Hilla University College Journal For Medical Science

Manuscript 1082

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Mustafa Ali H. Khalaf

Israa Ketab H. Al-Yasiri

Karrar Abdil-Aziz A. Beg

ORIGINAL STUDY

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The Role of Interleukin 2 and Interleukin 15 in Diagnosis and Prognosis of Celiac Disease in Children

Mustafa Ali H. Khalaf [a,*], Israa Ketab H. Al-Yasiri [a], Karrar Abdil-Aziz A. Beg [b]

[a] Dept. of Microbiology, College of Medicine, Jabir ibn Hayyan University for Medical and Pharmaceutical Sciences, Najaf, Iraq
[b] Dept. of Paediatrics, College of Medicine, Jabir ibn Hayyan University for Medical and Pharmaceutical Sciences, Najaf, Iraq

Abstract

Background: Celiac disease (CD) is an autoimmune condition that affects the small intestine, triggered by the intake of gluten and related prolamines in genetically predisposed individuals. The anti-tissue tTG-IgA transglutaminase test is a primary screening method for suspected celiac disease.

Materials and Methods: Forty-five children with celiac disease were enrolled in the study, with an age range between 2–14 years, and 43 healthy subjects as the control group in the Medical City Hospital in Baghdad. The diagnosis of the disease was confirmed by Anti tissue transglutaminase (tTG) IgA and IgG antibodies, IL-2, and IL-15 using the enzyme-linked immunoassay technique (ELISA).

Results: The results showed that serum levels of anti-tTG (IgA, IgG) antibodies were significantly higher in patients with celiac disease than in the control group. The serum anti-tTG IgA level was 23.06 ± 15.6 in the patient group, while the control group was 8.69 ± 0.61. Also, the serum anti-tTG IgG level was 104.10 ± 61.1 in the celiac group, and the healthy control group was 7.41 ± 0.57. In addition, IL-2 and IL-15 serum levels were increased in the celiac disease groups with highly significant differences. The IL-2 level was 26.33 ± 6.59 in the patient group, and in the healthy control groups were 6.55 ± 0.30, P value (P = 0.004). Furthermore, the serum IL15 level was 42.31 ± 7.42 in the celiac patient group and 12.70 ± 0.71 in the healthy control group, P value (P < 0.0001).

Conclusion: The elevated Interleukin-2 (IL-2) and Interleukin-15 (IL-15) levels in patients with celiac disease have been suggested as biomarkers and therapeutic targets (immunotherapies) for celiac disease.

Keywords: Celiac disease, Interleukin-2 (IL-2), Interleukin-15 (IL-15)

1. Introduction

Celiac disease (CD) is an autoimmune disorder affecting the small intestine in genetically predisposed individuals that is triggered by the intake of gluten and related prolamines. It is characterized by human leukocyte antigen (HLA)-DQ2 or HLA-DQ8, celiac-specific antibodies, several combinations of small intestinal damage, and gluten-dependent clinical symptoms [1, 2].

The prevalence of celiac disease is around 1–2% of the global population [3]. In genetically predisposed individuals, particularly those with HLA-DQ2 and HLA-DQ8. The ingestion of gluten found in wheat, rye, kamut, and barley results in induced autoimmune response. This immune response lead to increases both intestinal and extraintestinal changes [4].

The T-lymphocyte is the predominant cause of celiac disease by triggering the autoimmune response when modified peptides from gliadin to CD4+ T helper cells by HLA molecules DQ2 and DQ8, then both T-lymphocytes and B cells are activated. This signalling initiates the secretion of antibodies against gluten and tissue transglutaminase (tTG) in the

Received 8 August 2025; revised 16 August 2025; accepted 17 August 2025.
Available online 31 December 2025

* Corresponding author.
E-mail addresses: mustafa.a.hussein@jmu.edu.iq (M. A. H. Khalaf), israa_alyasiri@jmu.edu.iq (I. K. H. Al-Yasiri), k.alibeg@jmu.edu.iq (K. A.-A. A. Beg).

https://doi.org/10.62445/2958-4515.1082

lamina propria, hence activating self-reactive T helper cells [5].

The ESPGHAN guidelines in 2012 recommend using the anti-tissue tTG-IgA test as the primary screening method for suspected celiac disease, with the total IgA test to exclude selective IgA deficiency, antitissue tTG-IgA has a high sensitivity and specificity, also its lower cost compared to the EMA IgA antibody test [6]. The anti-tissue transglutaminase (tTG) -IgG may be used in patients with IgA deficiency [7].

Interleukin-15 (IL-15) is a member of the type I cytokine family. It stimulates T lymphocytes and natural killer NK) cells and contributes to the persistence of CD8+ memory T cells [8].

The gliadin-induced secretion of IL15 by macrophages, dendritic cells, and epithelial cells subsequently activates cytotoxic CD8+ intraepithelial lymphocytes independently of T cell receptor (TCR) specificity [9].

Interleukin-15 (IL-15) performs several biological functions important for the preservation and activity of various cell types. Despite its expression being tightly controlled, high IL-15 expression has been documented in numerous organ-specific autoimmune diseases. Celiac disease is an intestinal inflammatory disorder induced by gluten ingestion and characterized by the elevated expression of IL-15 in the intestinal mucosa. Because of its overexpression in both the lamina propria and the gut epithelium, it influences different cell types and affects several immunological components and pathways, thereby disrupting intestinal immune homeostasis [10].

Also, IL-15 can impact immune cells that affect multiple immune cells, including intraepithelial lymphocytes (IELs), enhancing IFN-γ synthesis and cytotoxic activity, consequently result in enterocytes apoptosis [11].

IL-2 is a primary cytokine released by Th1 cells that has a function in activation-induced cell death and the maintenance of regulatory T cells [12]. IL-2 is secreted by T-lymphocytes, which are white blood cells integral to immunological function. In celiac disease, some T-lymphocytes become sensitized to gluten. Upon detecting gluten, a T-cell first secretes high quantities of IL-2 to initiate an autoimmune response [13].

Interleukin-2 (IL-2) is identified as one of the initial cytokines activated after gluten exposure in patients with celiac disease that plays an important role in the adaptive immunological pathogenesis of celiac disease (CD) [14].

IL-2 and IL-15 have structural similarities, they share two receptor subunits, CD122 (β chain) and CD132 (γ(c) chain); Although the expression patterns and physiological functions of their respective receptors are distinctly different [15].

The aim of this study was to investigate Inflammatory cytokines play roles in the pathogenesis of celiac disease and to introduce new diagnostic markers in patients with celiac disease for easy, fast, low-cost, and non-invasive diagnosis.

2. Materials and methods

2.1. Study design

The study started with sample collection from September 2024 February 2025 following standard health and safety measures. It was a case-control study, the subjects of the study were attended to at the children welfare teaching hospital/Medical City in Baghdad.

2.2. Study participants and groups

The study comprised an age group of 2 to 14 years with a total of 88 participants. The participants were divided into two groups:

Patients Group:

This group includes 45 patients diagnosed with celiac disease (CD) by a specialist. The patients were selected from those who attended the children welfare teaching hospital in Medical City/Baghdad Governorate. All the patients, 45 were classified as having active CD, and all of them were newly diagnosed.

Control Group:

The control group included 43 apparently healthy individuals. Their ages matched those of the patients in the CD group, and they had no history of celiac disease or other gastrointestinal diseases.

2.3. Samples collection

Demographical and clinical data, including name, age, sex, duration of a gluten-free diet, time diagnosis, and living status, were gathered through interviews conducted with patients and/or their parents using a questionnaire. The subjects of the study were attended to at the children welfare teaching hospital/Medical City in Baghdad.

The collection of blood samples utilized disposable syringes to withdraw 5 ml of venous blood consecutively from child patients, as well as from control subjects. The blood was aspirated into gel tubes, which were then marked with barcodes. Afterward, the samples (gel tubes) underwent centrifugation at

a speed of 4000 revolutions per minute (rpm) for a duration of 10 minutes in order to separate the serum. After obtaining each sample, a volume of 1/2 to 1 ml of the liquid portion (serum) was drawn out and transferred into labeled Eppendorf tubes that were labelled. These tubes were then stored at a temperature of –20 °C until they were needed.

2.4. Statistical analysis

Analysis of Variance (ANOVA) and the T test were then applied to compare means, and where a P-value < 0.05 was considered statistically significant. For categorical variables, the chi-square test was used to analyze relationships. All reported results were expressed as the mean ± SE. A P-value less than 0.05 denoted statistical significance. Correlation between numeric variables was explored using Pearson's correlation coefficient between numeric variables was explored using Pearson's correlation coefficient.

Antitissue transglutaminase (tTG) IgA,IgG antibodies tests

The autoantibodies of tTG IgA, tTG-IgG in serum this tests has been achieved according to the manufacturing company (Aeskulisa/Germany) [16, 17].

Determination of Interleukin IL2 & Interleukin 15 Level in Serum:

The levels of (IL-2 and IL-15), in the blood of celiac patients and control participants, were quantitatively assessed using ELISA kits from the SUNLONG Human Bioassay Technology Laboratory (China) by strictly following the manufacturer's procedure [18, 19].

Ethical Approval

The study was approved by the research ethics committee of Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences, Faculty of Medicine, as well as by the Ministry of Health. Prior consent was sought from patients or their parents (in the case of children) by having them complete an enrollment questionnaire.

3. Results

3.1. Determination of anti-tissue transglutaminase (tTG)-IgA antibody

The serum anti-tTG IgA levels, which are given as 123.06 ± 15.6 in the patient group, compared to the control group, were 8.69 ± 0.61. The data revealed a highly significant variation between the patients and the healthy control group levels of IgA anti-tTG <0.0001 (HS), as seen in (Table 1).

3.2. Determination of anti-tissue transglutaminase (tTG)-IgG antibody

The serum anti-tTG IgG level was 104.10 ± 61.1 in the celiac group, while the healthy control group was 7.41 ± 0.57. The results found a highly significant difference between the patient and healthy control groups (P <0.001), as shown in (Table 1).

3.3. Detection of Interleukin 2 (IL2) in celiac patients and control groups

The serum IL2 level was 26.33 ± 6.59 in the celiac group and 6.55 ± 0.30 in the healthy control group. The results show a highly significant statistical difference between the patients and healthy control groups, P value (P = 0.004), as shown in (Table 2).

Table 1. Determination of the serum levels of the anti-tissue transglutaminase (tTG) IgG antibody and anti-tissue transglutaminase (tTG)-IgA antibody in celiac patients and healthy control groups.

	Control group		Patients group			
Parameters	Mean ± SE	Range	Mean ± SE	Range	T test	P value
anti tTG -IgA Ab	8.69 ± 0.61	16.04	123.06 ± 15.6	303.1	7.12	<0.0001 (HS)
anti tTG- IgG Ab	7.41 ± 0.57	12.45	104.10 ± 61.1	371.5	5.98	<0.0001 (HS)

HS: High significant difference at P < 0.01.

Table 2. Determination of the serum levels of Interleukin 15 (IL-15) and Interleukin 2 (IL2) in celiac patients and control groups.

	Control group		Patients group			
Parameter	Mean ± SE	Range	Mean ± SE	Range	T test	P value
IL15	12.70 ± 0.71	12.7	42.31 ± 7.42	301.8	3.97	>0.0001 (HS)
IL2	6.55 ± 0.30	7.01	26.33 ± 6.59	288.02	2.99	0.004 (HS)

HS: High significant difference at P < 0.01.

3.4. Detection of Interleukin-15 (IL-15) in celiac patients and control groups

The serum IL-15 level was 42.31 ± 7.42 in the celiac patient group, and 12.70 ± 0.71 in the healthy control group. The results show a highly significant difference between the patients and control groups regarding the IL15, P value (P<0.0001), as shown in (Table 2).

Data in the Table 3 show [3]:

Serum levels of anti-tTG- IgA have a positive correlation with tTG-G antibody. This correlation is statistically highly significant, with p-values of p (<0.0001) and data show a positive relationship (r = 0.774). Serum levels of IL-15 showed a positive correlation with tTG-IgA. The correlation coefficient is 0.241 but there was no statistical significance, p value p(= 0.11). A negative correlation was observed between IL15 and tTG- IgG. Furthermore, IL2 has a negative correlation with anti-tTG-IgG. There was not a statistically significant result (p = 439) and the correlation coefficient value was (–0.118).Moreover, the data demonstrate a positive correlation between IL-2 and IL-15. There was a statistically significant result. The p-value is (p < 0.0001) and a correlation coefficient value of 0.836. In addition, IL-2 has a positive correlation with anti tTG-IgA. the correlation coefficient value was 0.113, but it was not statistically significant (p = 0.461).

Table 3. Correlation between immunological markers.

parameters	R and P value	tTG-IgA	tTG-IgG	IL15	IL2
tTG-IgA	R	1			
	P	0			
tTG-IgG	R	0.774	1		
	P	0*	0		
IL-15	R	0.241	–0.023	1	
	P	0.11	0.882	0	
IL-2	R	0.113	–0.118	0.836	1
	P	0.461	0.439	0*	0

* Significant correlation.

3.5. Distribution of patients with celiac disease and control according to BMI

In the control group (n = 43), 53.48% had a BMI less than 18.5, 46.51% had a BMI between 18.5 and 25, and none (0%) had a BMI between 25 and 30. Within the control group, there was no significant (P > 0.05) statistical difference between the "Less than 18.5" and "18.525" BMI categories, but significant (P < 0.05) differences were observed when comparing the "2530" category with either "Less than 18.5" or "18.525" (Fig. 1). Conversely, in the patient group (n = 45), 71.11% had a BMI less than 18.5, 26.66% had a BMI between 18.5 and 25, and 2.22% had a BMI between 25 and 30. Within the patient group, the distribution across BMI categories showed a significant (P < 0.05) statistical difference among the three categories of BMI (Fig. 2). Overall, there is not significant statistical difference among BMI distributions between the celiac group in comparison to healthy control group, p value = 0.109, (Table 4).

4. Discussion

The antitissue tTG-IgA transglutaminase test is a primary screening method for suspected celiac disease. This result was compatible with the study conducted in Iraq by Aljamrawy et al. who found that the serum levels of anti-tissue Transglutaminase (tTG) IgA were significantly increased in patients with celiac disease in comparison to control group [20].

In this finding, the positive result with celiac group, due to the anti-tissue Transglutaminase (tTG) IgA test now used as the preferred test for screening of the celiac disease because its high sensitivity, high specificity and all the patients in first stage of disease with newly diagnosed.

This finding is consistent with the Iraqi study conducted by Majeed et al. who found that the anti-tTG IgG was significantly elevated in patients with celiac disease than in healthy groups [21].

Table 4. Distribution of patients with celiac disease and control according to the body mass index (BMI).

Variable	Category	Study groups		Calculated P value
		Patients(n = 45)	Control (n = 43)	
BMI	Less than 18.5	32(71.11)	23(53.48)	0.109(NS)
	18.5–25	12(26.66)	20(46.51)	
	25–30	1(2.22)	0(0)	
	Total	45(100)	43(100)	
	P value	<0.0001	<0.0001	

NS: No significant difference at P < 0.05, Less than 18.5 underweight, 18.5–25 normal weight, 25–30 (over weight).

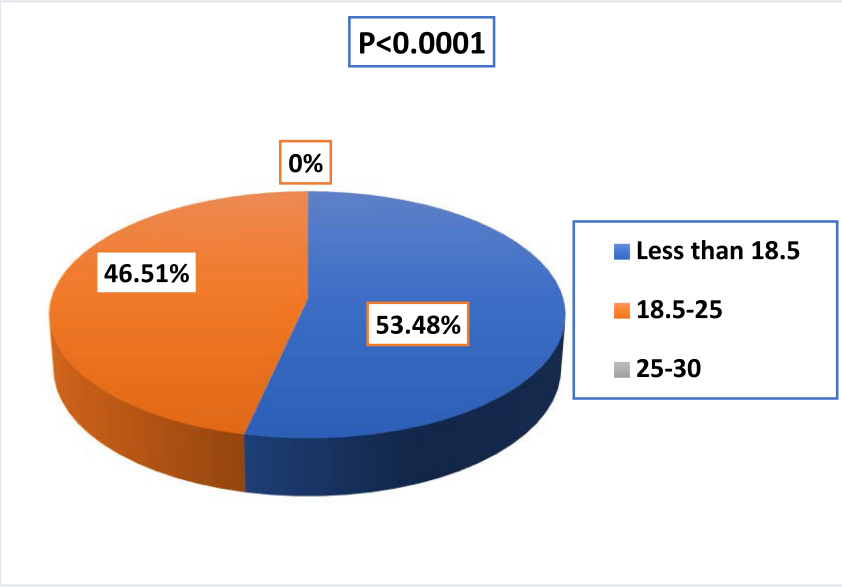

Fig. 1. Distribution of control group according to the BMI.

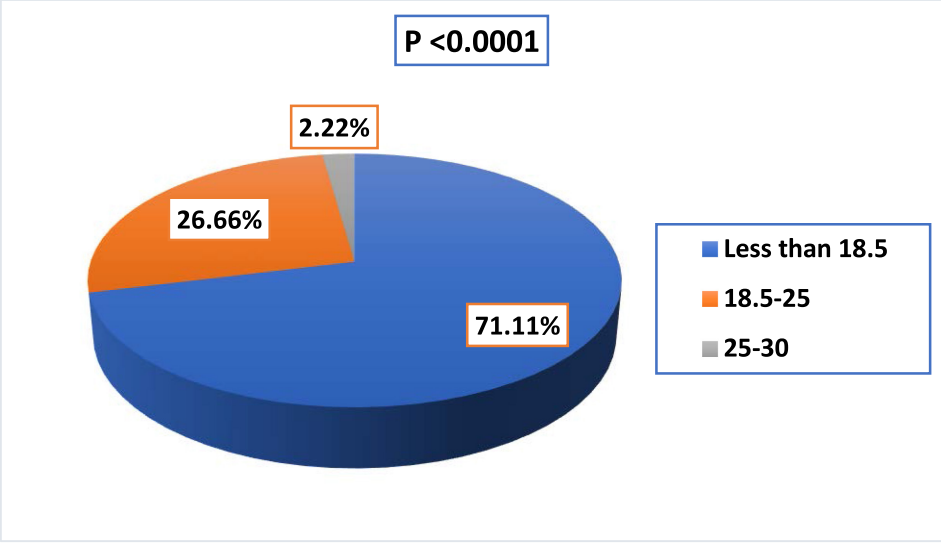

Fig. 2. Distribution of patients with celiac according to the BMI.

In this study, the high incidence of patients testing positive for Anti-tTG IgG, may be attributed to its high sensitivity and specificity in this study, confirming its diagnostic effectiveness.

Recent study is similar to Iraqi study conducted by Eidan and Mubark who found that the IL-2 level had significantly higher in patients with celiac disease in comparison with controls [22].

Also our findings were compatible with Iraqi research conducted in Najaf province by Abd and Hamad, which found that the IL-2 level had a highly significant difference in patients than healthy groups [23].

In this study, the high IL-2 serum levels in patients with celiac disease were due to IL-2 being the first cytokine produced by T lymphocytes in celiac disease; all patients were newly diagnosed, and the patients were on a gluten-containing diet.

This result agrees with a study done in Iraq by Eidan and Mubark, who found that the Interleukin-15 (IL-15) levels were significantly high in patients with celiac disease in comparison to the healthy control group [24].

Another study conducted in Iraq by Mohammed and Jassem revealed a high significance of IL-15 in untreated celiac patients compared with that in the healthy control group [25].

In this study, the elevated levels of interleukin-15 (IL-15) in patients with celiac disease, particularly during the early stages of the disease, indicate its potential use as a biomarker for early diagnosis and disease monitoring.

These results were compatible with the study conducted by Aljamrawy et al. who found that there is a positive correlation between tissue transglutaminase (tTG) IgA and IgG antibodies [20].

Also, the current study is consistent with the study conducted in Iraq by Alrazaaq, who showed a positive correlation of interleukin-2 (IL-2) with Anti tTG-IgA, while there was a negative correlation between IL-2 and anti-t TG IgG [26].

In addition, this research agrees with research conducted in Iraq by AL-Jarrah and Alattabi, revealed that there was a positive correlation between serum IL15 level and serum tTG (IgA), while there was a negative correlation between serum IL15 and serum tTG (IgG), A small size sample and the undetectable levels of IL15 revealed by the study might influence this correlation [27].

On the other hand, the findings from the study conducted by Ragab et al. showed that no significant correlation between serum IL-15 levels and anti-tTG IgA [28]. This finding was compatible with a study conducted by [29] who found that 18.3, 28.8, and 25.8% of the children had a short stature, low body weight, and low BMI, respectively.

In contrast to this result, the study conducted by van der Pals et al. [30] showed that the majority of the children with screening-detected celiac disease had a normal weight.

This finding indicates that the patients with celiac disease at the time of examination (with newly detected celiac disease) had a significantly low BMI (underweight), because the CD patients have malnutrition, which results in loss of body weight.

5. Conclusion

According to the results of this study, the increase in IL-15 and IL-2 concentrations in patients with celiac disease reflects that IL-2 and IL-15 both lead to intestinal inflammation and tissue damage by activating innate and adaptive immune responses. Moreover, IL-2 shares many structural and functional properties with IL-15. Elevated IL-15, IL-2 in recently diagnosed patients may serve as potential biomarkers in the diagnosis and prognosis of celiac disease. There is a positive association between anti-tTG-IgA and anti-tTG-IgG antibodies; also, there is a positive correlation between IL-2 and IL-15. On the other hand, there is a negative correlation between IL-15 and anti tTG-IgG. As well as, there is a negative correlation between IL-2 and anti tTG-IgG.

Financial funding

This research received no external funding.

Conflicts of interest

The authors declare no conflict of interest.

Ethical approval

Ethical approval statement is mentioned at the end of the "Materials and methods" section (above).

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