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EVALUATION OF CYTOKINES IL-12 AND IL-15 IN PATIENTS WITH AUTOIMMUNE CELIAC DISEASE IN NAJAF PROVINCE: IMPLICATIONS FOR IMMUNE RESPONSE

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Abstract:
Celiac disease (CD) is a common immune-mediated intestinal disease. In genetically susceptible people, it is caused by gluten proteins found in barley, wheat, and rye. Screening for CD requires anti-gliadin antibodies (AGA), although high AGA levels are found in many immune-mediated skin diseases without gastrointestinal symptoms. This study aimed to determine the levels of "anti-tissue transglutaminase antibodies and anti-gliadin antibodies," "IL-12," and "IL-15," in patients with celiac disease and controls. This case-control study was conducted at , Al-Sadr Medical City, Al-Hakim Hospital and Gastroenterology Consultation Clinic in Najaf Governorate. Data were collected between December 2023 and April 2024, A sample of 60 people was drawn. The study included two groups of 30 patients with celiac disease (CD) and 30 healthy controls. Patients with CD were defined by their physicians and were previously diagnosed by a gastroenterologist. Patients and controls were defined by serological tests (anti-tTG antibodies and anti-gliadin antibodies. Patients with celiac disease displayed markedly elevated levels of anti-tTG IgA, with a mean of 141.122 ± 111.115, compared to 6.568 ± 4.367 in the control group. Similarly, anti-tTG IgG levels were significantly higher in patients (82.324 ± 77.815) than in controls (7.213 ± 5.634). For anti-gliadin antibodies, the results were consistent. Patients had an average of 6.121 ± 2.958 for anti-gliadin IgA, while the control group had much lower levels at 1.799 ± 1.137. while The data indicate significant differences in cytokine levels between celiac disease patients and healthy controls. For IL-12, patients exhibited a mean level of 30.121 ± 9.849, while controls had a mean of 19.217 ± 2.274. In the case of IL-15, patients showed a mean level of 25.279 ± 6.971 compared to controls, who had a mean of 13.589 ± 1.973. In comparison to controls, who had IL-12 levels of 51.16 ± 2.56 for men and 44.28 ± 3.36 for females, celiac disease patients had lower levels, with males having 30.12 ± 4.21 and females having 20.97 ± 2.11. Patients with celiac disease reported considerably higher levels of IL-15: 77.91 ± 7.34 for females and 88.97 ± 8.41 for males. On the other hand, the mean for females was 29.87 ± 3.67 while the mean for control men was 49.86 ± 2.98.

Keywords: Cytokines, IL-12, IL-15, Autoimmune, Celiac Disease, Immune Response, Najaf Province.

Introduction

Celiac disease (CD) is a prevalent condition characterised by an immune-mediated enteropathy. It occurs in individuals who are genetically predisposed and is triggered by the consumption of gluten proteins found in some cereals including barley, wheat, and rye (Kumar et al., 2024). Those with a genetic predisposition may have inflammatory bowel disease because the digestive enzymes normally used in the intestines are unable to properly break down the gliadin protein found in certain meals (1).

Gluten is a complicated mixture of very similar proteins that are insoluble in water but dissolve in alcohol. This substance stands out because of its high amino acid content material, along with glutamine and proline. These amino acids help to present it a distinct resistance to protease destruction in the digestive tract (2).

Celiac ailment has grow to be extra not unusual in current decades, with an predicted global incidence fee of 1% to at least one.5%(3). This might be because of changes in environmental variables that impact the frame's reactivity to nutritional gluten and due to enhancements in diagnostic approaches that have elevated sensitivity (4). In Saudi Arabia, the CD fee is 3.2%, that's the very best among Arabian countries (5).

A wide variety of cytokines are implicated inside the persistent inflammatory tactics that characterize celiac disease (6). Cytokines are small proteins with extremely low molecular weights that feature as messengers among cells in the immune machine (7). They are necessary for developing a coordinated immune response because they coordinate the pastime of various cellular kinds in various locations of the body. (8).

Cytokines and celiac disease have been the subject of a great deal of study. It was only very recently, however, that the precise cytokine release in reaction to gluten ingestion and its association with symptomatology were clarified (9). Celiac disease patients' clinical reactions to gluten are significantly impacted by T cell activation, as seen by the first increase in blood interleukin- 2 (IL-2) levels after gluten consumption (10).

Crucial biological processes depend on the pro-inflammatory cytokine interleukin-15 (IL-15), which is produced by several cell types (11). A pro-inflammatory chemokine, interleukin-15 (IL-15), is essential for the maintenance and function of many different types of cells (12). Some prior investigations have indicated that Celiac Disease (CD) is typified by a pronounced Th1 immune response (13). Conversely, Interleukin-12 (IL-12), which serves as a pivotal cytokine within the Th1 response, has been documented to exhibit diminished levels in intestinal biopsies obtained from patients with CD (14), whereas alternative research reports heightened levels in pediatric patients diagnosed with CD, which significantly decreased following the implementation of a gluten-free diet (GFD) (15). The observed reduction in serum IL-12 concentrations among individuals with CD may suggest that the Th1 polarization of CD4+ T lymphocytes in the context of CD immunopathology is likely influenced by intricate immunological and molecular mechanisms alongside various contributing factors (16). This phenomenon may be characterized by both elevated and down-regulated cytokine concentrations within the serum, specifically pertaining to the distinct category of Th cytokines (17) .

2. Material and Method

2.1 Study Design and Setting:

This case-control study was conducted at, Al-Sadr Medical City, Al-Hakim Hospital and Gastroenterology Consultation Clinic in Najaf Governorate. Data were collected between December 2023 and April 2024

2.2 Study Population

Study Sample A sample of 60 people was drawn. The study included two groups of 30 patients with celiac disease (CD) and 30 healthy controls. Patients with CD were defined by their physicians and were previously diagnosed by a gastroenterologist. Patients and controls were defined by serological tests (anti-tTG antibodies and anti-gliadin antibodies.

2.3 Ethical Approval

Informed consent was received from Jabir Ibn Hayyan Medical University's Research Ethics Committee and the Ministry of Health, as well as ethical approval from all patients and volunteers who completed the registration questionnaire.

2.4 Exclusion criteria:

Patients with other autoimmune disorders, pregnancy, central nervous system diseases, and cardiovascular diseases, as well as those suffering from parasitic or bacterial digestive system problems.

2.5 Sample Collection

Five milliliters of blood were collected from each patient at Al-Hakim Hospital, Sadr Medical City, and Gastroenterology Consultation Clinic in Najaf Governorate by puncturing a vein in a sterile and simple tube. We allowed the blood samples to clot at room temperature and then centrifuged them at 3000 rpm for 5 minutes. The serum of each sample was separated and transferred to an Ependrof tube, which was quickly stored at -20°C before use. Multiple studies were performed on the serum, including examination of IL-12, IL-15, anti-gliadin antibodies (IgA, IgG), and anti-tTG (IgA, IgG).

2.6 Immunologic Investigation

The laboratory investigations for this study comprised an immunological examination of cytokines linked with Celiac disease, specifically interleukin 12 (IL-12) and interleukin 15 (IL-15), as well as a serological assay for Celiac disease-related antibodies. Anti-transglutaminase (t TG) antibodies were present in both the test and control samples, as were antibodies targeting gliadin IgA. Cytokine testing was carried out utilizing the sandwich enzyme linked immunosorbent assay (ELISA) method using antibodies.

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3.Result

3-1 Measurement level of Anti-tTG and Anti-gliadin

The results from the comparison of anti-gliadin antibodies and anti-tissue transglutaminase (anti-tTG) antibodies between celiac disease patients and control individuals reveal significant differences. Patients with celiac disease displayed markedly elevated levels of anti-tTG IgA, with a mean of 141.122 ± 111.115, compared to 6.568 ± 4.367 in the control group. This indicates a substantial increase, highlighting the immune response related to gluten exposure. Similarly, anti-tTG IgG levels were significantly higher in patients (82.324 ± 77.815) than in controls (7.213 ± 5.634). For anti-gliadin antibodies, the results were consistent. Patients had an average of 6.121 ± 2.958 for anti-gliadin IgA, while the control group had much lower levels at 1.799 ± 1.137. The difference was also pronounced in anti-gliadin IgG, with patients showing 8.131 ± 3.781 compared to controls. the elevated antibody levels in celiac disease patients suggest a strong autoimmune response associated with gluten ingestion, significantly differentiating them from healthy controls. Each parameter exhibited a statistically significant p-value of less than 0.001

Table (1): A comparison of the levels of anti-gliadin antibodies and anti-tissue transglutaminase (anti-tTG) antibodies was performed between celiac disease patients and control persons.

	GROUPS	Mean ± SD	t.test	P. value
Anti-tTG IgA	Patients	141.122±111.115	4.813	**<0.001**
	Control	6.568±4.367		
Anti-tTG IgG	Patients	82.324±77.815	5.982	**<0.001**
	Control	7.213±5.634		
Anti-gliadin IgA	Patients	6.121±2.958	7.895	**<0.001**
	Control	1.799±1.137		
Anti-gliadin IgG	Patients	8.131±3.781	6.621	**<0.001**
	Control	2.490±1.881		

3-2 Measurement of the levels of cytokines (IL-12, IL-15)

The data indicate significant differences in cytokine levels between celiac disease patients and healthy controls. For IL-12, patients exhibited a mean level of 30.121 ± 9.849, while controls had a mean of 19.217 ± 2.274. The t-test result of 4.987, with a P value of <0.001, suggests a highly significant increase in IL-12 levels among patients. In the case of IL-15, patients showed a mean level of 25.279 ± 6.971 compared to controls, who had a mean of 13.589 ± 1.973. The t-test value of 6.538, along with a P value of <0.001, further confirms the significant elevation of IL-15 in patients. These findings highlight the role of IL-12 and IL-15 in the immune response associated with celiac disease. The elevated cytokine levels may reflect an active

inflammatory process, indicating their potential significance in the diagnosis and understanding of the disease's pathophysiology.

Table (2): Comparison of the levels of cytokines (IL-12, IL-15) in celiac disease patients and control individuals

	GROUPS	Mean ± SD	t.test	P. value
IL_12	Patients	30.121±9.849	4.987	**<0.001**
	Control	19.217±2.274		
IL_15	Patients	25.279±6.971	6.538	**<0.001**
	Control	13.589±1.973		

3-3 Measurement of the levels (IL-12, IL-15) distributed by gender

In comparison to controls, who had IL-12 levels of 51.16 ± 2.56 for men and 44.28 ± 3.36 for females, celiac disease patients had lower levels, with males having 30.12 ± 4.21 and females having 20.97 ± 2.11. Patients with celiac disease reported considerably higher levels of IL-15: 77.91 ± 7.34 for females and 88.97 ± 8.41 for males. On the other hand, the mean for females was 29.87 ± 3.67 while the mean for control men was 49.86 ± 2.98. These results show clear cytokine-level patterns, suggesting a greater inflammatory response in celiac patients relative to healthy people, as well as significant gender disparities.

Table (3) : Serum levels of IL-12 and IL-15 in celiac disease patients and controls, distributed by gender

Interleukins	Mean ± Standard Error			
	Celiac Disease Patients (No.=30)		Controls (No.=30)	
	Males	Females	Males	Females
IL-12	30.12±4.21	20.97±2.11	51.16±2.56	44.28±3.36
IL-15	88.97±8.41	77.91±7.34	49.86±2.98	29.87±3.67

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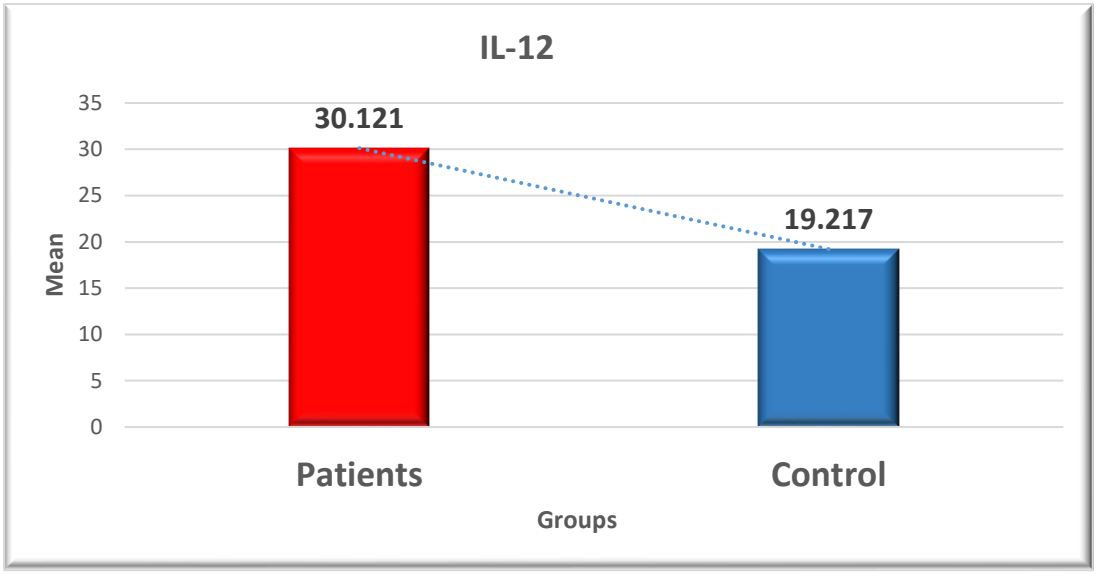

Figure (1): Comparison between the IL-12 levels of celiac disease patients and control participants

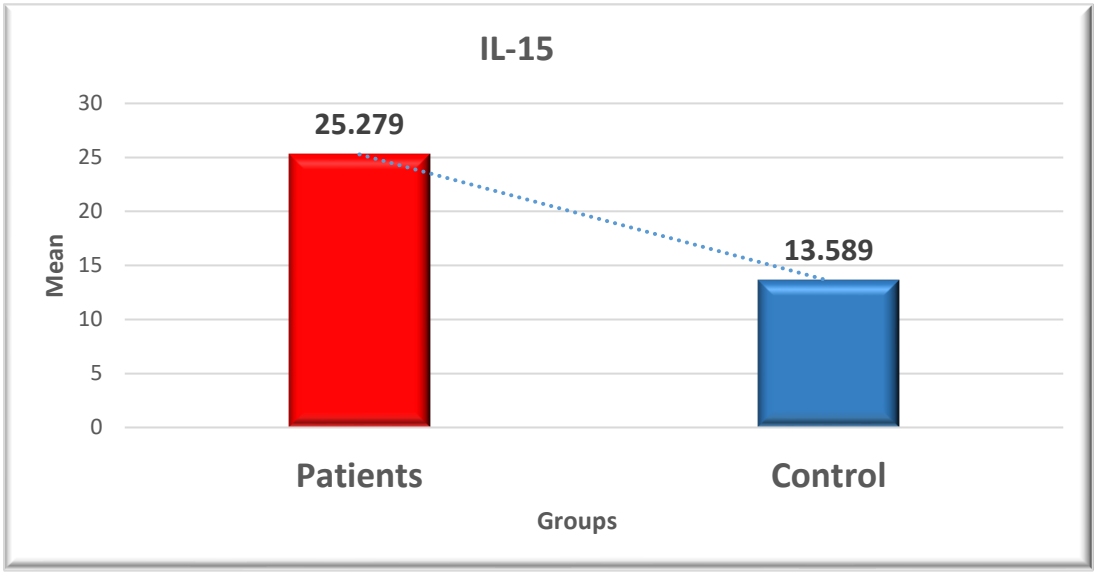

Figure (2): Comparison of the levels of IL-15 in celiac disease patients and control people

5. Discussion

The results of the current study reveal significantly higher levels of anti-tTG and anti-gliadin antibodies in patients diagnosed with celiac disease compared to control subjects. The mean anti-tTG IgA level of 141.122 ± 111.115 is particularly noteworthy, as it confirms the results from a study by (18), which showed a similar pattern in celiac disease patients, thus highlighting the diagnostic accuracy of anti-tTG IgA as a primary marker for celiac disease. Furthermore, the anti-tTG IgG levels in our patients (82.324 ± 77.815) further support the idea that both IgA and IgG isotypes are important in the diagnostic framework, especially in cases where IgA

deficiency is a concern. This observation is consistent with the (19), who emphasized the usefulness of measuring both isotypes to improve diagnostic sensitivity. In addition, the high levels of anti-gliadin antibodies (6.121 ± 2.958 for IgA and 8.131 ± 3.781 for IgG) in your results reflect the conclusions of (20), who suggested that while anti-gliadin antibodies are less specific compared to anti-tTG antibodies, they can still provide valuable insights, especially in cases of gluten sensitivity. Overall, the current study contributes to the growing body of evidence supporting the role of these serum markers in the diagnosis of celiac disease. The significant differences highlighted in the study results reinforce the need to integrate both anti-tTG and anti-gliadin antibodies into clinical practice to achieve a comprehensive diagnostic approach.

The results of the present study indicate a significant increase in the levels of cytokines IL-12 and IL-15 in celiac disease patients compared to the control group. In particular, the mean level of IL-12 was 30.121 ± 9.849 in patients versus 19.217 ± 2.274 in controls, which is consistent with (21), which showed increased levels of IL-12 in celiac disease patients, suggesting that IL-12 plays a pivotal role in the inflammatory response associated with this condition. Similarly, IL-15 levels were significantly higher in patients (25.279 ± 6.971) than in controls (13.589 ± 1.973). This is consistent with the work of (22), which highlighted the importance of IL-15 in the pathogenesis of celiac disease, pointing to its role in the activation and proliferation of intraepithelial lymphocytes, which are hallmarks of the immune profile of the disease. The elevated levels of both IL-12 and IL-15 in this study reinforce the understanding that these cytokines are an integral part of the inflammatory environment in celiac disease. Their presence may not only indicate active disease but may also serve as potential biomarkers for monitoring disease progression and response to treatment. These findings emphasize the need for further research into therapeutic targeting of these cytokines to modulate immune responses in celiac disease, as suggested by recent studies exploring cytokine-based interventions. Analysis of IL-12 and IL-15 levels in patients with celiac disease reveals sex-based differences. In the present study, mean IL-12 levels were significantly higher in male patients (30.12 ± 4.21) than in female patients (20.97 ± 2.11). This disparity is notable as it suggests that male patients may have a more pronounced inflammatory response, which is consistent with findings (23) that high IL-12 levels are associated with severe immune activation, and their results reinforce the idea that there are sex-specific mechanisms involved in the causation of celiac disease. Similarly, IL-15 levels also showed gender differences, with male patients showing a mean of 88.97 ± 8.41 compared to 77.97 ± 7.34 in females. This is consistent with (24), who emphasized the role of IL-15 in the activation of intraepithelial lymphocytes, suggesting that males may have a higher immune activation that could contribute to the disease. These findings highlight the importance of considering gender in the context of celiac disease research. Hormonal influences, as suggested (25), may play a crucial role in modulating immune responses, leading to these observed differences. Understanding these sex-specific cytokine profiles could improve our approach to diagnosis and treatment, allowing for more personalized treatment strategies that take into account these differences in immune response. Overall, the marked

differences in IL-12 and IL-15 levels between male and female patients underscore the complexity of celiac disease.

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