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Serum IL-21 and IFN-γ Levels in Celiac Disease and Their Association with Biochemical Indicators

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Abstract

Background : Celiac disease (CD) is an autoimmune disorder that is mainly specific to the small intestine and is caused by an abnormal T-cell-mediated immune response to dietary gluten which involves mucosal inflammation and villous atrophy. Activation of the systemic immunology has the potential of causing extra-intestinal symptoms. Objective: The levels of serum IL-21 and IFN-g of the celiac disease patients were determined in this study and the levels were compared to the level of biochemical parameters like hemoglobin, ferritin, calcium, and vitamin D3. Methods: A total of 70 clinically proven celiac patients (9-74 years old) were recruited in Wasit hospitals at a time period of March 2024 to April 2025. Venous blood samples were collected using the enzyme-linked immunosorbent test (ELISA), centrifuged and analyzed with regard to the levels of IL-21 and IFN-g. Measurement of biochemical parameters was done using automated analyzers in accordance to the conventional protocols. Results: Celiac patients had much higher serum concentrations of IFN-g and IL-21 in comparison to healthy controls ($p < 0.001$). All the levels of vitamin D3, calcium, iron, ferritin, and hemoglobin were lowered significantly ($p < 0.05$). These biological markers were found to have negative relationships with cytokines. Conclusion: In celiac disease, immune activation is manifested by increased levels of IL-21 and IFN-g, which can be one of the causes of intestinal damage and nutrient loss. Also, findings indicate immunopathological and not diagnostic relations between biochemical deficits and cytokine imbalance..

Highlight :

- Stress strongly predicts insomnia in celiac patients.
- Persistent symptoms also contribute to insomnia.
- Diet quality is not related to insomnia.

Keywords : Celiac Disease , Anti-Tissue Transglutaminase, cytokines, IL-21, IFN-γ.

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Introduction

People who are genetically susceptible, eating gluten triggers an inflammatory immune response, leading to celiac disease (CD), an immune-mediated enteropathy. Intestinal damage is caused by a series of events that begin with an immune response, which is triggered by both innate immunity, which is mediated by cells similar to natural killer (NK) cells, and adaptive immunity, which is driven by T cells. The HLA molecules represented by the aforementioned alleles can provide CD4+ T-cells with immunogenic peptides derived from the inadequate digestion of dietary gluten fractions. This mechanism is particular to CDs. Tissue transglutaminase (tTG) deamidates the gluten fractions that have not been fully digested, making them immunogenic. Gluten (gliadin and glutenin) from wheat and related storage proteins in other cereals (e.g., rye's secalin and barley's hordein) might aggravate CD symptoms. (1). It is characterized by a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, and enteropathy(2). Although the likelihood of additional autoimmune disorders (ADs) in celiac disease (CeD) patients is high, little is known about the causes and risk factors for ADs. (3). In recent decades, celiac disease (CD) has transitioned from a rare condition to one of the most prevalent lifelong disorders, with an estimated prevalence of 0.7–2.9% in the general population; this frequency is elevated among females, individuals with autoimmune disorders, and relatives of those affected by CD(4).The clinical manifestations Gluten exposure in persons with celiac disease presents a wide array of features, collectively defining this illness as a multisystem ailment rather than only an isolated intestinal disease(5).

The predominant symptoms consist of weight loss, persistent diarrhea, and abdominal discomfort (6). The inflammation and duodenal dysfunction associated with active celiac disease have distinct detrimental effects on the stomach(7). Intestinal inflammation in CD is driven by an autoimmune response to gluten, leading to the damage of the intestinal epithelium and villous atrophy (8). Moreover, celiac disease has been associated with various autoimmune disorders, mental conditions, and certain malignancies(9) . The predominant manifestation of celiac disease is chronic diarrhea, characterized by frequent episodes of watery, loose stools .Iron deficiency (ID), with or without anemia, in celiac disease (CD) can be attributed to many etiologies. The principal mechanism is malabsorption due to destruction of the small intestinal mucosa, namely villous atrophy(10,11).

Simultaneously, decreased iron consumption, frequently resulting from stomach distress and diarrhea, together with hidden gastrointestinal bleeding, are contributing causes. Celiac disease can also lead to deficits in other minerals and vitamins, including folic acid and vitamin D(12). The objective of this research is to analyze the most recent evidence of immunological markers while assessing their effects and the influence of age and gender on patients with Celiac disease.

Subjects and study design:

The study encompassed 70 patient samples with Celiac disease, 24 male and 46 female , comprising 30 healthy subjects as control group . participants aged between 6 to 74 years old , as well as control in the same age range .within the limited period from 2024-March, to 2025 April.

Sample collection:

Seventy Serum samples are obtained from persons diagnosed with Celiac disease and 30 healthy subjects constituting the control group. Serological tests for tissue transglutaminase (tTG), anti-gliadin antibodies (AGA), anti-tissue transglutaminase antibodies (ATG), and anti-endomysial antibodies (ATA) are conducted on all patients and the control group. Serum was taken from peripheral blood collected from individuals with informed consent. All participants are recruited from hospitals in Wasit, Iraq.

Statistical Analysis:

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 26 and Microsoft Office Excel 2010 using independent-Sample T Test analyze, . Numeric data were presented as mean and standard deviation after the Kolmogorov-Smirnov normality test, which determined the distribution of variables as normal or non-normal

Result

Seventy participants with a diagnosis of celiac disease provided valid replies, which were compared to thirty healthy people as a control group. As can be shown in (Table 1), the bulk of CD patients were 24 men (34.3%) and 46 females (65.7%), indicating that more were included in this study. In the present study, there were significantly more CD females than CD males.

Table (1): Gender comparison between CD patients and control groups.

Study groups	Gender		Total	p-value
	Male	Female		
CD patients	24 (34.3 %)	46 (65.7%)	70	0.075 ¥ NS
Control	16 (53.3 %)	14 (46.3%)	30	
Total	40 (40.0%)	60 (60.0%)	100	

¥: Chi-square test; **NS**: not significant at $P > 0.05$

The current study detected CD patients age ranging between (6-74) years. as indicated by the results in (Table 2) . the mean age of individuals with coeliac disease was 38.82 ± 9.61 years, while the mean age of control subjects was 39.40 ± 8.65 years. The mean age difference between the two groups was not statistically significant (P = 0.884) . Patients with Celiac disease included 20 (28.6) patients under 30 years old, 16 (22.9%) patients between 30 and 39 years old, 8 (11.4%) between 40 and 49 years old, and 26 (37.1%) patients over 50. The control group included 7 (23.3%) people under 30 years old, 10 (33.3%) people between 40 and 39 years old, 5 (16.7%) people between 40 and 49 years old, and 8 (26.7%) people over 50 years old. The frequency distribution of patients and control subjects by age group did not differed significantly (P = 0.528).

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Table (2) Comparison of age between CD patients and control groups.

Age	CD patients	Healthy control	p-value
Mean ± SD	**38.82 ± 9.61**	**39.40 ± 8.65**	0.884 † NS
Range	**6–74 years**	**18– 62 years**	
< 30 years, *n* (%)	**20 (28.6)**	**7 (23.3%)**	0.528 ¥ NS
30-39 years, *n* (%)	**16 (22.9%)**	**10 (33.3%)**	
40-49 years, *n* (%)	**8 (11.4%)**	**5 (16.7%)**	
≥ 50 years, *n* (%)	**26 (37.1%)**	**8 (26.7%)**	

n: number of cases; **SD**: standard deviation; †: Independent T test; ¥: Chi-square test; N**S**: non-significant at *p>0.05*.

CD was diagnosed based on the results of the serologic enzyme-linked immunosorbent test (ELISA). The results of the current study showed that the anti-tissue transglutaminase (tTG) serum level was IgG ≥ 9 U/ml were considered as positive and with concentrations > 9 U/ml were considered as negative. Serum level of (tTG)IgG among CD patients and control is shown in (Table 3). The present results showed that the mean serum tTG-IgG levels in CD patients and the control group were 9.3 ± 3.17 and 5.48 ± 1.5, respectively, and that these differences were significant. Regarding qualitative results, There existed a substantial disparity. (p > 0.05) between the 29 (41.4%) CD patients and the 1 (3.3%) control group who had a positive tTG-IgG.

as well as , Anti-gliadin antibodies (AGA) with concentrations ≥ 12 U/mL were deemed positive, whereas those with concentrations > 12 U/mL were deemed negative. The current findings indicate that the mean serum level of AGA were 7.19 ± 2.6 in patients with CD and 5.05 ± 1.48 in healthy controls, with a statistically significant difference seen. In terms of qualitative data, 14 (20.0%) of patients with coeliac disease tested positive for anti-gliadin antibodies (AGA), but all healthy controls were negative for both AGA, and the difference was statistically significant (p > 0.05), as shown in Table(3). The present data suggest that the average levels of serum ATG and serum ATA were elevated in people with Celiac disease in comparison to healthy controls, with a statistically significant difference.

Table (3): serum levels some parameters among CD patients and Healthy control

Parameters	CD patients	control	*P*
Anti-tissue transglutaminase (tTG) IgG (U/mL) (Quantitative results)			
Mean ± SD	9.3 ± 3.17	5.48 ± 1.5	0.001 † S
Range	3.70 –21.30	2.10 –10.70	
Anti-tissue transglutaminase (tTG) IgG (U/mL) (Qualitative results)			
Positive, *n* (%)	29 (41.4%)	1 (3.3%)	0.001 ¥ S
Negative, *n* (%)	41 (58.6%)	29 (96.7%)	
Anti-Gliadin Antibody (AGA) (U/mL) (Quantitative results)			
Mean ±SD	7.19 ± 2.6	5.05 ± 1.48	0.037 † S
Range	2.80 –18.30	2.5 –10.90	
Anti-Gliadin Antibody (AGA) (Qualitative results)			
Positive, *n* (%)	14 (20.0%)	0	0.008 ¥ S
Negative, *n* (%)	56 (80.0%)	30 (100.0%)	
ATG level (U/mL) (Quantitative results)			
Mean ± SD	21.76 ± 5.9	5.75 ± 1.6	0.001

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Range	4.50 –39.80	2.90 –10.10	† S
ATA level (U/mL) (Qualitative results)			
Mean ± SD	19.09 ± 65	6.64 ± 2.2	0.001 † S
Range	4.00 –52.90	3.40 –10.30	

n: number of cases; ¥: Chi-square test; †: Independent T test; NS: not significant at P > 0.05.

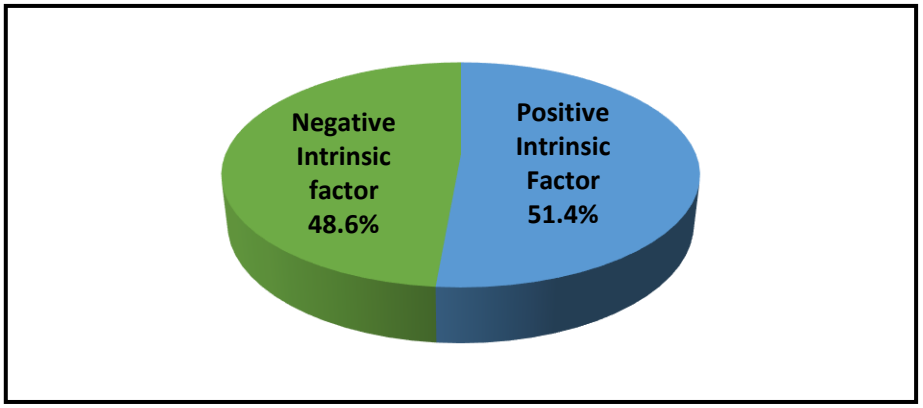

Figure (1): The distribution of CD patients based on intrinsic factor positivity

This study compared cytokine IL-12 , IFN-γ and Biochemical biomarker based on the results of the Intrinsic Factor, and the findings are shown in (Table 4). The current results indicate that No significant difference was seen between celiac disease patients with positive intrinsic factor and those with negative intrinsic factor (p<0.05).

Table (4): Serum level of Cytokines IL-21, IFN-γ and Biochemical biomarker in CD patients according to intrinsic factor**.**

Parameter	IF Positive(n=36)	IF Negative (n-34)	p-value
IL-21 (pg/mL)	435.6 ± 47.4	461.2 ± 37.9	0.116 †
IFN-γ (pg/mL)	33.5 ± 4.6	35.5 ± 4.9	0.178 †
Hemoglobin (g/dL)	11.27 ± 0.95	10.75 ± 1.7	0.134 †
Iron (µg/dL)	10.71 ± 2.1	10.58 ± 3.1	0.964 †
Ferritin (ng/mL)	5.0 ± 1.6	5.59 ± 1.8	0.479 †
Calcium (mg/dL)	8.15 ± 1.0	8.22 ± 1.1	0.708 †
Vitamin D3 (ng/mL)	22.52 ± 5.1	23.50 ± 4.8	0.413 †

IF: intrinsic factor SD: standard deviation; †: Independent T test; **: significant at $P < 0.05$

As shown in (Table 5), Celiac disease patients had significantly higher serum level of IL-21 (448.2 ± 44.7 pg/mL) than control groups, (160.4 ± 31.62 pg/mL , p=0.001). Similarity, elevated serum concentration of IFN-γ in Celiac disease patients compered control group, (34.46 ± 4.84 pg/mL) (6.37 ± 1.27 pg/mL , p=0.001), respectively. In contrast , Biochemical and hematological biomarker decreased in Celiac disease patients compered control group , The findings of the present investigation indicated that the mean hemoglobin levels were (11.2 ± 1.4) g/dl in CD patients and (14.46 ± 1.8) g/dl in the healthy control group, respectively. A significant reduction in Hemoglobin concentration was seen in CD patients compared to the control group, with a probability threshold of P < 0.001, as presented in the (Table5).As well as , the mean values of iron parameters (Iron and Ferritin) in CD patients and controls. The mean iron levels in CD patients were lower than those in the healthy control group, and the difference was very significant (P < 0.001). The mean iron levels in CD patients were (10.63 ± 3.2 µg/dL) and (43.89 ± 6.47 µg/dL),

respectively. Ferritin levels in CD patients were (5.28 ± 1.44 ng/mL) and in the healthy control group were (29.82 ± 5.66 ng/mL), respectively; the mean levels were lower in CD patients than in the healthy control group, and the difference was highly significant (P < 0.001). The mean calcium levels in individuals with CD were significantly lower than those in healthy controls (P < 0.001). The average values were (8.18 ± 1.1 mg/dL) in CD patients and (12.07 ± 1.5 mg/dL) in healthy controls, respectively. The vitamin D3 levels in celiac disease (CD) patients and the healthy control group were (22.99 ± 3.91) and (26.13 ± 5.78 ng/mL), respectively; the mean values in CD patients were significantly lower than those in the healthy control group (P=0.035).

Table (5): Serum level of Cytokines IL-21, IFN-γ and Biochemical biomarker in CD patients compered to healthy control **.**

Parameter	CD patients (n=70) Mean ± SD	Control (n=30) Mean ± SD	p-value
IL-21 (pg/mL)	448.2 ± 44.7	160.4 ± 31.62	0.001** †
IFN-γ (pg/mL)	34.46 ± 4.84	6.37 ± 1.27	0.001** †
Hemoglobin (g/dL)	11.2 ± 1.4	14.46 ± 1.8	0.001**
Iron (µg/dL)	10.63 ± 3.2	43.89 ± 6.47	0.001**
Ferritin (ng/mL)	5.28 ± 1.44	29.82 ± 5.66	0.001**
Calcium (mg/dL)	8.18 ± 1.1	12.07 ± 1.5	0.001**
Vitamin D3 (ng/mL)	22.99 ± 3.91	26.13 ± 5.78	0.035**

SD: standard deviation; †: Independent T test; **: significant at $P < 0.05$.

To determine the diagnostic accuracy of comparing IL-21, IFN-γ concentrations in patients with coeliac disease to healthy control subjects, receiver operating Receiver Operating Characteristic (ROC) analysis was performed. The outcomes are presented in (Table 6) and Figure 2. The threshold for IL-21 was >320.15, with sensitivity, specificity, positive predictive value, negative predictive value, and area under the curve all at 100.0% and 1.000 (1.000–1.000). The present data indicate that IL-21 is considered an excellent diagnostic marker. Also , The IFN-γ cutoff value was determined to be more than 18.35, Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve are all recorded at 100.0%, 100.0%, 100.0%, and 1.000 (1.000–1.000), respectively. Current data indicate that IFN-γ is considered a highly efficient diagnostic marker.

Table (6): Sensitivity and specificity of IL-21 and IFN-γ level in Celiac disease patients

Biomarker	Cut-off value	Sensitivity %	Specificity %	PPV %	NPV %	AUC (95% CI)
IL-21 (pg/mL)	> 320.15	100.0 %	100.0 %	100.0 %	100.0 %	1.000 (1.000- 1.000)
IFN-γ (pg/mL)	> 18.35	100.0 %	100.0 %	100.0 %	100.0 %	1.000 (1.000- 1.000)

CI: Confidence interval, AUC: Area under curve.

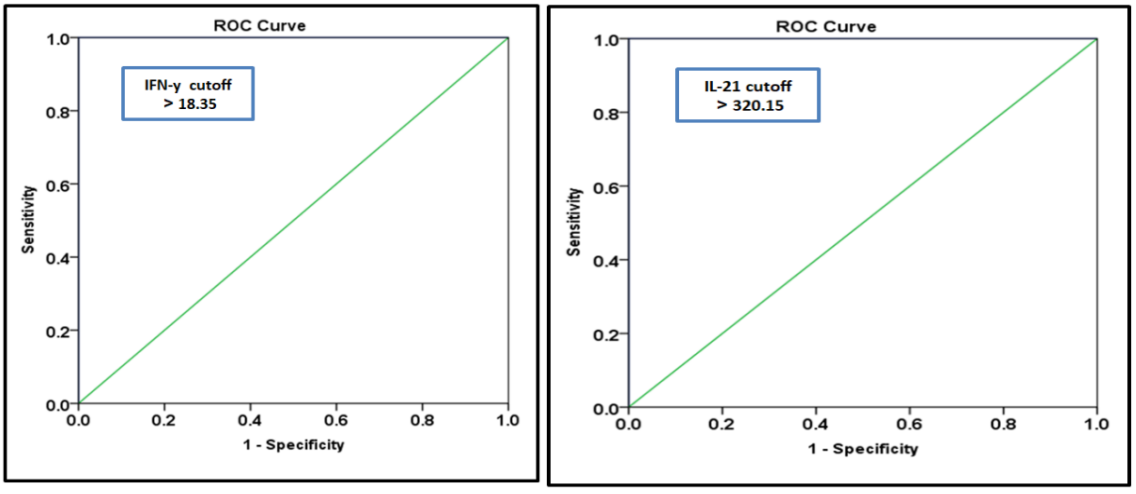

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Figure (2): Curve study of IL-21 and IFN-γ for determining potential diagnostic cutoff values**.**

(Tables 7) displayed the relationship between immunological and other markers in patients with coeliac disease. In patients with CD, there is a significant negative correlation between IL-21 levels and various levels of calcium (r=-0.306 and p=0.010), vitamin D3 (r=-0.342 and p=0.004), ferritin (r=-0.359 and p=0.003), HB (r=-0.316 and p=0.008), tTG-IgG (r=-0.252 and p=0.035), IFN-y levels, and vitamin D3 (r=-0.258 and p=0.031).

Table(7): Correlation between immunological and other parameters in patients with to Celiac disease

Parameters	Parameters			
	IL-21		IFN-γ	
	r	*P*	*r*	*P*
IL-21(pg/mL)	1			
IFN-y (pg/mL)	0.112	0.358	1	
Calcium (mg/dL)	-0.306	0.010*	-0.052	0.667
Vitamin D3 (ng/mL)	-0.342	0.004*	-0.258	0.031*
Iron (µg/dL)	0.130	0.284	0.137	0.259
Ferritin (ng/mL)	-0.359	0.003*	0.136	0.261
Hemoglobin (g/dL)	-0.316	0.008*	-0.130	0.283
tTG-IgG (U/mL)	-0.252	0.035*	-0.115	0.343
AGA (U/mL)	-0.113	0.354	-0.206	0.087
ATG (U/mL)	-0.082	0.501	0.058	0.633
ATA (U/mL)	0.107	0.378	-0.029	0.809

***r*: correlation coefficient.**

Discussion

Celiac disease (CD) is a persistent autoimmune disease marked by the injuries and inflammation of the mucosal small intestine of genetically predisposed people due to gluten intake. The pathophysiology of CD is associated with both innate and adaptive immunological responses; the outcome of pathophysiology includes villous atrophy, crypt hyperplasia, and poor nutritional absorption (10,11). Although the elimination of diagnostic methods and the serological screening have improved diagnostic techniques, the prevalence of CD continues to rise among the adult and pediatric age groups in the world (12-14).

In the present study, serum IL-21 and IFN-g levels were significantly increased in the group of patients with celiac disease compared to their healthy controls. These cytokines mediate the Th1-mediated immunity that causes intestinal inflammation in CD (47-49). The leading contributor of IL-21 is an activated CD4 T cell that induces the B-cell differentiation and the plasma-cell activation that heighten mucosal inflammation and sustain the autoimmune process (50,51). Furthermore , The IFN-g released by Th1 and intraepithelial lymphocytes promotes Macrophage stimulation and enhances antigen presentation leading to additional epithelial damage (52-55). The fact that a combination of imbalance between pro- and anti-inflammatory mediators contributes to the emergence of a disease is justified by the simultaneous rise in the levels of both cytokines (56-58).

Immunological activity and dietary inadequacies have significant relationship indicated by reported negative correlation with the levels of cytokine with the biochemical indicatures, including hemoglobin, ferritin, calcium, and vitamin D3 levels. Micronutrient absorption is diminished by the chronic intestinal inflammation that damages the absorptive mucosa (15,16). Iron deficiency anemia, as per the previous research, may occur in 50% of adult patients and is among the most common extra-intestinal signs of undiagnosed CD (11,17,18). Chronic malabsorption of iron, calcium and vitamin D has been attributed to villous atrophy and a continuing immune activation of the mucosa (19,20). Our own data supports the mechanistic relationship between immunological dysregulation and malabsorption as patients with lower hemoglobin and ferritin levels also had higher levels of IL-21 and IFN-g.

As has been shown, vitamin D deficiency, which is another prevalent metabolic disorder in CD, modulates the immunological responses by regulating the T-cell growth and preventing the production of Th1 cytokines (43-45). In this way, a vicious cycle between mucosal inflammation and nutritional insufficiency could be developed, and the drop in vitamin D levels, which was observed in our study, may contribute to the increase in the expression of IL-21 and IFN-g. This finding correlates with previous studies that found that immune activation via cytokines causes intestinal disease and systemic metabolic alterations (46,48,49).

Moreover, our results are consistent with previous studies that established the presence of high IL-21 levels in intestinal samples of patients with active CD, which was associated with anti-tTG antibody titers and villous damage (50). The extent of mucosal lesions has also been continuously linked to an increased IFN-g expression, which goes back to normal after a strict compliance with a gluten-free diet (55,57). At this point, the combination of findings confirms that IL-21 and IFN-g are diagnostic biomarkers not per se but indicators of immunological activation and disease activity (13,14,49).

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In clinical terms, Il-21 and IFN-g surveillance on serum can help illuminate the immunopathology of the CD patients, particularly those who remain symptomatic or do not have full recovery of mucosa despite dietary control (28,37). They can be associated with biochemical manifestations to obtain a more detailed understanding of the relationship between immunological dysfunction and such systemic manifestations as anemia and bone demineralization (38-42). It seems possible that in refractory cases paying attention to such cytokine pathways can be a beneficial adjunct measure to promote the healing of the mucosa and the normalization of nutritional balance (47,50,56).

Finally, the serum IL-21 and IFN-g were high in this study, which needs to be emphasized as the significance of cytokine-mediated immune activation in the development of celiac disease. The process of cytokine expression and biochemical markers is negatively correlated, and it highlights the impact of immune-mediated gut damage on the overall health and nutrition absorption of the system. They are consistent with new developments in immunological concepts that indicate that celiac disease is not a confined intestinal condition but a systemic immune-metabolic dysfunction (10,19,22,49,58).

Conclusion

This paper demonstrates that elevated serum concentration of IL-21 and IFN-g, which are serum markers of an active Th1-mediated immune response with intestinal inflammation and villous destruction, are predictive of celiac disease. Collectively, It has been proposed that nutritional deficiency may result directly due to immunological dysregulation by the decreased absorption rate as observed by the established low biochemical correlations between cytokine concentrations and biochemical indices, such as hemoglobin, ferritin, calcium, and vitamin D3. As well as, these data prove the idea that the celiac disease is a systemic immune-metabolic disease characterized by chronic inflammation which exceeds the gut. Also, the assessment of IL-21 and IFN-g can provide valuable data on activity and severity of the disease, which proves their potential application as immunopathological, but not diagnostic, biomarkers.

Ethical Approval:

Accordingly, written informed consent was taken from all participants before any intervention.

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