 years. No significant differences between groups existed concerning the average age ($P = 0.794$) and sex ratio ($P = 0.823$).

The levels of IL-15 in serum were found to be higher in the celiac group than the control group in the current study (148.6 ± 28.4 pg/mL vs. 42.3 ± 11.7 pg/mL, $P = 0.001$) and the difference represents a fold increase of 3.5. IL-15 is an inflammatory cytokine produced mainly by intestinal epithelial cells following stimulation by gluten peptides and it has been identified as the main effector of innate immunity in activating the cytotoxic function of IELs through the NKG2D/MICA pathway [19]. Previous studies involving ELISA analysis on subjects suffering from celiac disease have also shown significantly increased levels of IL-15 in their sera with an AUC value of 0.922, supporting the use of IL-15 as a blood marker for diagnosis [20]. These results also concur with previous studies where inhibition of IL-15 through antibodies has resulted in the resolution of villous atrophy as well as normalization of IEL counts in a primate model of gluten sensitivity enteropathy [21].

Despite this, however, there are inconsistencies within the current body of research concerning the degree of plasma IL-15 elevation, which might arise from differences in disease activity, distribution of patient HLA haplotypes, as well as the use of medications at the time of sample acquisition [22]. The fact that only untreated patients were included in the study cohort prior to beginning a gluten-free diet means that cytokine concentrations in this case can be considered unaffected by any interventions and, thus, are true reflections of the innate immune response. In recent years, moreover, new evidence has shown that IL-15 is involved in suppressing the regulatory function of Tregs and stimulating production of granzyme B by IELs; as a result, the immune system becomes even more reactive to gluten ingestion [23].

Serum levels of IL-21, similar to IL-15, were elevated in patients with celiac disease as compared to healthy controls (312.4 ± 54.7 pg/mL vs. 87.6 ± 21.3 pg/mL, $P = 0.001$) by a factor of 3.6. IL-21, mainly released by CD4+ T follicular helper cells, performs multiple immunomodulatory functions through JAK1/STAT3 signaling pathways, inducing the maturation of B cells into antibody-producing plasma cells that secrete anti-tTG IgA and anti-DGP IgG antibodies specific for celiac disease [24]. Systemic elevation of IL-21 parallels previous mucosal studies, where IL-21 expression was found to be highly elevated in duodenal biopsy specimens in patients with untreated celiac disease as compared to those receiving gluten-free diets, indicating its involvement in mediating mucosal inflammation in response to gluten exposure. In addition, serum levels of IL-21 along with other pro-inflammatory cytokines have been correlated with anti-tTG titers and degree of villous atrophy in celiac disease patients, potentially suggesting their utility as biomarkers [10].

The ROC curve method was used to assess the diagnostic potential of serum IL-15 and IL-21 in the differentiation of patients with celiac disease from those who did not have the disorder. The analysis showed that IL-15 possessed very good diagnostic properties with the optimal cutoff point of >85.5 pg/mL, resulting in sensitivity, specificity, and area under the curve of 92.5%, 95.0%, and 0.981 (95% confidence interval = 0.956–1.000) respectively. Similarly, the diagnostic accuracy of IL-21 was found to be much higher, as its cut-off was >178.3 pg/mL with a corresponding sensitivity, specificity, and area under the curve of 95.0%, 97.5%, and 0.994 (95% confidence interval = 0.985–1.000), respectively. This indicated an excellent discriminatory power of this cytokine. Overall, IL-15 and IL-21 seem to be extremely valuable non-invasive biomarkers for celiac disease that may even surpass currently utilized markers like anti-tTG IgA [18], [25].

Both IL-15 and IL-21 showed significant and progressive increases in concentrations in the sera as the histopathological grade of Marsh-Oberhuber classification increased (P = 0.001 for each test), revealing that there is a direct relationship between the systemic increase of cytokines and intestinal mucosal lesions. The two cytokines also showed significant positive correlation coefficients to the grade of Marsh-Oberhuber lesions (r = 0.841 for IL-21; r = 0.782 for IL-15), which suggests that the degree of elevation of systemic cytokines qualitatively represents the amount of mucosal lesion. In addition, a significant positive correlation coefficient was revealed between serum IL-15 and IL-21 levels (r = 0.614, P = 0.001), representing systemic evidence of the functional linkage between the two cytokines as an integrated axis of pro-inflammation [24]. New therapies for celiac disease focus on a gluten-free diet as the key treatment strategy, with pharmacologic treatments that block the IL-15 signaling pathway, including Janus kinase inhibitors and IL-15 antagonists, being explored for resistant cases [26]. The detection of patients with high levels of IL-15 and IL-21 in their blood may help in categorizing those who are prone to diseases, leading to an improvement in precision medicine practices in celiac disease.

Some methodological strengths of this study are worth mentioning. Firstly, only treatment-naïve patients were included in the study to ensure that cytokine levels measured would correspond to unmodified immune system activity. Secondly, using the Marsh-Oberhuber classification as the reference criterion provides an objective tool for assessing disease severity. However, there are also some weaknesses in this research. For instance, the sample size is rather limited; it is single-centered, and there is no comparison of cytokine levels in tissue and serum samples. Furthermore, a cross-sectional study design does not allow for analyzing cytokine dynamics following the start of a gluten-free diet. Altogether, higher levels of IL-15 and IL-21 in sera are found among untreated patients with celiac disease; both cytokines have high diagnostic accuracy, with AUC values being above 0.98. Therefore, an IL-15/IL-21 pro-inflammatory axis seems to play an important role in the pathogenesis of celiac disease..

Conclusion

In this research, high concentrations of IL-15 and IL-21 were found in serum of untreated celiac patients from Iraq. Both cytokines demonstrated a significant association with celiac disease severity based on Marsh-Oberhuber histopathology. The close relationship between these pro-inflammatory molecules confirms the importance of immunological pathways involving IL-15/IL-21 and including epithelial cell response to innate factors as well as the enhancement of adaptive immunity in celiac disease pathogenesis. However, this discovery does not exclude the application of histopathological evaluation for diagnosis. Multicenter research is necessary to assess further diagnostic possibilities of IL-15 and IL-21 in celiac disease. Future multicenter longitudinal research needs to be conducted to confirm the validity of their use and clarify changes after a gluten-free diet.

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