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The Impact of Interleukin-21 and 23 Serum Level and Gene Expression in Celiac Disease among Sample of Iraqi Patients

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ABSTRACT

Background: Celiac disease (CD) is an autoimmune disease characterized by chronic inflammation that essentially affects the small intestine and is caused by food that contain gluten. This research aimed to explore the impact of interleukin 21 (IL-21) and interleukin 23 (IL-23) on development the Celiac disease in Iraqi patients.

Methods: Forty adults have CD were enrolled in the study with age range between 15-60 years with an average age of 36.60±2.03 years. The diagnosis of the disease was confirmed by serological examinations and intestinal endoscopy in Gastroenterology and Liver Teaching Hospital in the Medical City Hospital in Baghdad and a forty healthy subjects were included in this study as control group with age range between 16-59 years with an average age of 32.22±1.97 years. The blood serum were examined for Anti tissue transglutaminase (tTG) IgA and IgG (IgA ant-tTG and IgG ant-tTG) and anti-DGP IgA (IgA ant-DGP) antibodies as well as IL-21 and IL-23 by using enzyme-linked immunoassay technique ((ELISA). Also, the gene expression of IL-21 and IL-23 in peripheral blood of Iraqi CD patients was determined by using quantitative real-time PCR (qRT-PCR) assay.

Result: The results showed that serum levels of anti-tTG (IgA, IgG) and anti-gliadin (IgA) antibodies were significantly higher in CD patients than in control (25.13±1.12, 19.86±1.13, and 32.01±2.36 vs. 12.62±1.04, 6.83±0.82 and 21.59±1.44 U/ml, respectively; $p<0.01$). In addition, IL-21 and IL-23 serum level was increased in CD patients with high significant differences (490.02±20.46 and 565.22±22.84 vs 106.87±4.23 and 256.03±13.52 pg/ml; $p<0.01$), respectively. The results of gene expression revealed the level of IL-21 and IL-23 was increased significantly by 3.33±0.35 and 2.83±0.27 fold change in CD patients in comparison to control.

Key words: Celiac disease, Gene expression, Interleukin-21, Interleukin-23.

INTRODUCTION

Celiac disease (CD) is an immune-mediated disorder caused by gluten in genetically susceptible individuals (Husby *et al*., 2012). It is characterized by the presence of chronic inflammation of the small bowel's mucosa and submucosa, and is clinically characterized by the presence of diverse systemic manifestations, it is also called celiac sprue, gluten-sensitive enteropathy, or non-tropical sprue (James *et al*., 2001). The gluten which is a protein found in wheat and other grains such as barley and rye (Julio *et al*., 2016). Celiac disease show gastrointestinal symptoms like diarrhea, cramping, bloating, flatulence, nausea, and electrolyte imbalance (Murray *et al.,* 2004). There are certain non-classical symptoms of CD, such as iron deficiency anemia (Obaid *et al*., 2020), increased transaminases, constipation, ataxia, lethargy, osteoporosis and dyspepsia (Oxentenko *et al*., 2019). The diagnosis of CD is difficult because symptoms can include diarrhea, weight loss, constipation, recurrent abdominal pain and extra-intestinal symptoms like osteoporosis, elevated transaminases, anemia, autoimmune disorders, recurrent miscarriages, and aphasia stomatitis. Alternatively, there may be no symptoms at all (Briani *et al*., 2008, Krishnaja and Ukkuru 2016). Over the last 20 years, the diagnostic accuracy of serology for CD has progressively increased with the development of highly reliable tests, such as the detection of IgA tissue transglutaminase and IgG anti-deamidated gliadin peptide antibodies (Volta *et al*., 2011). Interleukin-21 (IL-21), regulates both innate and adaptive immune responses and it not only has key roles in anti-tumor and antiviral responses but also exerts major effects on inflammatory responses that promote the development of autoimmune diseases and inflammatory disorders (Spolski *et al*., 2014). The IL-21 cytokine is produced by follicular T-helper cells that help B cells in making antibodies during T cell-dependent responses (O'Shea *et al*., 2010). It has been suggested that IL-21 produced by gluten-reactive T cells enhances both IL-17 and IFN-γ production in the intestinal mucosa. Interleukin-21 may have a wider role in CD pathogenesis as this cytokine also targets several other cell types such as CD8+ T cells and natural

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killer cells. Interleukin-21 also has a role in B-cell differentiation and plasma cell function and could possibly contribute to the production of celiac disease-associated antibodies (De Nitto *et al*., 2009). Notably, the IL-21 locus is a genetic risk factor for CD (DA van *et al*., 2007). Interleukin-23 (IL-23), which belong to the IL-12 family of cytokines, have a key role in intestinal homeostasis and inflammation and are implicated in the pathogenesis of inflammatory bowel disease. Upon their secretion by antigen-presenting cells (dendritic cells and macrophages) in response to microbial pathogens it exert both pro-inflammatory and anti-inflammatory receptor-mediated effects. Interleukin-23 is composed of two subunits, p19 and p40, which are shared with another cytokine called IL-12, these subunits combine to form the IL-23 heterodimer (Oppmann *et al*., 2000). Interleukin-23 binds to a receptor complex primarily found on T cells, known as the IL-23 receptor (IL-23R), which consists of two subunits, IL-23R and IL-12R β1 (Parham *et al*., 2002). The main function of IL-23 is to promote the differentiation and expansion of a specific subset of T cells called Th17 cells Th17 cells are known to produce various pro-inflammatory cytokines, including IL-17, which plays a critical role in inflammation and autoimmune diseases, IL-23 acts as a key regulator in the differentiation and maintenance of Th17 cells (Langrish *et al*., 2005). Interleukin-23 has been implicated in the pathogenesis of several autoimmune diseases, including psoriasis, rheumatoid arthritis, inflammatory bowel disease (such as Crohn's disease and ulcerative colitis) and multiple sclerosis (Duerr *et al*., 2006, Nandhini *et al.,* 2006). The aim of this study to elucidate the role of the IL-21 and IL-23 in celiac disease patients.

MATERIALS AND METHODS

Patients and control

Serum samples were obtained from 40 Iraqis patients with celiac disease (CD) and 40 healthy individuals as a control group, age range (15-60) year which enrolled in this study. The present study was carried out at Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq, This study began in July 2021 and the practical part was completed in April 2022. The theoretical part and writing of the research began and the research was finally completed in January 2024.

Immunological parameters

The blood serum were examined for Anti tissue transglutaminase (tTG) IgA and IgG (IgA ant-tTG and IgG ant-tTG) and Anti-DGP IgA (IgA ant-DGP) antibodies as well as IL-21 and IL-23 by using enzyme-linked immunoassay technique (ELISA). (Generic Assays GmbH, Germany and Fine Test, China; respectively) and instructions of the manufacturer were followed. We coated the ELISA microplate with an antibody specific to human immunological parameters studied. The specific antibody was introduced into the wells of the ELISA microplate containing the samples. Human biotinylated detection antibodies for immunological parameters studied were added to each microplate well, along with an Avidin-Horseradish peroxidase (HRP)-conjugated antibody. Then we left the dishes to settle for a while. The preserved components were rinsed away. In each well, a substrate solution was introduced. It is only possible to see the blue color in wells containing human immunological parameters studied, biotinylated detection antibodies and Avidin-HRP conjugate. The addition of a stop solution terminated the reaction of the enzyme with the substrate, giving the result a yellow color. Optical density (OD) is determined using spectrophotometry at a wavelength of 450 nm. Proportional to the concentration of human immunological parameters studied, the OD value is determined.

Fold change gene expression of IL-21 and IL-23

Gene expression of Interleukin-21 and IL-23 was determined by the reverse transcription quantitative polymerase chain reaction (RT-qPCR) method. Total RNA was isolated from peripheral blood using ready-to-use reagent (FavorPrep Total RNA Mini Kit, Favorgen / Korea). The isolated RNA was reverse-transcribed and the gene expression of IL-21 and IL-23 was assessed using GoTaq®1-Step RT-qPCR System kit (Promega, USA), and instructions of the manufacturer were followed. Forward and reverse primers for IL-21 (5′- GCAAAGTCAG GATTATTTCCCCA -3′ and 5′ - TCCTGGCCTC TTGGT TTGTC -3′, respectively). Forward and reverse primers for IL-23 (5′- CCCAAGGACTCAGGGACAAC -3′ and 5′- TGGAGGCTGCGAAGGATTTT -3′, respectively) and the housekeeping gene GAPDH (5′- AATGGGCA GCCGTT AGGAAA- 3′ and 5′- AATGGGCAGCCGTTAGGAAA -3′, respectively) were according to published sequences. The primers were designed using primer design web-based service and validated using the Primer-BLAST-NCBI database. The expression of IL-21 and IL-23 was expressed as a fold change in the level of the target gene expression, which was normalized to the expression level of the housekeeping gene GAPDH (endogenous control) and relative to the target gene in control subjects (calibrator) (Livak and Schmittgen, 2001).

Ethical clearance

The Ethical Committee (CSEC/0922/0100 on September 27, 2022) of the Department of Biology, College of Science, University of Baghdad, approved the research after receiving written informed consent from all participants.

Statistical analysis

All of the statistical analysis was carried out with the help of the SPSS version 24.0 software. The data were expressed as the mean±SE. The T Student tests were additionally utilized to compare the two numerical or categorical parameters. If the P values were less than 0.05, then the differences were determined to be statistically significant (Dipper *et al*., 2009).

RESULTS AND DISCUSSION

The results showed that serum levels of anti-tTG (IgA, IgG) and anti-gliadin (IgA) antibodies were significantly higher in CD patients than in control (25.13±1.12, 19.86±1.13 and 32.01±2.36 vs. 12.62±1.04, 6.83±0.82 and 21.59±1.44 U/ml, respectively; p<0.01) (Table 1). These results are in agreement with previous results showing that CD is associated with an increases in anti-tissue and anti-gliadin (Catassi *et al*., 2010). The results of (Dipper *et al*., 2009) found that an anti -tTG is the marker of choice for CD mass screening and helpful in identifying patients who can benefit from a gluten-free diet and follow-up. Although the gold standard for detecting CD is a duodenal biopsy, it has been shown that anti-tTG antibody together with an anti-gliadin antibody is a sensitive marker for CD (Hopper *et al*., 2008). The findings of this study and other studies indicate the diagnostic potential of both antibodies in CD when conducting screening surveys for the disease in children and adolescents (Aldaghi *et al*., 2016). According to (Al-Assaf *et al*., 2021) Serum levels of anti-tTG and anti-gliadin IgA antibodies were significantly higher in CD children than in control children. Study of (Aldaghi *et al.,* 2016) approved a statistically significant correlation between IgA anti-tTG level and histopathological finding which that showed a statistically significant relation was observed between the serological titer and positive pathologic results in patients with suspected CD.

The IL-21 and IL-23 serum level was increased in CD patients with high significant differences (490.02±20.46 and 565.22±22.84 vs 106.87±4.23 and 256.03±13.52 pg/ml; p<0.01), respectively (Table 2). In addition, the results of gene expression revealed the level of IL-21 and IL-23 was increased significantly by 3.33±0.35 and 2.83±0.27 fold change in CD patients in comparison to control (Table 3).

Like many autoimmune diseases, cytokines and signaling proteins play roles in CD pathogenesis (Aghamohamadi *et al*., 2020). The immune response in celiac disease involves the adaptive, as well as the innate and is characterized by the presence of anti-gluten and antitransglutaminase 2 antibodies, lymphocytic infiltration in the epithelial membrane and the lamina propria and expression of multiple cytokines and other signaling proteins (Briani *et al*., 2008). Production of the cytokine interferon-γ (IFN-γ) by gluten-specific CD_4+ T cells is thought to play a role in intestinal epithelial damage (Nilsen *et al*., 2008, Hameed *et al.,* 2023). However, other CD_4+ T cell–derived cytokines, in particular interleukin-21 (IL-21), have recently been suggested to contribute to the pathogenesis of CD (Fina *et al*., 2008, Ezz *et al*., 2023). It was found that IL-21 produced by gluten-reactive T cells enhances both IL-17 and IFN-γ production in the intestinal mucosa. The IL-21 may have a wider role in celiac disease pathogenesis as this cytokine also targets several other cell types such as CD8+ T cells and natural killer cells. The IL-21 also has a role in B-cell differentiation and plasma cell function and could possibly contribute to the production of celiac disease-associated antibodies (De Nitto *et al*., 2009, Hasan *et al.,* 2024). Notably, the *IL-2*/IL-21 locus is a genetic risk factor for celiac disease (DA van Heel *et al.,* 2007; Hasan *et al.,* 2024).

The results of Table 4 and 5 showed the correlation coefficient-r between IL-21 and IL-23 concentrations as well as between fold changes levels of IL-21 and IL-23 and other studied parameters.

Pathogenesis of CD and is critical for Th17-mediated immune response activation (Hisamatsu *et al*., 2016, Al-Khuzaay *et al.,* 2024). The heterodimeric cytokine interleukin-23 (IL-23 or IL23A/IL12B) is a member of the IL-12 family produced by dendritic cells and macrophages, which has a role in the pathogenesis of several tissue-specific autoimmune diseases. According to the result of studies greater peripheral blood mononuclear cell (PBMC) production of IL-23 caused by gliadin can contribute to the IL-23 has been implicated in the pathogenesis of several tissue-specific autoimmune diseases. Currently, celiac disease (CD) is the only autoimmune disease in which both the major genetic [95% HLA-DQ2 (+)] and etiologic factors (dietary glutens) for susceptibility are known. We demonstrate that wheat gliadin induces significantly greater production of IL-23, IL-1beta and TNF-alpha in PBMC from CD patients compared with HLA-DQ2 (+) healthy controls, strongly advocating a role for IL-23 in the pathogenesis of CD (Harris *et al*., 2008; Sarah *et al*., 2021; Hasan *et al*., 2024).

According to (Dickey *et al*., 2008., Al-Jumaily *et al*., 2023). The expression of IL-15, IL-17A, IL-23A, GzmB, TBX21 and TNFAIP3 genes in peripheral blood mononuclear cells of patients with celiac disease showed a significant increase compared with the control group. Among them, TNFAIP3, IL23A and GzmB have better resolution and diagnostic value in differentiating patients with celiac disease from healthy controls. According to (Ren *et al*., 2021).

Table 1: Serum levels of anti-tTG (IgA and IgG) and anti-gliadin (IgA) antibodies in CD patients and control group.

Groups	Mean±SE (U/ml)		
	IgA anti-Ttg	IgG anti-tTG	IgA anti-gliadin
CD patients	25.13±1.12	19.86±1.13	32.01±2.36
Control	12.62±1.04	6.83±0.82	21.59±1.44
T-test	3.054 **	2.755 **	5.501 **
P-value	0.0001	0.0001	0.0003

** (P≤0.01).

IL-21 has a role in the autoimmune illnesses lupus and psoriasis. Furthermore, there is a correlation between mucosal damage, anti-TG2 IgA autoantibodies, and higher blood IL-21 levels in celiac patients (Iervasi *et al*., 2020., Mais *et al.,* 2022). The ROC curve was able to differentiate between CD patients and healthy controls based on the levels of IL-15, IL-17A, IL-23A, GzmB and TNFAIP3 mRNA in peripheral blood (Khalkhal *et al.,* 2022, Yadav *et al.,* 2022).

Table 2: Serum levels of IL-21 and IL-23 in CD patients and control group.

Groups	Mean±SE (pg/ml)	
	IL-21	IL-23
CD Patients	490.02±20.46	565.22±22.84
'Control	106.87±4.23	256.03±13.52
T-test	41.606 **	5.860 **
P-value	0.0001	0.0001

** (P≤0.01).

Table 3: Fold change gene expression of IL-21 and IL-23 in CD patients and control groups.

Groups	Mean±SE	
	IL-21	IL-23
CD Patients	3.33±0.35	2.83±0.27
Control	1.00±0.00	1.00±0.00
T-test	0.815 **	0.702 **
P-value	0.0067	0.0081

** (P≤0.01).

Table 4: Correlation coefficient between IL-21 and IL-23 at serum levels and parameters studied of CD patients.

Parameters studied	Correlationcoefficient-r	
	IL-23	IL.21
IgA anti-tTG	-0.08 NS	0.17 NS
IgG anti-tTG	-0.14 NS	0.07 NS
IgA anti-gliadin	0.10 NS	-0.08 NS
IL-21	0.02 NS	-0.18 NS
IL-23	0.18 NS	-0.15 NS

* (P≤0.05), NS: Non-significant.

Table 5: Correlation coefficient between fold change gene expression of IL-21 and IL-23 and parameters studied of CD patients.

Parameters studied	Correlation coefficient-r	
	IL-21	IL-23
IgA anti-tTG	0.06 NS	-0.19 NS
IgG anti-tTG	-0.12	-0.28 NS*
IgA anti-gliadin	0.15 NS	0.08 NS

* (P≤0.05), NS: Non-significant.

Conclusion

Based on the findings of this research, it can be inferred that the variations in concentrations of IL-21 and IL-23 at serum levels have the potential to serve as a risk factor for the development of CD. Furthermore, these findings also imply that IL-21 and IL-23 genes have a role in the advancement of CD. Also, the results of current study indicate the concentrations of IL-21 and IL-23 in patients may be helpful predictors in the early detection of CD as well as serve as a prognostic indicator for the development of CD. A deeper understanding of these interactions, on the other hand, will be necessary to make the most effective use of a new medicines in the treatment of CD.

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Conflict of interest

The authors declare that they have no possible conflicts of interest.

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