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MOLECULAR AND IMMUNOLOGICAL STUDY OF CELIAC DISEASE IN SAMPLES OF IRAQI PATIENTS

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Abstract

Aim: Evaluation of Human Leukocyte Antigen (HLA) DQ2/DQ8 in celiac disease (CD) patients compared to healthy controls, and to investigate the presence of specific immunological markers (IL-15, IL-21, and TNFα) in the human serum samples from the study groups, also to detect HLA-DQ2 and HLADQ8 in relation to interleukins IL-15, IL-21, and TNF in CD patients.

Methods: A total of 90 individuals participated in this study, comprising 50 patients clinically and serologically diagnosed with celiac disease and 40 apparently healthy individuals serving as the control group. The participants were carefully selected and matched for age and gender where possible. Genotyping for HLA-DQ2 and HLA-DQ8 was conducted using the polymerase chain reaction (PCR) technique, which allows for accurate detection of specific alleles associated with genetic susceptibility to CD. In addition, enzyme-linked immunosorbent assay (ELISA) was employed to determine the serum concentrations of IL-15, IL-21, and TNF-α in both patient and control groups. Statistical analyses were performed to assess the significance of differences observed between the groups and to explore the relationship between HLA genotypes and cytokine expression levels.

Results: The individuals in the case study group were aged between 1 and 60 years. In terms of gender, the patient group consisted of 18 (36.0%) males and 32 (64.0%) females. Among 50 patients with celiac disease, 76.0% had HLA-DQ2, 20.0% had HLA-DQ8, and 14.0% had both. The majority of alleles encoded for HLADQ2 were significant in CD patients when compared with controls. The serum concentrations of IL-15, TNF-α, and IL-21 in the sick group were statistically significant with P-values of 0.001, 0.018, and 0.0001, respectively.

Conclusion: The HLADQ2 genotype is the most common HLA genotype among celiac patients in Iraq, followed by HLADQ8. Serum levels of IL-15, IL-21, and TNF-α were considerably elevated in individuals with CD compared to the control group.

Keywords: Celiac disease, HLA-DQ2, HLA-DQ8, IL-15, IL-21, TNF-α, Iraq, Immunogenetics.

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1. Introduction

Celiac disease, also known as gluten-sensitive enteropathy or non-tropical sprue, is a systemic autoimmune condition that affects genetically predisposed individuals and occurs in about 1% of the global population due to gluten consumption. The immune response is activated by the α-gliadin portion of gluten found in wheat, barley, and rye grains in the small intestine, causing mucosal injury and villous architectural loss. The immune reaction extends beyond the small intestine and can lead to other extraintestinal symptoms throughout the body. Genetic predisposition is crucial in the development of the condition; lifelong gluten intolerance requires the presence of HLA-DQ2 or HLA-DQ8. Having HLA-DQ2 or HLA-DQ8 haplotypes is essential for developing CD. [1] Celiac disease presents with a variety of clinical symptoms. Despite being common, most patients go undiagnosed for an extended period due to the fact that only a small number exhibit the typical malabsorptive signs. [2] Comorbidities include of immune-mediated disorders, dermatitis herpetiformis as a skin symptom, dental enamel hypoplasia (DED), and recurrent oral aphthous lesions (RAS) as oral symptoms. Gluten ataxia is a gluten-induced disorder characterized by the gradual degeneration of Purkinje cells. The common genetic background allows for the simultaneous presence of CD and other autoimmune disorders. Refractory coeliac disease is an uncommon and severe consequence of coeliac disease. Most patients develop ulcerative jejunitis, followed by enteropathy-associated T-cell lymphoma, which virtually exclusively occurs in celiac patients. [3]

2. Materials and Methods

The present cross-sectional study aims to analyze the expression levels of specific immunological markers (IL15, IL21, and TNFα) and identify haplotype alterations in HLA typing in patients with CD selected from Medical City Baghdad between November 2022 and February 2023. Fifty patients from the advisory clinic for digestive hospital and the pediatric teaching hospital in the medical city Baghdad participated in the study. The age groupings range from 1 to 60 years. All patients had severe malabsorption, diarrhea and abdominal pain and positively to serological test (anti-tTG Ab and anti-endomysial Ab). Forty controls were included in the study at the premarital examination unit in the medical city Baghdad.

2.1 Human interleukins

Serum human IL-15, IL-21 and TNFα levels measurement, where ELISA kits (CUSABIO\USA) from was used for quantitative determination of human ILs concentration in serum.

2.2 Determination of Celiac disease associated HLA haplotypes using Celiac Strip

HLA genotyping was performed using the polymerase chain reaction-sequence specific oligonucleotide probe (PCR-SSOP) method for HLA-DQ2, HLA-DQ2, HLA-DQ8. The Celiac Strip kit by Operon Immuno & Molecular Diagnostics in Spain can identify the presence or absence of haplotypes encoding HLA-DQ2 and HLA-DQ8, which are the primary HLA haplotypes linked to celiac disease (table 1). The test was carried out in three phases. Genomic DNA was isolated from nucleated cells in a whole blood sample, followed by amplification using PCR techniques. The final step included hybridization and development of the PCR result to identify and evaluate genes. [4]

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2.2.1 Extraction and amplification of genomic DNA

The blood samples were processed for genomic DNA extraction following the aseptic conditions outlined in the ReliaPrep™ Blood gDNA Miniprep System methodology by Promega, U.S.A. The DNA samples were amplified using a thermocycler from Analytic Jena GMBH, Germany.

3. Results

Age and Gender Distribution

The patient (CD) and control groups were well-matched in terms of age and gender. There was no statistically significant difference in the age distribution (p=0.988), mean age (p=0.560), or gender ratio (p=0.883) between the groups. This means any differences found in later tests are likely due to the disease itself and not because the groups were fundamentally different from the start. **(Table 2).**

Distribution of HLA Haplotypes

This table shows the core genetic finding in **Table 3** of the study. A highly significant majority (76%) of celiac patients carried the **HLA-DQ2** gene, compared to only 22.5% of healthy controls (p=0.0001). The **HLA-DQ8** gene was more common in patients (20%) than controls (7.5%), but this difference was not statistically significant (p=0.094). This confirms that HLA-DQ2 is the primary genetic risk factor for celiac disease in this Iraqi population.

HLA Haplotypes by Gender:

Among those who were positive for the risk genes (DQ2, DQ8, or both), there was no significant difference in the distribution between males and females in either the patient or control groups (all p-values > 0.05). This indicates that while the disease is more common in females, the underlying genetic susceptibility is not gender-specific. **(Table 4).**

Detailed HLA Alleles

This deep dive into the specific components (alleles) of the HLA-DQ2 and DQ8 genes shows that almost all individual alleles (e.g., DQA1*05, DQB1*02, DRB1*03) were found at a significantly higher frequency in celiac patients than in controls. This reinforces that the entire HLA-DQ2 haplotype is a strong genetic marker for the disease (Table 5).

Celiac patients had significantly elevated levels of all three pro-inflammatory cytokines compared to healthy controls:

- **IL-15:** Much higher in patients (p=0.001).
- **TNF-α:** Significantly higher in patients (p=0.018).
- **IL-21:** Highly significantly higher in patients (p=0.0001).

This indicates a state of intense immune system activation in celiac disease. As shown in Table 6.

Among celiac patients, those who carried the **HLA-DQ2** gene had significantly higher levels of **IL-15** (p=0.045) and **TNF-α** (p=0.047) than those who did not carry it. This suggests a link between the main genetic risk factor and a more pronounced inflammatory response.(Table 7)

In contrast to DQ2, there was **no significant relationship** between carrying

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the **HLA-DQ8** gene and the levels of any of the three cytokines in celiac patients. This implies that the inflammatory response is more tightly linked to the DQ2 genotype. (Table 8)

Table 9 evaluates the potential of these cytokines as diagnostic blood tests for celiac disease:

- **IL-21** was the best candidate, with high sensitivity (88%), specificity (80%), and overall accuracy (84.4%). This means it is very good at correctly identifying both celiac patients and healthy individuals.
- **IL-15** showed fair diagnostic performance with 71.1% accuracy.
- **TNF-α** was a poor standalone diagnostic test with only 58.9% accuracy.

5. Discussion

5.1 Age and gender distribution of participants.

The results of this study align with findings from several research studies conducted in Iraq and elsewhere. One study on celiac disease in Iraqi patients revealed that 37.5% of the patients were under 10 years old. [5] The age of patients in this study ranged from one year to 60 years, indicating that age-related bias was avoided during patient recruitment. The mean and mode age for all patients and each gender was 19.9±15.4 years. The study results align with previous findings. [6-7] There is no definitive explanation for the age-dependency of celiac disease. However, it is generally observed that the prevalence of celiac disease in children is higher compared to other age groups. This could be attributed to factors such as early introduction of gluten, lack of ongoing breastfeeding, environmental influences during infancy, delayed onset of celiac disease in adulthood, infections, or genetic predisposition. [5,7] Despite using a random sampling technique for patient recruitment, the study population consisted of 64% females and 36% males, resulting in a female to male ratio of 1.9. This occurrence may be explained by the reported female predominance in CD, Hameed and his research colleagues also reported that CD is more in women than in men. [5] Typically, females have a higher prevalence of autoimmune diseases. [8]

5.4 Detection of celiac disease-associated HLA haplotypes (HLA DQ2 and HLA DQ8and HLA DQ2&8) among the patients and the control group.

Multiple studies have shown that the HLA region significantly influences the genetic susceptibility to many systemic autoimmune disorders, such as CD. The HLA class II -DQ area contains genes that encode two alleles, HLA-DQ2 and HLA-DQ8, which are part of the major histocompatibility complex (MHC) II and are associated with hereditary susceptibility to CD .[9] APCs with MHC class II of the HLA-DQ2 or HLA-DQ8 genotype can identify the complex created when gliadin interacts with tissue transglutaminase, leading to immune response activation. This is the foundation of the pathophysiology of CD. [10] Studies from different populations have examined the expression of two genotypes in both individuals with CD and healthy populations. In European region, the prevalence of HLA-DQ2 ranged from 79% to 86% in patients with celiac disease and from 24% to 32% in the general population. For HLA-DQ8, the values were between 10% and 21% in celiac disease patients and 15% to 17% in the general population. [11-12] In Asia, HLA-DQ2 was found in 80-83% and HLA-DQ8 in around

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25% of patients with celiac disease. In the Asian general population, HLA-DQ2 was found in 22-35% of individuals and HLA-DQ8 in 3-22%. [13-15] HLA-DQ2/DQ8 heterozygotes have the lowest risk of getting celiac disease, with only a 3% chance despite a prevalence of 25-35% in the general population. The investigation revealed that 76.0% of the patients were HLA-DQ2 homozygotes, 20.0% were HLA-DQ8 homozygotes, and only 14.0% had HLA-DQ2/DQ8 heterozygosity. In a prior research of 74 CD patients, the prevalence of HLA-DQ2 homozygosity was 79.7%, HLA-DQ8 homozygosity was 8.1%, and HLA-DQ2/DQ8 was 10.8%. [13] Therefore, the HLA-DQ2 homozygosity forms the most predominant genotype in CD patients. [15] The complex molecular mechanism linking HLA-DQ2 homozygosity to the development of CD is not fully understood. However, it is proposed that HLA-DQ2 provides potent antigenic recognition sites for gliadin antigens and has a greater capacity to bind to a wide variety of gluten peptides on the MHC class II of APCs in individuals with this genotype. This may lead to an increased susceptibility to developing gluten-related enteropathy. This study's findings align with the majority of published global investigations, indicating that over ninety percent of individuals with celiac disease exhibit the HLA-DQ2 heterodimer. [17] Also, HLA DQ8 is less strongly associated with CD in the Middle East and North American countries. [18] The results of this study align with those of Çakır and colleagues, who investigated the accuracy of HLA-DQ genotyping and IgA anti-tissue transglutaminase for diagnosing celiac disease in Turkish children. Their findings revealed that 79.3% of celiac children had the DQ2 genotype, while 17.9% had the DQ8 genotype. [19]

5.5 Distribution of HLA haplotypes positive (HLA DQ2, HLA DQ8& HLA DQ2&8) among the study groups according to gender.

To compare the connections between females and males and determine if the parental origin of high-risk haplotypes has an influence. Female patients were shown to have a higher frequency of carrying haplotype DQ2 or DQ8 and DQ2\8 dimers compared to male patients in the study. The correlation between DQ status and gender indicates an influence of the HLA loci on the disease's gender bias. This finding was consistent with the research conducted by Maria C. Mazzilli, B.Sc, in 2007 in Italy and Hameed WS et al. in Iraq. Disagreed with Ahmet Basturk and colleagues in Antalya, Turkey, as well as M. Fernández-Mestre and colleagues in Venezuela on the same topic. [5,17,20]

Detection of CD-associated HLA haplotypes

According to the results of the present study the reports HLA typing information for DQ2 & DQ8 genes in patients with CD and healthy persons firstly regarding the alleles encoded to DQ2, the most common DQA1 alleles were DQA1*05 (82.0%), the most frequent genotype that significantly increased frequency for 41 CD patients, followed by DQB1*02 (78.0%) and DQB1*03 (70.0%) compared to healthy persons (37.5%, 30% and 20%) respectively, results similar to those published. [21-22] This result is similar with Brazilian study by Sliva et al. that revealed statistically significant increase of except DQB1*0302 were the less frequent genotype that the result was no significant P value (0.193). There are studies in regarding this subject that almost agree with the current study. [23] Some investigators have reported in disagreement with such suggestion. [24-25] It is important to consider that several factors such as infections,

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individual dietary habits, genetic make-up, and ethnicity may contribute to this difference.

5.8 Detection of serum IL-15, TNF-α and IL-21 in the patients group and healthy.

IL-15: Serum cytokine elevations in these patients resemble those found in the small intestinal mucosa, while others may originate from sources outside the intestine. Several in vitro investigations have shown that gluten (gliadin) triggers continuous production of pro-inflammatory cytokines of Th type 1. [26] The Th1 response to dietary gluten in the small intestine mucosa is likely responsible for the infiltration of lymphocytes and monocytes in the lamina propria. While having similar effects, Th-1 and Th-2 cytokines mediate distinct functions. The Th-1 response enhances cell-mediated immunity and pro-inflammatory responses, while Th-2 cytokines mainly impact the humoral immune response and help reduce inflammatory processes. Both responses have been noted in CD. [27] The expression of IL-15 varies among different forms of celiac disease, including active, potential, and individuals adopting gluten-free diet. Prior research has verified that individuals with active celiac disease exhibit elevated levels of IL-15 in the mucous membrane of the small intestine. [28] The current investigation found a notably higher amount of IL-15 in patients with CD compared to other groups investigated. Additionally, Abed et al, [27] confirmed the significantly increased level of IL-15 in patients with untreated CD compared with those in the control group. Tamara Vorobjova demonstrated that IL-15 levels were markedly elevated in patients with CD compared to the control group. These levels were also found to be strongly associated with the severity of small intestinal mucosa damage based on the Marsh classification. [27] The current study contradicted Fatemeh Heydari et al's findings in Iran, which demonstrated an insignificant relationship in serum levels between the study groups. [29]

TNF-α: The present investigation found that TNFα levels were higher in patients with effective CD compared to healthy individuals. Gliadin peptides have been demonstrated to stimulate increased TNF-α production by peripheral blood monocytes from Activated CD patients compared to monocytes from Healthy controls or GFD patients in vitro. Similar results have been found worldwide by several estimating methods, including the ELISA method, in countries including Iraq, Iran, and Poland. [27,30] John Sanil Manavalan and colleagues demonstrated that cytokines produced from APC, such as TNF- α, consistently increased in active CD patients compared to those following a gluten-free diet. [26] The USA discovered higher levels of IL15RA and IL-21 expression in duodenal tissues of untreated celiac disease patients compared to controls. IL15 and IL-21 synergistically stimulated intestine intraepithelial cytotoxic T cells. Specifically, they enhanced their transcriptional activity, proliferation, and cytolytic activity. According to a study by Erika Iervasi et al, untreated CD patients have significantly greater IL-21 concentrations in their serum compared to controls, while treated CD patients had low levels of IL-21 [31]. Gluten may act as an autoantigen that stimulates the production of IL-21. Longitudinal research has shown that following a gluten-free diet leads to a significant decrease in the levels of this cytokine. [32-33] IL-21 is known to boost the release of enzymes that break down the extracellular matrix by stromal cells, attractant molecules by epithelial cells, and counteract the immune-suppressing functions of Treg cells [34]. This could provide additional insight into the

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relationship between serum IL-21 levels and mucosal injury. The duodenal mucosa damage progresses gradually over time, culminating in the complete destruction of villi, known as villous atrophy. In this study, for the first time, the possible diagnostic performance of cytokines for CD and the healthy control were evaluated. Our findings demonstrated that IL-21 had the highest sensitivities, specificities, positive and negative predictive values and ACU (84.4) respectively for the detection of the CD patients followed by IL-15 and TNFa and with values ACU (71.1 and 58.9) fair and poor respectively (Table 9).

Correlation of HLA DQ with interleukins (IL-15, TNF-α and IL-21)

There has been a positive correlation between the HLADQ2 of CD patients with IL-15, TNF-α and negative with IL-21(Table 7). Also in the present study, no relation observed between HLADQ8 and immunological markers (cytokines). Histologically, by damage to the intestinal mucosa; and serologically, by the presence of anti-tTG2 Ab, anti-EMA, and/or DGP antibodies. [3] As is well-known, the main genetic determinant in CD involves HLA molecules, specifically the HLADQ2 and/or HLA-DQ8 heterodimers. [35] The physicochemical characteristics of HLA-DQ molecules and their ability to bind certain deamidated peptides by tTG2 are crucial in activating an immune response to gluten, leading to celiac disease. During the adaptive immunological response, gluten-derived peptides that have been altered by tTG2 are displayed by antigen-presenting cells in the mesenteric lymph nodes to CD4 + T cells, in association with HLA-DQ2 and/or DQ8. The Th1-type response results in the generation of IFN-γ and inflammation in the intestines. Earlier researches have shown considerably levels of the serum interleukins were relation with HLA the patients of the CD. [36-37]

At the same time some research has discrepancies these findings. [29,38] this is probably because of the sample size of patients. The conflicting results described by different authors may be due to the heterogeneity of the groups investigated clinical group diversity, the ELISA sensitivity difference, besides differences in the activity indices of the illness, and the variety of management approaches. Probably the main reason due to the small number of positive samples for HLADQ8 in the study.

6. Conclusion

The HLADQ2 genotype is the most common HLA genotype among celiac patients in Iraq, followed by HLADQ8. Celiac disease is more prevalent in children than in other age groups, and it affects females more than males, similar to many autoimmune illnesses. Three proinflammatory cytokines selected for the examination of their role in the immunopathogenesis of CD are IL-15, IL-21, and TNF-α. Each participant exhibited a notable rise in their serum levels among those with CD in comparison to the control group.

AUTHOR CONTRIBUTIONS

All authors have read and approved of the final manuscript.

CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

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ТАБЛИЦЫ

Table 1. Haplotypes that encode the HLA-DQ2 and HLA-DQ8

HLA region	**Specific haplotype**
HLA - DQ2 cis	DQA1*05 - DQB1*02 - DRB1*03
HLA - DQ2 trans HP2	DQA1*05 - DQB1*0301 - DRB1*11/DRB1*12 DQA1*02 - DQB1*02 - DRB1*07
HLA - DQ8	DQA1*03 - DQB1*0302 - DRB1*04

Table 2. The Age and gender distribution of both patients and control groups.

		Patient CD (N=50)		**Control (N=40)**		**P value**
		No	%	No	%	
Age (years)	<10years	16	32.0	12	30.0	0.988
	10---	14	28.0	10	25.0	
	20---	8	16.0	7	17.5	
	30---	5	10.0	5	12.5	
	=>40years	7	14.0	6	15.0	
	Mean±SD (Range)	19.9±15.4 (1-60)		21.8±16.1 (1-59)		0.560
Gender	Male	18	36.0	15	37.5	0.883
	Female	32	64.0	25	62.5	
*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.						
#Significant difference between two independent means using Students-t-test at 0.05 level.						

Table 3. Distribution of CD-associated HLA haplotypes among the patients and the control group.

HLADQ		**Celiac disease (N=50**		**Control (N=40)**		**P value**
		No	%	No	%	
DQ2	Positive	38	76.0	9	22.5	0.0001*
	Negative	12	24.0	31	77.5	
DQ8	Positive	10	20.0	3	7.5	0.094
	Negative	40	80.0	37	92.5	
DQ2&DQ8	Positive	7	14.0	2	5.0	0.157
	Negative	43	86.0	38	95.0	
***Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.**						

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Table 4. Distribution of HLA haplotypes positive among the study groups according to gender.

Positive		**Celiac disease**		**Control**		**P value**
		No	%	No	%	
DQ2	Male	15	39.5	3	33.3	0.733
	Female	23	60.5	6	66.7	
DQ8	Male	3	30.0	2	66.7	0.252
	Female	7	70.0	1	33.3	
DQ2&DQ8	Male	2	28.6	1	50.0	0.571
	Female	5	71.4	1	50.0	
***Significant difference between percentages using Pearson Chi-square test (χ^2-test) a 0.05 level.**						

Table 5. Detection of CD-associated HLA haplotypes among the patients and the control group.

Alleles		**Celiac disease(N=5**		**Control(N=40)**		**P value**
		No	%	No	%	
DQ2 cis						
DQA1*05	Positive	41	82.0	15	37.5	0.0001*
	Negative	9	18.0	25	62.5	
DQB1*02	Positive	39	78.0	12	30.0	0.0001*
	Negative	11	22.0	28	70.0	
DRB1* 3	Positive	35	70.0	8	20.0	0.0001*
	Negative	15	30.0	32	80.0	
DQ2 trans HP 1						
DQA1*05	Positive	43	86.0	14	35.0	0.0001*
	Negative	7	14.0	26	65.0	
DQB1*0301	Positive	23	46.0	10	25.0	0.040*
	Negative	27	54.0	30	75.0	
DRB1*11	Positive	25	50.0	9	22.5	0.007*
	Negative	25	50.0	31	77.5	
DRB1*12	Positive	9	18.0	-	-	0.005*
	Negative	41	82.0	40	100.0	
DQ2 trans HP2						
DQA1*02	Positive	21	42.0	8	20.0	0.026*
	Negative	29	58.0	32	80.0	
DQB1*02	Positive	31	62.0	10	25.0	0.0001*
	Negative	19	38.0	30	75.0	
DRB1*07	Positive	13	26.0	4	10.0	0.054
	Negative	37	74.0	36	90.0	
DQ8						
DQA1*03	Positive	27	54.0	10	25.0	0.005*
	Negative	23	46.0	30	75.0	

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DQB1*0302	Positive	10	20.0	4	10.0	0.193
	Negative	40	80.0	36	90.0	
DRB1*04	Positive	25	50.0	6	15.0	
	Negative	25	50.0	34	85.0	0.001*
***Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.**						

Table 6. Detection of serum IL-15, TNF-α and IL-21 in the patients group and healthy.

	Celiac disease (N=50)	**Control (N=40)**	**P value**
IL-15 (pg/mL)	15.076±15.076 (1.9 78.30)	6.666±6.227 (1.12-32.0	0.001#
TNF-α (pg/mL)	10.406±2.106 (6.7-14.9)	9.451±1.528 (7.42-12.9	0.018#
IL-21 (pg/mL)	22.445±6.547 (14.0-39.0	15.759±4.635 (11.7 31.30)	0.0001#
#Significant difference between two independent means using Students-t-test at 0.0 level.			

Table 7. Relationship between HLA DQ2 with IL-15, TNF-α and IL-21 in patients groups.

	Mean ± SE		
DQ2	IL-15 (pg/mL)	TNF- α (pg/mL)	IL-21 (pg/mL)
Negative	9.153±5.685	9.463±1.736	20.277±6.370
Positive	16.947±12.626	10.704±2.145	23.130±6.534
P-value	0.045	0.047	0.191
#Significant difference between two independent means using Students-t-test at 0.05 level. NS: Non-Significant			

Table 8. Relation of HLA DQ8 with interleukins (IL-15, TNF-α and IL-21) among celiac diseases group.

	Mean ± SE		
DQ8	IL-15 (pg/mL)	TNF- α (pg/mL)	IL-21 (pg/mL)
Negative	15.801±16.227	10.248±2.051	22.103±6.263
Positive	12.179±9.211	11.040±2.317	23.812±7.799
T-test	11.41 NS	3.29 NS	4.74 NS
#Significant difference between two independent means using Students-t-test at 0.0 level. NS: Non-Significant.			

Table 9. Diagnostic performance of IL-15, TNF-α and IL-21 tests according to the optimal cutoff values for ROC and the manufacturer.

Test Result Variable	Sensitivit	Specificity	PPV	NPV	FN%	FP%	Accuracy rate
IL-15 (pg/mL) ≥6	74.0	67.5	74.0	67.5	26.0	32.5	71.1
TNF-α (pg/mL) ≥9	72.0	42.5	61.0	54.8	25.0	57.5	58.9
IL-21 (pg/mL) ≥17	88.0	80.0	84.6	84.2	12.0	20.0	84.4

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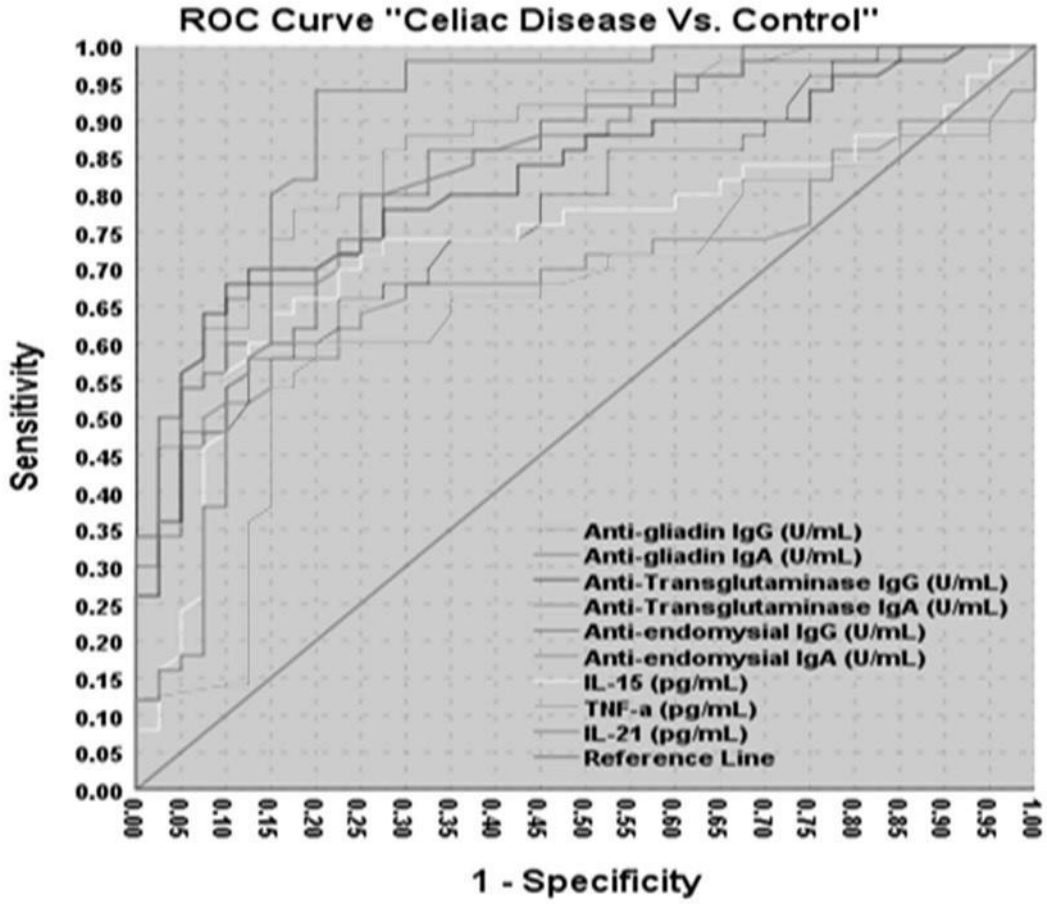
РИСУНКИ

Figure 1. ROC curve analysis of IgG and IgA classes for each Ttg, gliadin, endomysial and interleukins (IL-15, TNF-α & IL-21) in celiac disease patients showing area under the curve.



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ТИТУЛЬНЫЙ ЛИСТ_МЕТАДАННЫЕ

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Блок 3. Метаданные статьи

MOLECULAR AND IMMUNOLOGICAL STUDY OF CELIAC DISEASE IN SAMPLES OF IRAQI PATIENTS

Сокращенное название статьи для верхнего колонтитула:
MOLECULAR AND IMMUNOLOGICAL ASPECTS OF CELIAC DISEASE

Keywords: Celiac disease, HLA-DQ2, HLA-DQ8, IL-15, IL-21, TNF-α, Iraq, Immunogenetics.

Оригинальные статьи.
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Reference sequence number	Authors, title of a publication	Reference's URL
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