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Research Article

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The Assessment of Interleukin-15 and Interleukin-21 Gene Expression and Serum Level in Celiac Disease Prevalence Patients

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Abstract: Eighty-four Iraqi patients (Male & Female) with celiac disease with an age range (3-65) were investigated. Samples were taken from the Al Karama Teaching Hospital period October 2020 - to June 2021. Data sheet was filled according to questionnaire format, which include name, age, smoking, family history & alcohol intake. The patient's diagnosis was based on some serological test (AGA, tTG) the patients were clinically subdivided into treated and untreated. Twenty-seven controls were selected randomly from apparently healthy individual (31 male & 53 female). This study aims to evaluate evaluated some genetic & immunological parameters; her were: 1-Determine whether the gene expression and the serum level of interleukins (15 and 21) have an effect on the functioning of the immune system in individuals with celiac disease. 2-Evaluated CD gene expression of treated (gluten-free diet) and naive (untreated) CD patients compared with healthy individuals. The study methodology was based on an ELISA test kit provides a quantitative in vitro assay for human autoantibodies of the IgA class against tissue transglutaminase in serum or plasma. Result shown in Table (2) indicate that the majority of patients in celiac disease were diagnosed in females more than males; in the other hand, 66,67% of the confirmed treated Patients with celiac disease were females, while 33.33% were males, 40% confirmed untreated Patients from celiac disease were males when they were females 60 %, moreover furthermore 62.96% control female group more than 37.04% male control group with a significant difference (P-value 0.03661). The collected data showed that 23 (76.67%) patients had no clinical history of celiac disease in the untreated group; on the other hand, only 7 (23.33%) had a clinical history of the patient in the same group, while 1 (3.7%)) The group of patients treated with no clinical history more than 26 (96.30%) and the patient in the same group had a clinical history, as well as 20 (74.07%) patients, had no history of celiac disease in the control group 7 (25.93%) Patients with a family history of celiac disease in the control group, with highly significant difference * (P≤0.05), ** (P≤0.01), (P-value 0.0001). he casuistic in table (3) showed the serum of 84 patients, including 30 with celiac disease without treatment tTG-IgA (5.41 ±0.27 b), tTG-IgG (3.76 ±0.27 b),27 patients treated group tTG-IgA (10.65 ±1.84 a), tTG-IgG (7.31 ±1.46 a) and 27 healthy as controls tTG-IgA (3.62 ±0.19 b), tTG-IgG (2.72 ±0.17 b), with P-value tTG-IgA (0.0001), tTG-IgG (0.0007), within significantly high difference ** (P≤0.01). The variants represented IL-15 and IL-21 gene expressions of celiac patients untreated, treated group, compared with that in the healthy control group individuals. The results showed that of IL-21 expression in untreated patients was (27.22 ±0.23 a) tables (6) and (7), while treated group (27.37 ±0.24 a), more than control group (25.04 ±0.53 b), with high significant P-Value (P≤0.01). At the same time, the expression of the IL-15 gene in untreated patient was (29.50 ±0.40), while treated group (29.63 ±0.26a) and control group (29.04 ±0.81), with non-significant P-Value (P≤0.706).

Keywords: CD, IgA, IgG, enteropathy, gastrointestinal.

INTRODUCTION

Coeliac disease (CD) is a complex immune-mediated illness that is sparked by dietary gluten-sensitive enteropathy as well as progresses over time in genetically predisposed people susceptible persons during their lifetime (Singh. *et al.*, 2018). CD is associated with small intestine inflammation and villous atrophy, autoimmune & multifactorial gastro-intestinal disorder in children and adults define as Gluten-sensitive enteropathy is a long-term condition (Spijkerman. *et al.*, 2016). When a person consumes gluten, the small intestine is harmed, actually results in gastrointestinal complaints, malnutrition, small bowel mucosal damage, and malignancies (Fueyo-Díaz. *et al.*, 2019). Celiac illness is a type of celiac disease that It's also known as celiac sprue, gluten sensitivity enteropathy, or non-tropical sprue. (Bai and Ciacci 2017). The clinical spectrum of Celica disease can include extra-intestinal symptoms like anemia, fatigue, and dermatitis herpetiformis. These factors make CD difficult to diagnose, and only (0.1-0.3) % of all CD patients are properly diagnosed (Lionetti and Catassi, 2011). The discovery of tissue transglutaminase as an autoantigen, establishing the autoimmune origin of celiac disease, was a watershed moment in the disease's history.

As a result, the autoimmune character of this condition is confirmed. (Caio. *et al.*, 2019). The disease can occur at any age, with a variety of symptoms/manifestations (Al-Toma. *et al.*, 2019). Although the clinical manifestations of CD vary, the majority of patients experience gastrointestinal issues such as stomach pain, bloating, diarrhea, vomiting, changed bowel habits, short stature, and constipation. (Majeed, 2021; Semwal. *et al.*, 2018). Celiac disease is characterized by a reaction in the small intestine induced by gluten, which is a protein found in wheat as well as other grains such as barley and rye (Tovoli. *et al.*, 2015). Clinical spectrum of CD includes, the clinical spectrum of CD includes the following, typical or classical,

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atypical or non-classical, and silent. Gastrointestinal difficulties such as abdominal distention, chronic diarrhea, loss of appetite, malabsorption, and failure to grow properly in children are common symptoms. This usually begins between the ages of six months and two years. The most prevalent symptoms are non-classic, especially in children over the age of two. (Celiloğlu, Karabiber, and Selimoğlu, 2011; Salazar, Jennyfer M. García-Cárdenas, and Paz-y-Miño, 2017; Semwal. *et al.*, 2018).

MATERIAL AND METHOD

Patients Sample

Eighty-four Iraqi patients (male & female) with celiac disease with the age range (3 - 65) were investigated. Samples were taken from the Al Karama Teaching Hospital period October 2020 - to June 2021. datasheet was filled according to questionnaire format, which include name, age, smoking, family history & alcohol intake.

Study Design

Blood Sample Collection

A sufficient amount (5 mL) was collected from each subject under aseptic precautions using disposable latex gloves and syringes. The collected blood was divided into two parts, the first part (2 mL) of the blood placed in the EDTA tube for genetic test, and second part (3 mL) was allowed to clot in a serum tube naturally at room temperature and then separated by centrifugation at (1500 rpm) for 10 minutes patients & controls use for serological test were screened for the serum level of IL-15 & IL-21 antibodies. All samples were (blood in EDTA tube & serum) labeled by a serial number and the person's name, and then frozen at -20ºC And aboutt Serological tests applied for diagnosis of CD is Accordingly, celiac disease patients had been diagnosed by serological tests (tTg) as a useful test to exclude celiac disease, in the 2020 in the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), new guidelines for diagnosis of celiac disease and Anti-tissue Transglutaminase antibodies (IgA & IgG): This test carried out to detect the Anti-tissue Transglutaminase antibodies, IgA and IgG by ELISA with double well.

Study Period

Samples were taken from the Al Karama Teaching Hospital period October 2020 - to June 2021.

Aim of Study

1-Determine whether the gene expression and the serum level of interleukins (15 and 21) have an effect on the functioning of the immune system in individuals with celiac disease.

2-Evaluated CD gene expression of treated (gluten-free diet) and naive (untreated) CD patients compared with healthy individuals.

RESULTS AND DISCUSSION

Table 1: Distribution of sample study according to Age

Factors		Untreated No= 30	Treated No= 27	Control No= 27	P-value
Gender No (%)	Male	12 (40.00%)	9 (33.33%)	10 (37.04%)	0.03661 *
	Female	18 (60.00%)	18 (66.67%)	17 (62.96%)	
Family history No (%)	Yes	7 (23.33%)	26 (96.30%)	7 (25.93%)	0.0001 **
	No	23 (76.67%)	1 (3.70%)	20 (74.07%)	
Age (year)	Mean ± SE	27.20 ±2.72	28.00 ±3.79	37.11 ±3.21	0.0639 NS
* (P≤0.05), ** (P≤0.01).					

Result shown in Table (2) indicate that the majority of patients in celiac disease was diagnosed in females more than males; in the other hand, 66,67% of the confirmed treated Patients with celiac disease were females, while 33.33 % were males, 40% confirmed untreated Patients from celiac disease were males when were females 60 %, moreover furthermore 62.96% control female group more than 37.04% male control group with significant difference (P-value 0.03661).

The combination of two X chromosome potentially contains over 1000 genes relating to the immune system, leaves women at greater risk for autoimmune diseases, while the Y chromosome only has about a hundred, and women produce more immunoglobulins than men (Libert, Dejager, and Pinheiro 2010).

There are some matched studies which showed that females dominate among patients (Mohammed Abbas Waheed 2009) found the ratio of females to males was (1.5:1).

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The result of this study are in agreement with some worldwide studies that relatively indicating the highest females compared with males (Joshi. *et al.*, 2019), found that 48 % celiac disease patients were females and 27% males.

Also, (Mukherjee. *et al.*, 2010) showed the clinical diagnosis of celiac disease was not the same in men and women; the disease is not only more frequent in women 79% than in men 21% but also more severe and more rapid, 1:3.8

Consistent finding were reported by (Elsurer. *et al.*, 2005), who recorded the clinical features of CD in the Turkish population, indicated 65% of patient were females and 35% males and reported with a predominance of females.

On other hand, (Ageep, 2012) observed that the ratio of females to males among celiac disease patients was nearly equaled.

In Europe and the United States, CD prevalence appears to be higher in females than in males (Wagtmans. *et al.*, 2001), while in Asia, the opposite has been observed (Leong, Lau, and Sung 2004; Prideaux. *et al.*, 2012).

Females with an age of 25–29 years and particularly those older than 35 years, are more prone to CD compared to their male counterparts. An increased risk of up to 40% has been observed (Shah. *et al.*, 2018); older males (>45 years) appear to have a 20% higher incidence rate of UC compared to women (Shah. *et al.*, 2018).

Most gender differences in celiac disease are physiological and have females-predominant associated with diseases; however, in general, men have indirect evidence of greater malabsorption than females (Bai. *et al*., 2005).

Table 2: Distribution of sample study according to Gender

Factors		Untreated No= 30	Treated No= 27	Control No= 27	P-value
Gender No (%)	Male	12 (40.00%)	9 (33.33%)	10 (37.04%)	0.03661 *
	Female	18 (60.00%)	18 (66.67%)	17 (62.96%)	
Family history No (%)	Yes	7 (23.33%)	26 (96.30%)	7 (25.93%)	0.0001 **
	No	23 (76.67%)	1 (3.70%)	20 (74.07%)	
Age (year)	Mean ± SE	27.20 ±2.72	28.00 ±3.79	37.11 ±3.21	0.0639 NS
* (P≤0.05), ** (P≤0.01).					

Concerning family history, the families are an important factor in celiac disease testing qualification. As a consequence, the clinical history of a first-degree relative, such as with a parent, brother, or sister, should be known (Paavola. *et al*., 2021; Uenishi. *et al*., 2014). The information from each patient registered used in this study shown in table 3.

The data that was gathered showed 23 (76.67%) patients did not have clinical history of celiac disease in untreated group on other hand, only 7 (23.33%) there was a clinical history of the patient in the same group, while 1 (3.7%) patients treated group there was no clinical history than 26 (96.30 %) the patient in the same group had a clinical history, as well 20 (74.07%) patients was no history of celiac disease in the control group further 7 (25.93%) patients with family evidence history of celiac disease in control group, with highly significant difference * (P≤0.05), **(P≤0.01), (The P-value 0.0001).

That was in agreement with previous Arab Country's study (El-Metwally. *et al.*, 2020). Evidence proposed that only around (10% to 15%) of the positive patient carrying the family history.

Previous study by (Rubio-Tapia. *et al.*, 2008) show an elevated prevalence of celiac disease (CD) in family members (FMs) and being a sibling (odds ratio, 2.5; 95% confidence interval, 1.1–5.8) are high-risk factors for CD.

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Table 3: Distribution of sample study according to the Family histor

Factors		Untreated No= 30	Treated No= 27	Control No= 27	P-value
Gender No (%)	Male	12 (40.00%)	9 (33.33%)	10 (37.04%)	0.03661 *
	Female	18 (60.00%)	18 (66.67%)	17 (62.96%)	
Family history No (%)	Yes	7 (23.33%)	26 (96.30%)	7 (25.93%)	0.0001 **
	No	23 (76.67%)	1 (3.70%)	20 (74.07%)	
Age (year)	Mean ± SE	27.20 ±2.72	28.00 ±3.79	37.11 ±3.21	0.0639 NS
* (P≤0.05), ** (P≤0.01).					

In the current research, all patient sera were stored at -18 C° until analyzed, and TGA antibodies were determined with human recombinant tissue transglutaminase. Were measured with the Celikey assay (AESKU. GROUP, GmbH, Germany) according to the manufacturer's instructions.

As a result, the current study used tTG tests with the ELISA method to diagnose celiac disease in 84 suspected patients of various ages and genders.

The transglutaminase enzyme (tTG) is the target antigen of autoantibodies found in the serum of patients with celiac disease (CD). Also, tTG activity either results in cross-linking of proteins by formation of a covalent bond between aglutamine in one protein and a lysine in another or the conversion of glutamine into glutamic acid (Tonutti and Bizzaro 2014).

There were numerous studies that backed the strategy of using anti-tTG antibody; in fact, it has been found that the intestinal mucosa is rich in proteins able to act as glutamine-acceptor substrates; these findings are compatible with the hypothesis that tTG catalyzes the formation of gliadin (glutamine-donor)–protein complexes, thus generating a novel self-antigen responsible for the autoimmune responses in CD (Johny. *et al.*, 2020).

The result concerning serum levels of anti-tissue transglutaminase IgA (AtTG IgA) and of anti-tissue transglutaminase IgG (AtTG IgG) in out of 57 patients and control are shown in Tables 2 & 3.

Table 4: Comparison between patients and control groups in Anti-tissue transglutaminase-IgA and Anti-tissue transglutaminase-IgG

Group	Mean ± SE	
	Anti-tissue transglutaminase-IgA	**Anti-tissue transglutaminase-IgG**
Patients (no. 57)	7.89 ±0.94	5.44 ±0.73
Control (no. 27)	3.62 ±0.19	2.72 ±0.17
T-test	2.765 **	2.154 **
P-value	0.0028	0.011
** (P≤0.01).		

Table 5: Comparison between difference groups in Anti-tissue transglutaminase-IgA and Anti-tissue transglutaminase-IgG

Group	Mean ± SE	
	Anti-tissue transglutaminase-IgA	**Anti-tissue transglutaminase-IgG**
Untreated (no. 30)	5.41 ±0.27 b	3.76 ±0.27 b
Treated (no. 27)	10.65 ±1.84 a	7.31 ±1.46 a
Control (no. 27)	3.62 ±0.19 b	2.72 ±0.17 b
LSD value	2.954 **	2.353 **
P-value	0.0001	0.0007
** (P≤0.01).		

Patient group which was significantly higher as compared than those of control subject (P ≤0.01).

(Dahlbom. *et al.*, 2010) found that high levels of IgA-tTG and IgG-tTG antibodies were associated with the grade of mucosal villous atrophy and more severe in clinical cases of CD. In addition, the combination of IgA-tTG and IgG-tTG tests would enable a non-invasive predication of small intestinal villous atrophy with high accuracy and might reduce the need for biopsy in patients with suspected CD.

However, ((Waheed. *et al.*, 2017) found that, out of 412 Iraqi CD patients, the proportions of

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patients who were sero-positive for IgA-tTG and IgG-tTG (156% and 16.75%, respectively).

Also, (Hameed 2012) out of in Kirkuk city/Iraq. reported 13.3% and 15% sero-positivity for IgA-tTG and IgG-tTG, respectively.

Moreover, an Egyptian study by (Abu-Zekry. *et al.*, 2008) found that both IgA and IgG-tTG were positive in 4.7% out of 150 suspected patients with CD.

The casuistic in table (3) showed the serum of 84 patients, including 30 with celiac disease without treatment tTG-IgA (5.41 ±0.27 b), tTG-IgG (3.76 ±0.27 b),27 patients treated group tTG-IgA (10.65 ±1.84 a), tTG-IgG (7.31 ±1.46 a) and 27 healthy as controls tTG-IgA (3.62 ±0.19 b), tTG-IgG (2.72 ±0.17 b), with P-value tTG-IgA (0.0001), tTG-IgG (0.0007), within significantly high difference ** (P≤0.01).

It has been reported that levels of cytokines in serum varies in response to inflammation and hence could be considered as useful molecular markers of different immunological disease, including celiac disease (Masaebi. *et al*., 2020).

Result shown in table 4 & 5 indicated that the serum level of IL-15 and IL-21.

As shown in the table, IL-21 serum levels were markedly raised in the majority of CD patients (688.14 ±63.28) higher than control group (659.20±98.74), with a significant T-test 155.26, a p-value of 0.021.

IL-15 serum level was considerably decrease in the almost all of the CD patients (148.98±21.51) less than with the healthy control group (218.20±42.44), non-significant T-test 103.59 with a P-value 0.186.

Table 6: Comparison between patients and control groups in IL-15 and IL-21/Serum

Group	Mean ± SE	
	Serum level IL-15 ()	**Serum level IL-21 ()**
Patients	148.98 ±21.51	688.14 ±63.28
Control	218.20 ±42.44	659.20 ±98.74
T-test	103.59 NS	155.26
P-value	0.186	0.021
NS: Non-Significant.		

Table 7: Comparison between difference groups in IL-15 and IL-21 /Serum

Group	Mean ± SE	
	Serum level IL-15 ()	**Serum level IL-21 ()**
Untreated	713.52 ±103.82	167.48 ±35.32 ab
Treated	658.34 ±66.06	127.26 ±21.69 b
Control	659.20 ±98.74	218.20 ±42.44 a
LSD value	273.44 NS	85.57 *
P-value	0.883	0.0495
NS: Non-Significant.		

CONCLUSION

The following are the results of the current study:
Most of the untreated patients group high positive titer tTG-IgA and tTG-IgG.

Low positive suggests that the immune response has just a minimal impact in CD based on history and age.
Treated group Gluten-free diet (GFD) for celiac patients may be reduce the risk of Celica diseases in concentration and activity.

Celiac disease was found in female significantly more than in male.

In sequence analysis of IL-21, it was found the majority high significant more than IL-15 non-significant.
IL-21abililty render effector in with gene expression more than in IL-15 shown to mediate loss of gene expression.

Diagnostic criteria should help physicians in avoiding misdiagnosis and missing cases of CD (i.e., seronegative patients with classic symptoms not undergoing biopsy) and preserve people from an unjustified GFD.

The titer of detected antibodies in the serum are correspondent to the degree of villus atrophy in the duodenum of celiac disease suspected cases.

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RECOMMENDATION

The treatment for CD is still primarily a GFD, which requires significant patient education, motivation, and follow-up.

The Mediterranean diet (MD) has been recommended to the general population by many scientific organizations as a healthy dietary pattern based on strong evidence that it improves digestive system health.

Drugs to lower intestinal permeability.

Modulation of pro-inflammatory intestinal cytokines with biological agents

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