Pro-inflammatory and Anti-inflammatory Cytokines Profile in Celiac Disease Patients

M.Sc. Zahraa Abbass Qasim[1]*, Prof. Dr. Hawraa Abdul Ameer Ali Al-Dahhan[2]

[1]Depart. of Pathological Analysis, Faculty of Science, Kufa University, Iraq.

[2] Depart. of Pathological Analysis, Faculty of Science, Kufa University, Iraq.

*Corresponding author, e-mail: hawraa.aldahan@uokufa.edu.iq

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Abstract:

Celiac disease is an autoimmune disorder that occurs when people with a genetic predisposition react to gluten, a protein found in wheat, barley, and rye. This reaction primarily affects the small intestine, causing a variety of symptoms. While the disease is most commonly associated with digestive issues, it can also cause problems in other parts of the body, leading to both intestinal and non-intestinal symptoms. This condition can affect both children and adults, triggering an immune response that results in damage to the intestine. The study aims to increase the role of (pro-inflammatory cytokines IL-6) and (anti-inflammatory cytokines IL-10 and IL-21) in the serum of CD patients. A total of 103 individuals of both sexes with an age range of 5-≥ 64 years were collected from (AL-Sadder Medical City in Najaf province). All patients were examined serologically by TTG antibody (IgG & IgA) and antigliadin (IgG & IgA) with a special ELISA kit for determination of CD patients. Interlukin-6, interleukin-10, and interleukin-21 were examined serologically in patients (103) and healthy persons as a control group. The findings of the current study indicate that there was no significant elevation of IL-6(30.98±24.58) and IL-10 (60.32 ± 17.97) in celiac disease patients comparable with control (27.4±15.66) and (51.6±18.64), respectively. IL-21 revealed a significant ($P \leq 0.001$) elevation (100.26±27.77) in CD patients compared to control (58.7±20.02).

Keywords: Pro-inflammatory cytokines, Anti-inflammatory cytokines, (IL-6), (IL-10), (IL-21), celiac disease.

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1. INTRODUCTION

CD is a long-term condition that affects the small intestine, this is a result of the immune system's reaction to gluten in the diet. This reaction is driven by T-cells, which trigger inflammation and damage to the intestine (1).

A lifelong gluten-free diet (GFD) is the sole available treatment; it eliminates the antigenic trigger and stops inflammation but does not repair the tolerance breakdown (2). Several adaptive and innate immunological disruptions that lead to a lack of gluten tolerance are the cause of celiac disease. Patients who carry the HLA-DQ2 or HLA-DQ8 genes have a strong dependence on a specific part of the immune system component referred to as the major histocompatibility complex (MHC) class II. This dependence suggests an imbalance in the body's adaptive immune response (1,3). Celiac disease is widely recognized as a T-cell-mediated condition. In this process, peptides from gliadin, a component of gluten, are modified by an enzyme called tissue transglutaminase. These modified peptides are then presented by immune cells to T helper lymphocytes in the tissue beneath the surface of the intestine (4). When activated, T helper cells macrophages secrete pro-inflammatory cytokines, which trigger the activation of lymphocytes within the intestinal lining. This immune response leads to the characteristic changes in the tissue that are seen in CD (5). Studies have shown that the levels of certain cytokines are linked to the activity of the disease. In active celiac disease (CD), T cells within the small intestine produce a Th1 cytokine called interferon (IFN), which plays a key role in the immune response (6). Intestinal epithelial cells also produce higher levels of interleukin-15, which affects the lymphocytes found within the lining of the intestine (7). The majority of research on

cytokine increases in CD has used entire biopsy samples from the duodenum or jejunum, and in situ, hybridization or immunohistochemistry techniques have been used to evaluate cytokine production at the local level within the small bowel mucosa (8,9). A few studies have quantified levels of serum cytokine using enzyme-linked immunosorbent assays (ELISAs). These studies have found that serum levels of (interleukin-6) were significantly elevated in patients with active celiac disease compared to healthy individuals. Additionally, interleukin-6 levels in the serum only decreased after patients had been on a GFD for a year (6). Several studies have demonstrated that IL-10, along with interferon-alpha (IFN-α), is produced in significantly greater amounts by intraepithelial lymphocytes (IELs) from patients with ACD compared to IELs from patients who are on a gluten-free diet or healthy individuals (7,3,10) The recruitment of immune cells to the inflamed tissue triggers fibroblasts to produce proteases that damage the tissue. Additionally, this process makes the effector CD4+ T cells less responsive to the immune-suppressing effects of regulatory T cells (7). Due to the increasing prevalence of celiac disease in recent years, we chose to conduct a thorough assessment of serum cytokine levels in individuals with celiac disease. We also aimed to evaluate how a GFD affects these levels of cytokine.

2. MATERIALS AND METHODS

Samples Collection

A total of 85 individuals of both genders participated in the study (July - December 2023), all samples were collected from the (Internal Medicine Unit at Al-Sadder Medical City) and (the Specialized Hospital for Gastroenterology and Hepatology) in Al-Najaf province. The patients' ages ranged from (5-60) years. Each patient participated in a completely general and clinical examination to diagnose CD using various serological tests (tissue transglutaminase- IgA, tissue transglutaminase -IgG, anti-gliadin antibody-IgA, and anti-gliadin antibody -IgG) and immunological tests (interleukin-6, interleukin -10, interleukin -21, and IFN-γ). A control group of 60 healthy individuals, matched by (age and gender) to the patients, was also included in the study. The samples of venous blood (5 ml) were obtained from both the patient group (85 individuals) and the control group (60 individuals) under sterile conditions and written consent. After centrifuging the samples at (3000 rpm/10 minutes), the serum was isolated and stored at (-80°C) until further analysis using ELISA.

Detection of serum Cytokines

The levels of (IL -6, IL -10, and IL -21). The serum levels were measured using (ELISA), according to the manufacturer's instructions for the ELISA kit (Solarbio, China). A double-antibody sandwich ELISA method was used to assess the concentration of the target proteins in the samples. The serum was added to wells that were pre-coated with monoclonal antibodies specific to the target proteins, and the mixture was incubated. After washing was performed to remove unbound enzyme, Chromogen Solution (A and B) were added. This caused the liquid to change color from blue to yellow due to the acidic reaction. The intensity of the (yellow color) was directly correlated with the concentration of the target protein in the samples changed from (blue to yellow) under acidic conditions. The color intensity was directly proportional to the concentration of the target protein in the samples.

Statistical analysis:

The data are presented as mean ± standard deviation (SD). All graphs were generated using (GraphPad Prism 9) for statistical analysis.

3. RESULT

Evaluation of Interleukin 6 (IL-6) Serum Levels

The data showed an elevated level of (IL-6) in patients with celiac disease, with a mean value of 30.98 ± 24.58, as shown in table and figure (1).

Table (1): Concentration of (IL-6) in patients and control groups.

Studied groups	No.	Mean ± S.D Pg/ml	*P* value
Patients	85	30.98±24.58	*P* ≥ 0.001
Control	60	27.38±15.66	

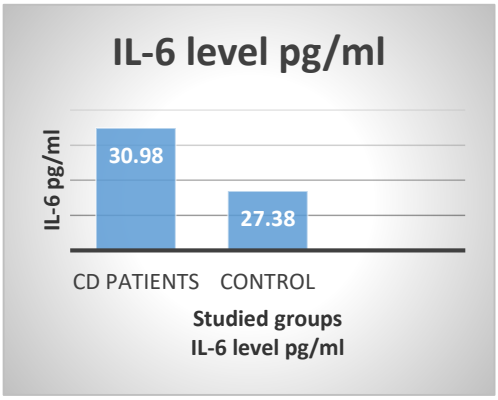


Figure (1): Concentration of (IL-6) in patients and control groups.

Determination of Anti-inflammatory cytokines

Evaluation of interleukin -10 Serum Levels

Table (2) and Figure (2) showed the results of the level of IL-10 in patients with CD (60.32 ± 17.97) were higher than those of control (51.58±18.64) with a significant difference ($P \leq 0.001$).

Table (2) Concentration of (IL-10) in patients and control groups.

Groups	No. of cases	Mean ± S.D Pg/ml	*P* value
Patients	85	60.32 ± 17.97	$P \geq$ 0.001
Control	60	51.58±18.64	

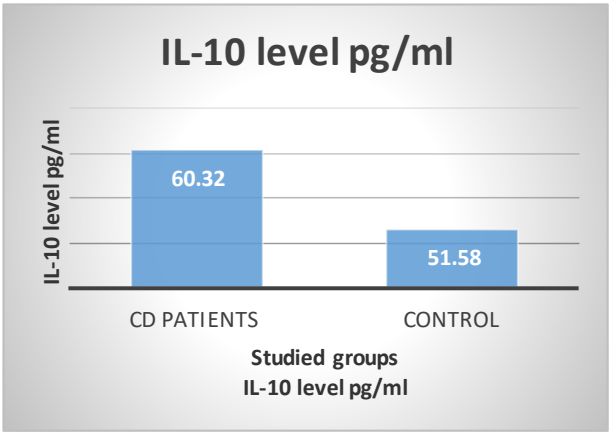


Figure (2): Concentration of (IL-10) in patients and control groups.

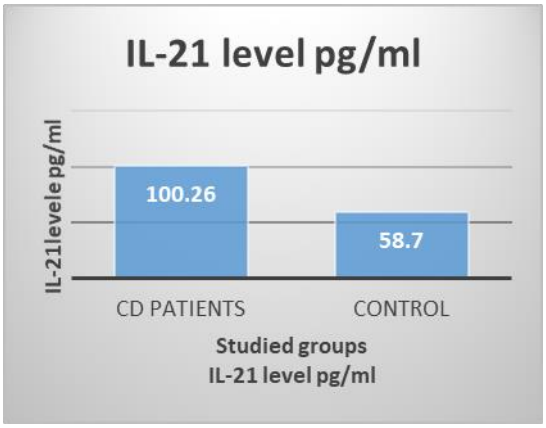
Evaluation of Interleukin 21(IL-21) Serum Levels

Recorded data outcome demonstrated that the levels of IL-21 in patients with CD (100.26±27.77) were higher than those of control (58.7±20.02) with a significant difference ($p \leq 0.02$) table and figure (3).

Table (3): Concentration of (IL-21) in patients and control groups.

Groups	No. of cases	Mean ± S.D pg/ml	*P* value
Patients	85	100.26± 27.77	P ≥00.01
Control	60	58.7±20. 02	

Figure (3): Concentration of (IL-21) in patients and control groups.



4. Discussion

Celiac disease (CD) is an autoimmune disorder elicited by gluten intake in individuals with a genetic predisposition. It is characterized by distinct serological and histological markers. Since primary prevention is not currently possible, celiac disease presents a significant public health concern. The condition can develop at any age, as it does not target any specific age group. For example, someone who tests negative for celiac disease at age 50 may still develop symptoms later, such as at age 65, since gluten intolerance can develop at any stage of life. IL-6 may be an important a target for therapies interventions aimed at specific cytokines in the treatment of celiac disease (11,12). Interleukin-6 levels were significantly increased in patients with celiac disease and indicated a positive correlation with the degree of inflammation associated with the disease (13).

Several national and local Iraqi studies have found a link between IL-6 levels and celiac disease (14-16). In this study, serum levels of IL-6 in patients with celiac disease were found to be slightly higher compared to the controls, although the increase was not statistically significant ($P \geq 0.001$).

The results of the present study show a non-significant increase in IL-6 levels in patients with celiac disease, which is inconsistent with the findings of other studies (15) that indicated that IL-6 levels a highly increased in patients with CD.

Other researchers (17), previous studies have found that IL-6 levels in celiac disease patients were significantly higher, with a mean serum level of 228.01, compared to other groups.

The lack of an increase in IL-6 levels in celiac disease patients in the present study may be explained by the fact that most of the patients were on a gluten-free diet or undergoing treatment, in contrast to other studies that focused on patients with active disease (new or relapsed cases).

Interleukin-6 plays a critical role in the immune response during ACD, especially in response to infections. Therefore, measuring IL-6 levels in celiac disease patients could be crucial for identifying those who do not respond to biopsy, helping to better manage their condition (18,9). The serum levels of IL-6 were observed and the values were significantly elevated in patients with active celiac disease compared to the control group. These levels only decreased after the patients had been on a GFD for one year (19). Interleukin-10 plays a key role in regulating T cell responses, particularly by promoting the production of (IL-1, IL-6, and TNF-α) by macrophages (20,21), as well as increasing the production of Th1 cytokines in the development of celiac disease. Additionally, several studies have reported a connection between IL-10 levels and celiac disease in patients (20,15,22), it was found that patients with ACD exhibited significantly increased mean serum levels of both interleukin-6 and interleukin-10 compared to the control group (19). The present study showed that IL-10 expression was higher in celiac disease patients who had significantly higher mean serum levels of both IL-6 compared to the controls, although this difference was not statistically significant ($P \leq 0.020$) (23). It has been reported that celiac disease patients exhibit increased levels of the anti-inflammatory cytokine IL-10 and higher lymphocyte infiltration. IL-10 plays an important role as an anti-inflammatory factor in regulating Th1 responses, and these cytokines are important for inducing HLA-G expression.

IL-21 is believed to play a crucial role in coordinating the mucosal inflammatory response in celiac disease. In this context, we review the current understanding of the expression and function of IL-15 and IL-21 in the development and progression of CD (24). It is now clear that IL-21 can also influence the replication and effector functions of (CD8+ T cells and NK cells).

The present data showed a significant increase ($p \leq 0.001$) in IL-21 levels in CD patients (100.26 ± 27.77) compared to the control group (58.7 ± 20.02). These results were inconsistent with the findings of other studies (16) one study found that there was no increase in interleukin-21 levels in newly diagnosed celiac disease patients, with minimal expression, and it was not considered a useful indicator. However, in patients with relapsed disease, there was a significant rise in IL-21 levels. In contrast, patients who were undergoing treatment or on a gluten-free diet showed no significant change. The differences between the results of the two studies may be attributed to factors such as the age of the participants and the stages of the disease.

5. CONCLUSION

The results of the current study demonstrated an increase in the levels of IL -6, IL -10, and IL -21 in all CD patients from Najaf Province, compared to the control group.

CONFLICT OF INTEREST: NIL.

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