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Assessment of Levels of Interferon Gamma (IFN-γ) and Interleukin-15 as markers in Patients with Celiac Disease

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ABSTRACT

Background: *Celiac disease (CD) is a long-term digestive disorder caused by the immune system that affects people who are inherently more likely to get it. The ingestion of gluten-containing meals or beverages triggers the condition in those who are sensitive. The study aimed to serum levels of Tissue Transglutaminase (tTG-IgA and tTG-IgG), Interferon gamma (IFN-γ) and Iterleukin-15 in patients with celiac disease.* ***Materials and methods:*** *This case-control investigation was conducted at Marjan Hospital, Imam AL-Sadiq Hospital, and Al-Imam Ali Hospital. A convenience sampling of 146 samples. The study consisted of 73 patients with celiac disease (CD) and 73 healthy controls. 5 mL of venous blood was donated by each participant and subsequently deposited in gel containers (6 ml). The tubed blood is centrifuged at 3000 rpm for 10 minutes. The serum for Tissue Transglutaminase (tTG-IgA and tTG-IgG), Interferon gamma (IFN-γ) and Iterleukin-15 are stored in Eppendorf containers at -20 ºC until it is thawed on the day of the ELISA.* ***Results:*** *The present study found that patients had significantly higher levels of IL-15 (23.374±7.016) compared to controls (16.632±3.680), p-value < 0.001. In addition, the results report that Interferon gamma level was 11.268±5.381 in patients with celiac disease are higher significantly than the controls 7.461±2.304 with P. value <0.001.* ***Conclusions:*** *There was a significant elevating in levels of Interferon gamma (IFN-γ) and Iterleukin-15 in patients with celiac disease. Furthermore, the study found that tTG IgA and IgG show a significant positive correlation. In addition, there was positive correlation between Interferon gamma with tTG (IgA and IgG), and IL-15.*

Keywords: *Celiac Disease, Interleukin-15, Interferon Gamma, Antibody.*

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INTRODUCTION

Celiac disease (CD) is a long-term digestive disorder caused by the immune system that affects people who are inherently more likely to get it (1). The ingestion of gluten-containing meals or beverages triggers the condition in those who are sensitive (2). There is significant variability in the geographical distribution. The prevailing idea suggests that CD is more prevalent in Western nations, affecting approximately 1% of the total population (3). There has been a significant rise in the number of reported cases each year, particularly among children, which can be attributed in part to the rising sensitivity of diagnostic tests, changes in feeding practices, and a higher incidence of digestive symptoms resulting from the condition (4). The preferred method for detecting or screening for celiac disease regardless of age, is the use of anti-tissue transglutaminase (tTG) as a single test. The tTG

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test has a sensitivity of approximately 95% for detecting untreated CD, and its specificity is also 95% or higher. The test's sensitivity for detecting celiac disease (CD) is highest with tTG, and the probability of obtaining a true positive result increases as the test titer rises (5).

Interleukin-15 is crucial in the development of Celiac disease. The increase in IL-15 expression in the lining of the intestines has become a characteristic feature of the CD. Deamidated gluten peptides, catalyzed by tissue transglutaminase, induce the release of inflammatory cytokines, particularly interleukin-15, and stimulate the generation of intraepithelial lymphocytes (6). IFN-γ production is a characteristic of HLA-DQ2- and HLA-DQ8-restricted T lymphocytes specific to "gluten" peptides seen in the small intestinal mucosa of individuals with celiac disease. It is believed to play a crucial role in starting the process of mucosal destruction (7). Blocking IFN- has been shown to prevent gluten-induced mucosal deterioration in CD mucosa biopsies kept in organ culture. CD4+ T cells in the mucosa of CD patients are committed to producing IFN-γ, which aligns with findings that the transcription factor T-bet, responsible for Th1 cell development, is increased in untreated CD patients' mucosa and normalizes after gluten elimination from the diet (8).

MATERIALS AND METHODS

Study design and setting:

This study is a case-control design conducted in Al-Imam Ali Hospital, Imam Al-Sadiq Hospital, and Marjan Hospital. The data was collected during a period from November 2023 to April 2024.

Study population:

A convenience sampling of 146 samples. The study comprised from two groups include 73 patients with Celiac disease (CD), 73 healthy controls. Patients with CD defined by physician as previously diagnosed by gastroenterologist. The patients and controls were defined by serological testing (tTG antibodies). As shown in table 1.

Table (1): The distribution of the study groups according to tTG antibody range

Test	Range	Status	Defined as	Reference
tTG antibody	< 12 U/mL	Negative	Healthy control	(9) (10)
	12-18 U/mL	positive	Eqivalent	
	>18 U/mL	positive	Patient with CD	

Inclusion criteria:

This study includes individuals all ages (age matched ± 5 years between study groups) and both sexes.

Exclusion criteria:

Subjects with diabetes mellites, pregnancy, ischemic heart disease, other autoimmune diseases, other kidney diseases, and other gastrointestinal disorders prior to the study were excluded from consideration.

Methods of sample collection:

The researcher developed a questionnaire to collect the age and sex of the study group

members after the interviews. Subsequently, 5 mL of venous blood was collected from each participant in the study in a sequential manner, and the blood was subsequently transferred to gel tubes (each containing 6 mL). The blood in containers is centrifuged at 3000 rpm for 10 minutes. Subsequently, the serum for Tissue Transglutaminase (tTG-IgA and tTG-IgG), Interferon-gamma (IFN-γ), and Iterleukin-15 is transferred to Eppendorf containers and stored in a deep freezer at -20 ºC until it is thawed on the ELISA working day.

Immunological Assays:

The enzyme-linked immunosorbent assay (ELISA) technique was utilized for the evaluation of Tissue Transglutaminase (tTG-IgA and tTG-IgG), Interferon-gamma (IFN-γ), and Iterleukin-15 serum levels, the biochemical kit used in the study for performed by a company AESKULISA.

Statistical analysis:

The data were gathered, arranged, analyzed, and presented using Microsoft Office Excel 2010 and the statistical package for social sciences, version 27, from IBM-SPSS software. Following the determination of the normality of the variables, the numerical data's mean, standard deviation, standard error, and range were shown using the Kolmogorov-Smirnov normality test. The two independent samples t-test may be used to assess the mean difference between any two groups if the variable is normally distributed. The chi-square test (X^2) may be used to examine associations between any two category variables. The level of significance was defined as a P-value of 0.05 or less, and the very significant level was indicated by a P-value of 0.01 or less (11).

RESULTS

Demographic characteristics of patients with celiac disease and control subjects.

Figure (1) demonstrates that the majority of patients (34.2%) were lie within age group (11-20 years), while most of controls were lie within age group (21-30 years) with 38.4%. There is no a statistically significant difference (p = 0.134) was found between patients and control groups. Figure (2) demonstrates that there is no a statistically significant difference (p = 0.715) was found between patient and control groups in terms of sex.

Levels assessment of the Tissue Transglutaminase (tTG-IgA and tTG-IgG), Interferon gamma and Interleukin-15.

In **Table (2)**, the results reveal that patients had significantly higher levels of TTG IgA antibodies (138.462 ± 131.105) compared to controls (7.689 ± 5.484), p-value < 0. 001. Regarding TTG IgG, the results reveal that patients had significantly higher mean levels (98.407±87.922) than controls (8.157±6.535), at statistically significant P. value <0.001.

Table (3) compares the levels of the patients with celiac disease and control subjects according to the levels IL-15 and Interferon gamma. The present study found that patients had significantly higher levels of IL-15 (23.374±7.016) compared to controls (16.632±3.680), p-value < 0.001**.** In addition, the results report that Interferon gamma level was 11.268±5.381 in patients with celiac disease are higher significantly than the controls 7.461±2.304 with P. value <0.001. **Table (4)** shows celiac disease patients' parameter correlation coefficients. The study found that tTG IgA and IgG show a significant positive correlation (r = 0.793, p < 0.001). Moderate positive correlations between TTG IgG levels and IL_15 (r = 0.572). In addition, there was positive correlation between Interferon gamma with tTG (IgA and IgG), and IL-15.

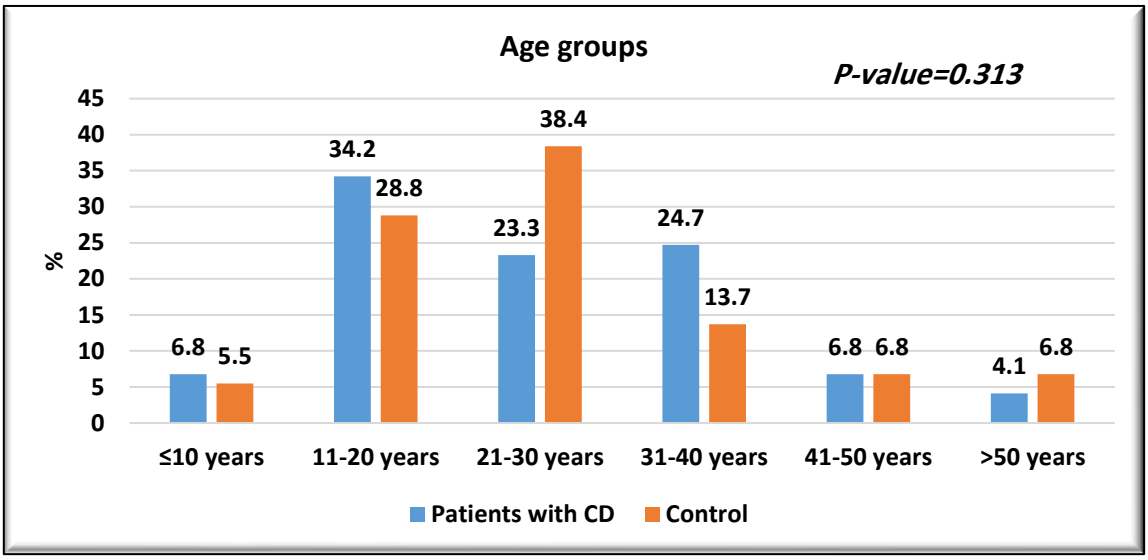

Figure (1): The distribution of the patients with celiac disease and control subjects according to age group.

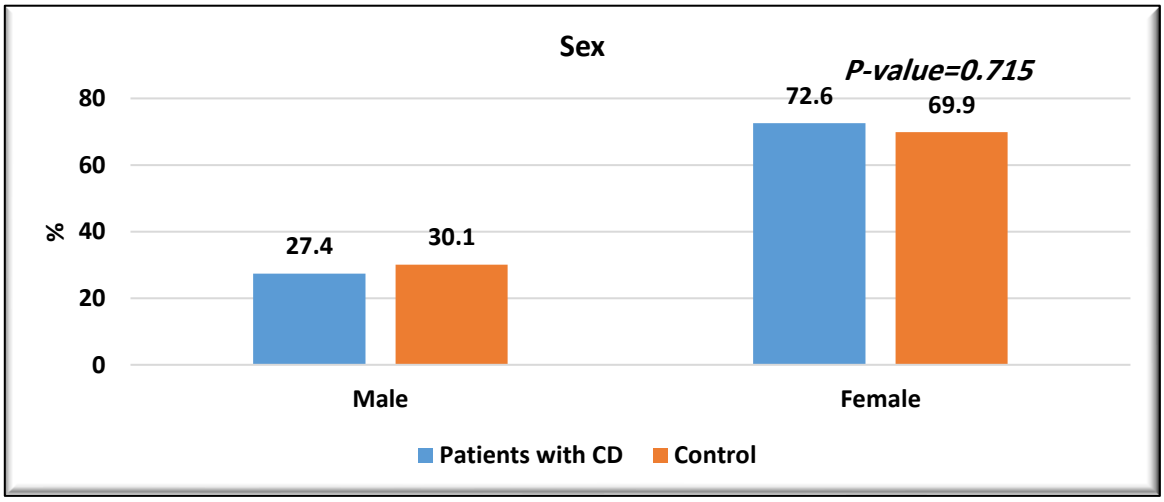

Figure (2): The distribution of the patients with celiac disease and control subjects according to sex.

Table (2): Comparison between levels of the patients with celiac disease and control subjects according to Ttg.

	GROUPS	Mean ± SD (U/ml)	SE	t. test	P. value
tTG IgA	Patients	138.462±131.105	15.345	8.515	<0.001
	Control	7.689±5.484	0.642		
tTG IgG	Patients	98.407±87.922	10.290	6.522	<0.001
	Control	8.157±6.535	0.765		

Table (3): Comparison between levels of the patients with celiac disease and control subjects according to the levels of IL-15 and Interferon gamma.

	GROUPS	Mean ± SD	SE	t.test	P. value
IL_15	**Patients**	**23.374±7.016**	**0.821**	**7.271**	**<0.001**
	Control	**16.632±3.680**	**0.431**		
Interferon-gamma	**Patients**	**11.268±5.381**	**0.630**	**5.558**	**<0.001**
	Control	**7.461±2.304**	**0.270**		

Table (4): Correlation between the studied parameters in patients with celiac disease

		tTG IgA	tTG IgG	IL_15	Interferon gamma
tTG IgA	r	1	**0.793****	**0.500****	**0.521****
	P. value		**<0.001**	**<0.001**	**<0.001**
tTG IgG	r		1	**0.572****	**0.424****
	P. value			**<0.001**	**<0.001**
IL_15	r			1	**0.605****
	P. value				**<0.001**

. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

In this study, there is no a statistically significant difference (p >0.05) was found between patient and control groups in terms of age and gender. These results are consistent with the previous study findings conducted by (12) reported there was matching between case and control regarding age and gender (P. value >0.05). This can explain that gender/age matching is essential for study design to control bias effects. Researchers can reduce the impact of gender/age-related variables by ensuring that both case and control groups have equal numbers of men and women. Gender/age matching reduces bias and improves study validity by allowing more accurate case-control comparisons.

The results of this study indicate that patients had significantly higher levels of TTG IgA antibodies (138.462 ± 131.105 U/ml) compared to controls (7.689 ± 5.484 U/ml), p-value < 0.001. These results agreed with the study findings done by (13) which revealed that the average level of TTG IgA were higher in people with CD. Also, these results supported by (14) (15). In addition, a study by (16) reported that TTG IgA specificity was significantly higher in patients with CD. Also, in Iraq, a study by Awadh and Hasan, (2022), which reported that Celiac disease patients had a significantly higher mean of tTg IgA than the healthy Group, 131.00 pg\ml vs. 1.99 pg\ml, respectively (P. value <0.05). This elevated can explain that patients with celiac disease have elevated tissue transglutaminase (TTG) IgA antibodies, indicating a small intestine immune response to gluten. The immune system mistargets TTG, an enzyme

that modifies gluten proteins, causing inflammation and tissue damage in celiac disease. A reaction creates blood-testable IgA antibodies against TTG. Celiac disease diagnosis needs elevated TTG IgA antibodies (18).

Patients exhibited considerably higher mean TTG IgG levels (98.407±87.922 U/ml) than controls (8.157±6.535 U/ml), with a P-value <0.001. These findings are congruent with (19), which found that isolated tTG IgG was 97% effective in CD diagnosis (172/178). We can discuss that celiac disease patients' immune systems perceive gluten for a threat and assault the small intestine. Intestinal TTG is targeted by this immune response, resulting in increased TTG IgG antibodies. This immune reaction damages the intestinal villi, hindering nutrient absorption and causing celiac disease symptoms (20). In this study, the present results found that patients had significantly higher levels of IL-15 (23.374±7.016) compared to controls (16.632±3.680), p-value < 0.001. the results of this study is in agreement with findings of the study done by Vorobjova *et al.*, (2019) reported that the significant increase of the serum level of IL-5 in CD patients compared to controls. Also, a study in Iraq by (22) revealed that there was a statistically significant ($p \leq 0.001$) increase in IL-15 serum levels between CD patients and seemingly healthy controls.

The results report that Interferon gamma level was 11.268±5.381 in patients with celiac disease are higher significantly than the controls 7.461±2.304 with P. value <0.001. these results agreed with (23) which found that the mean serum levels of IFN-γ ($p = 0.04$) were significantly different between the patients in the CD and control groups. In karbala, Iraq a study by Awadh and Hasan, (2022), which reported that celiac disease patients had a significantly higher mean of IFN-γ than the healthy group, 245.93 pg\ml vs. 52.69 pg\ml, respectively. Elevated IFN-γ levels in celiac disease patients result from a dysregulated immune response caused by gluten consumption. Gluten-containing meals cause celiac disease patients' immune systems to confuse gluten peptides for hazardous invaders, activating T-helper 1 (Th1) cells. The Th1 cells release IFN-γ, a pro-inflammatory cytokine that controls the immune response to perceived threats. In celiac disease, IFN-γ activates macrophages and stimulates inflammatory mediator synthesis, leading to inflammation and tissue destruction in the small intestine. Celiac disease patients have intestinal inflammation, villous atrophy, and clinical symptoms due to this chronic immunological reactivity (24) (23) (25).

CONCLUSIONS

There was significant elevating in levels of Interferon gamma (IFN-γ) and Iterleukin-15 in patients with celiac disease. Furthermore, the study found that tTG IgA and IgG show a significant positive correlation. In addition, there was positive correlation between Interferon gamma with tTG (IgA and IgG), and IL-15.

Ethical approval:

The study followed a protocol approved by a local ethics committee at University of Kufa/ Medicine College/department of medical microbiology and Babylon Health Directorate.

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