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SINGLE NEUCLEOTIDE POLYMORPHISM OF IL17A GENE IN CELIAC DISEASE IN IRAQI PATIENTS

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Abstract

Celiac disease (also termed gluten-sensitive enteropathy) was early on considered a food hypersensitivity disorder as it precipitates in genetically susceptible individuals by the ingestion of cereal gluten proteins. The study aimed to detect the genotypes and allelotypes of IL17 A gene and their possibility effective relationship in celiac disease through their polymorphism. Case-control study enrolled 250 blood samples collected from patient attended to the Marjan Teaching Hospital- Hilla and from October 2019 to May 2020. IL17A genotyping was performing for 55 patients with celiac disease and 30 healthy unrelated controls by means of the PCR-ARMS method. The results of PCR-ARMS for the IL17A rs (10484879) gene polymorphism show that TT genotype was significant differences in optional celiac type p-value (0.05*) with an OR (1.80) comparing with genotype of control subjects and The results showed no significant differences) with genotype celiac disease type active and silent in compared with control subjects Levels of IL17 are significantly increased in the group of patients with celiac disease in comparison with controls group in all types of celiac disease recorded the P-value = <0.001. According to these finding, IL-17A could have a role in the pathogenesis of refractory celiac disease.

Key words: polymorphism, IL17A gene, IL17 Elisa, Celiac disease.

Introduction

Celiac disease (CD) is an autoimmune illness that develops in susceptible individuals exposed to gluten with a prevalence of about 1% of the general population worldwide (Shannahan and Leffler, 2017). Previous literatures pointed that celiac disease is a lifelong autoimmune disease affecting about 1% of the population, although many cases of CD remain undiagnosed CD is caused by abnormal immune response, in genetically susceptible individuals, triggered by the ingestion of gluten proteins from wheat, rye and barley (Lohi *et al*., 2007; Catassi *et al*., 2010). Cytokine changes were correlated strongly with one another and the symptomatic patients had the highest elevations. Early elevations of IL-2, IL-17A, IL-22 and IFN-γ after gluten in patients with coeliac disease implicates rapidly activated T cells as their probable source. (Goel *et al.,* 2019). IL-17A is mainly expressed by an activated cluster of differentiation CD^{4+} T cells, which are classified as T helper 17 (Th17) cells (Lee *et al.,* 2012). IL-17 (IL-17A), also termed cytotoxic T-lymphocyte-associated antigen (CTLA-8), is a 155 amino acids pro-inflammatory cytokine, which belongs to the IL-17 cytokine family of six homologous proteins, designated IL-17A through IL-17F(Cua and Tato., 2010; Veldhoen., 2017). The role of IL-17A in the CD pathogenesis remains elusive although a few studies pointed out the cytokine implementation in the persistent inflammation, including in refractory CD (Monteleone *et al.,* 2010).

Interleukin (IL) 17A is well known as a potent inflammatory cytokine mainly produced by T helper 17 (Th17) cells and innate immune cells and intestinal Paneth cells (Gu *et al.,* 2013).

Materials and Methods

A totals of 225 patients who regularly admitted and clinically diagnosed as a ceoliac patients by medicals committee of ALkafeel hospital and Marjan Teaching hospital, from October 2019 to May2020.

The rang age of patient(6-80) years including both sex male (150) while the number of females (75) in addition to (30)samples were taken from apparently healthy in Babylon province as control.

Blood samples

The venous blood was collected from the patients

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and healthy persons using (5ml) syringes, the blood then separated to (2ml) with anticoagulant tube and (3ml) without anticoagulant. Blood samples without anticoagulant allowed clotting at room temperature then serum was separated by centrifugation at (3000rpm) for (5min). Within 2-3 hours after collection (lewis *et al*., 2001). Blood samples with anticoagulant were used for the DNA extraction using specific DNA extraction kit (Favorgen/Taiwan).

Immunological Tests

Determination of IL-17 (human IL17) concentration in human serum

Sandwich -ELISA technique was applied for detection of IL17 level in patient's sera and healthy control as the method using Bioscience Elisa kit and their instruction.Estimations of the cytokine values in the serum of the diabetes patients and the apparently healthy persons that used as the control group was done according to standard curve that created between standard concentration and their optical density, as shown as in Fig. 1.

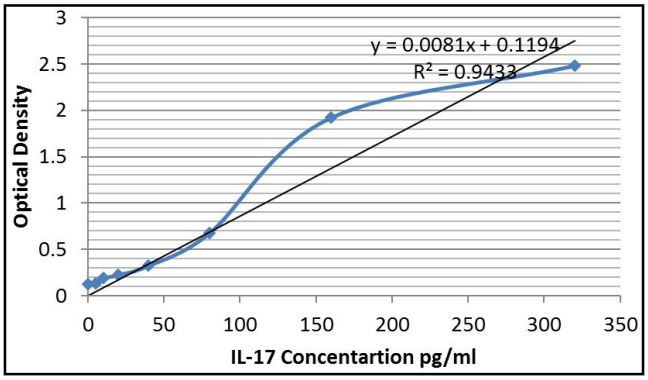

Fig. 1: Standard curve of IL-17concentration with optical density.

DNA Extraction and PCR assay

DNA extraction for human fresh blood samples of patients and healthy control human were performed according to the protocols recommended by manufacturer (Favorgen/Taiwan). For freezing samples thirty µl of Proteinase K (10 mg/ml) was combined with 200 µl of blood.

Estimation of DNA Concentration and Purity

DNA samples were examined for their purity by Spectrophotometer (Nanodrop) equipment, determination of DNA samples concentration depends on the absorption at wavelength (260 nm) level and sum up in to levels due to the principle of the equipment and thus after calibration the instrument used Elution Buffer, then (1 µl) of the DNA was enough for reading the result.

Preparation of Primers

The primers are dissolving according to the company's instructions, by adding Nuclease free water (320 or 300 µl) to the primer to form a stock. Twenty µl of each primer were diluted with (180µl) of nuclease-free water to get a final volume of (200µl) achieved, which was applied for amplification, while the originals primers were kept in -20ÚC and the procedure was continued with the stock primer.

Table 1: Primers sequences.

Primers	Sequences 5-3	Size(bp)	Reference
IL17Ars (1048 4879)	5' GAT ATG CAC CTC TTA CTG CAC TC -3' 5' GAT ATG CAC CTC TTA CTG CAC TT- -3' Antisense common Primer 5'-AGT TGT ACA GGC CCA GTG TA-3'	200bp	(kaur *et al.,* 2018)

Preparation of IL17AProduct

The IL17A PCR product is prepared of final volume of 25µl, by adding 2.5 µl DNA and 1.5 µl from forward 1and reverse primers in tube the same for forward 2 in another PCR tube that containing 15 µl master mix then 6µl nuclease-free water is added to getting the final volume (25µl) then the tube is entered to the thermal cycler machine, with the program includes 94 2min and 30 s and 35 cycles of 55for 30sec annealing and 72 for 5 min as extension due to (kaur *et al.,* 2018) with modification.

Detection of Amplified Product Using Agarose Gel Electrophoresis

The effective PCR amplification is confirmed by using agarose gel electrophoresis (Sambrook and Russell, 2001). Agarose gel was prepared by dissolving 1.6gm of agarose powder in 100ml of TBE buffer previously prepared (90 ml DW was added to 10 ml TBE buffer l0X, the final concentration is 1 X and pH: 8) on hot plate for approximately 10 min, allowed for cool to 50^0C and ethidium bromide at the concentrations of 0.5µg/ml was added.

The agarose was slowly poured into the gel-casting tray that contain the comb of wells, the comb is then gently removed from the tray, the tray was put in an electrophoresis chamber filled with a TBE buffer covering the gel's surface, 5µl of each PCR product was transferred to the wells of agarose gel, and 6µl of the ladder was added to one well. The electric current is allowed at 100 volts for 30min., then 70 volts for 30 min. The E-graph Gel documentation system was used for observation of DNA bands.

Statistical Analysis

All data were analyzed statistically by the SPSS applied mathematics software system (17; SPSS Inc., Chicago, IL) , P- values <0.05 were thought-about statistically important. OR and CI were also applied for results comparison.

Results

Distribution of interleukin 17A (rs10484879) gene polymorphism was detected by ARMS-PCR technique, at this locus there're three genotype GG, GT and TT, the current study for active type celiac disease revealed that The most prevalence of (rs10484879) IL17A GG genotype appeared in healthy control group so it considered the wild type (reference) whereas the least frequent of (rs10484879) IL17A genotype was TT, therefore it regarded as the variant (mutant) genotype.

Considering control group, the frequency distribution of (rs10484879) IL17A genotype GG, GT and TT in patients were 1(5.56%), 8(44.44%) and 9(50.0%) respectively. The results show no significant differences in genotype GT p-value (0.27) with an OR (0.29) and genotype TT p-value (0.13) with an OR (0.18) .Allelic distribution for celiac disease type active and control subjects showed that allele T was more frequency than G allele but there is no significant differences p- value (0.09) with an OR (0.50) between them. As shown in table 2.

Table 2: Genotype distribution of IL17Agene polymorphisms for celiac disease type active.

Genotype IL17Active	Patients	Control	P-Value	OR = (95%CI)
GG[a]	1 (5.56%)	6(20.0%)		
GT	8(44.44%)	14(46.67%)	0.27	0.29(0.03-2.87)
TT	9(50.0%)	10(33.33)	0.13	0.18(0.01-1.84)
Allele				
G	10	26	0.09	0.50(0.20-1.22)
T	26	34		

$P \leq 0.05$; OR = (95% CI); [a]reference.

Concerned with potional type of celiac disease the frequency distribution of (rs10484879) IL17A genotype GG. GT and TT were 0(0%), 8(50.0%) and 8(50.0%) respectively in patient. The current study genotype GT was no significant differences p-value (0.10) with an OR (1.57) and genotype TT was significant differences p-value (0.05*) with an OR (1.80) comparing with genotype celiac disease type potional and control subjects. While allelic distribution showed that allele G and T had no significant differences p- value (0.06) with an OR (0.43). As shown in table 3.

Table 3: Genotype distribution and odd ratio of IL17A gene polymorphisms for potential celiac disease.

Genotype IL17Active	Patients	Control	P-Value	OR = (95%CI)
GG[a]	0 (0%)	6(20.0%)		
GT	8(50.0%)	14(46.67%)	0.10	1.57(1.14-2.15)
TT	8(50.0%)	10(33.33)	0.05*	1.80((1.91-2.72)
Allele				
G	8	26	0.06	0.43(1.16-1.12)
T	24	34		

$P \leq 0.05$; OR = (95% CI); [a]reference.

In case of third type silent celiac disease and considering with control group, the distribution of (rs10484879) IL17A genotype GG. GT and TT were 1(4.55%), 11(50%) and 10(45.45.0%) respectively in patient The current study genotype GT was no significant differences p-value (0.16) with an OR (0.21) and genotype TT was no significant differences p-value (0.11) with an OR (0.54) comparing with genotypes of control subjects. In addition allelic distribution showed no significant differences for allele G and T, p- value (0.10) with an OR (0.54). as shown in table 4 Fig. 1, 2.

The results of IL17 genotype detection by (PCR-ARMS) among control and celiac disease subjects show

Table 4: Genotype and allelotype distribution of IL17Agene polymorphisms for silent type celiac disease patients.

Genotype IL17Active	Patients	Control	P-Value	OR = (95%CI)
GG[a]	1 (4.55%)	6(20.0%)		
GT	11(50%)	14(46.67%)	0.16	0.21(0.02-2.03)
TT	10(45.45.0%)	10(33.33)	0.11	0.16((0.01-1.64)
Allele				
G	13	26	0.10	0.54(0.24-1.25)
T	31	34		

$P \leq 0.05$; OR = (95% CI); [a]reference.

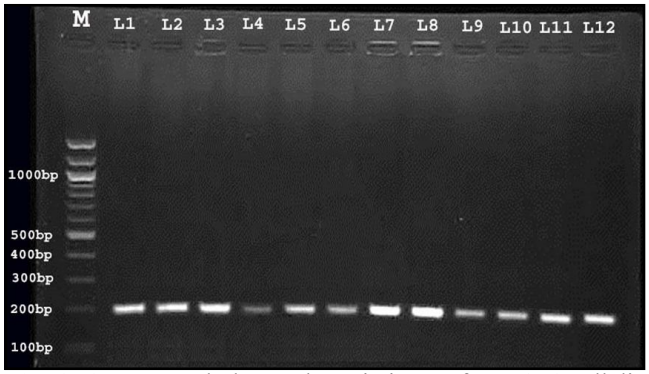

Fig. 1: Agarose gel electrophoresis image for IL17A allelic polymorphism gene amplicon product in human, M, marker 200bp, L1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 : refers to number of samples, and all appear in heterozygous G/T.

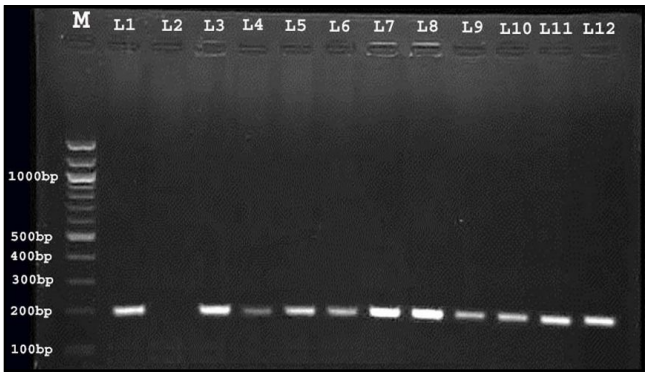

Fig. 2: Agarose gel electrophoresis image for IL17A allelic polymorphism gene amplicon product in human, M, marker 200bp, L3, 4, 5, 6, 7, 8, 9, 10, 11, 12 : refers to number of samples, and all appear in heterozygous G/T. and L1, 2,: refers to number of samples and all appear in homozygous G/G.

out of 55 samples patient 20 samples control the result in male gender Celiac disease type optional shows significant differences p-value (0.03*) comparing with control group the result in female gender Celiac disease types shows significant differences comparing with control group. As shown in table 5.

Table 5: Distribution of IL17AGenotype in celiac Disease types and Control According to Gender.

Gender	Disease type	(Mean ± SD)	Geno type	AN OVA	P-Values
Male	active	5.00±0.001	GT	5.17	0.1
		00±00	GG		
		11.33±4.04	TT		
	potional	9.00±1.41	GT	29.40	0.03*
		0.0±0.0	GG		
		8.00±0.001	TT		
	silent	0.0±0.0	GT	12.01	0.07
		0.00±00	GG		
		20.66±4.61	TT		
	Control	5.33±2.30	GT	0.86	0.46
		5.00±0.001	GG		
		9.00±5.83	TT		
Female	active	70.00±0.001	GT	17.12	0.02*
		0.0±0.0	GG		
		72.33±13.56	TT		
	potional	42.66±21.93	GT	6.89	0.02*
		0.0±0.0	GG		
		84.80±24.10	TT		
	silent	13.12±8.25	GT	26.65	<0.001*
		7.00±0.001	GG		
		54.0±5.65	TT		
	Control	16.66±13.70	GT	0.97	0.41
		4.00±0.001	GG		
		12.00±8.04	TT		

*P ≤ 0 .05.S.D. standard deviation.

Detection of IL17 concentration in patient sera

The concentration of IL17 in celiac disease type according to gender shows significant differences comparing with control group, the concentration in active type male samples was (8.50 ± 5.44) and in female samples was (68.00 ± 14.11) with *P- value* = <0.001* and the concentration in potional type shows highly significant differences comparing with control group male samples was (1.15 ± 8.66) and in female samples was (19.91 ± 35.57) with *P- value* = <0.0001* and the concentration in silent type shows highly significant differences comparing with control group male samples was (5.68 ± 19.66) and in female samples was (20.55 ± 48.76) with P- value = <0.0001*. as shown in table 6.

Table 6: IL17Concentration in Type of celiac disease Patients According to Gender.

Groups	Disease type	Gender		P value
		Male (Mean ± S.D)	Female (Mean ± S.D)	
patient	active	8.50±5.44	68.00±14.11	<0.001*
control		6.44±4.97	20.54±15.33	
patient	potional	1.15±8.66	19.91 ±35.57	<0.0001*
control		6.44±4.97	20.54±15.33	
patient	silent	5.68±19.66	20.55±48.76	<0.0001*
control		6.44±4.97	20.54±15.33	

*P ≤ 0.05. S.D. standard deviation.

Discussion

IL17A rs (10484879) distrupution

The results of PCR- ARMS for the IL 17 A (rs 10484879) polymorphism showed that genotype TT reveals significant differences in celiac disease type potential comparing with control subjects as shown in table 2, while this genotype reveals no significant differences in active and silent type celiac disease comparing with control table 3, 4.

These results match (KAUR *et al.,* 2018) study indicated decreased frequency of IL-17A (rs10484879) G allele (51.8 vs. 65.0%) in patients as compared to healthy controls while the TT was 29.5% in patient and 14.7% with OR = 2.7132 (1.465 5.027).

In agreement with some previous studies our polymorphism results of IL-17A shows that TT genotype and T allele of IL-17A rs10484879 was predominant, this led to increased susceptibility of IL-17 gene towards pathogenesis (Kirkham *et al.,* 2014). Contrasting the results conducted by (Bedoui *et al.,* 2018) rs10484879 was significantly higher in CRC (Colorectal cancer) patients than control subjects. Heterozygous rs10484879 [OR (95% CI) = 2.63 (1.64 – 4.21) was associated with

higher risk. During inflammation, IL-17A found to mediate pro-inflammatory responses (Ishigame *et al.,* 2009). Out of this findings, IL-17A acts synergistically or additively with other pro-inflammatory cytokines, including TNFso considered within the context of the local microenvironment (Kirkham *et al.,* 2014).

Another study with other rs showed no statistically significant association was observed between the IL-17 (-197A/G) polymorphism and CD (p>0.05). In addition, the symptoms and histopathological findings of children with CD were not related to either of the polymorphisms and percent of GG was (54.8%) in patient while in control was (51.8%) p-value = (0.613) (Akbulut *et al.,* 2017) Several studies have reported an association between the IL-17A (-197A/G) polymorphism and autoimmune diseases however, other studies have reported no such relation (Arisawa *et al.,* 2008: Yan *et al.,* 2012).

IL-17 concentration

The concentration of IL-17 in celiac disease type according to gender shows significant differences comparing with control group, the concentration in active type male samples was (8.50 ± 5.44), and in female samples was (68.00 ± 14.11) with P- value = <0.001*. The concentration in potential type shows highly significant differences comparing with control group male samples was (1.15 ± 8.66) and in female samples was (19.91 ± 35.57) with P- value = <0.0001* and the concentration in silent type shows highly significant differences comparing with control group male samples was (5.68 ± 19.66) and in female samples was (20.55 ± 48.76) with P- value = < 0.0001* in table 6 these results match with study conducted by (Velikova *et al.,* 2019) Three out of the twelve patients have shown high levels of serum IL-17A (average 103.2 ± 24.5 pg/ml) and nine patients had IL-17A below the detection limit of the kit. At baseline, all patients showed positive results for celiac-related autoantibodies. The baseline level of cytokine IL-17A was higher in patients with no decrease of anti-tTG antibodies and persistent symptoms after six months of gluten-free diet compared to patients with decreased antibodies after gluten-free diet (Velikova *et al.,* 2019). The present results match with study conducted by (Monteleone *et al.,* 2010) who showed high expression of IL-17A in active CD mucosa IL-17A than in patients on a gluten-free diet and controls (p = 0.001 and p , 0.001, respectively). To confirm these results, IL-17A was also evaluated in protein extracts prepared from biopsy specimens of CD patients and controls by ELISA.

IL-17A is mostly produced by T cells preferentially CD4+ and CD4+ CD8+ cells in active CD (Weaver *et al.,* 2011). Demonstrated that the primary source of IL-17 secretion is not gliadin specific CD4+ T cells but gluten-specific IL-17Aproducing cells (Faghih *et al.,* 2018). Contrasting result s the study conducted by (Mohsen, *et al.,* 2018) The results of IL17 level showed a decrease of IL17 in patients with type 1 diabetes compared to control group with no statistically significant difference (p> 0.05),. While these results did not match the results of the study conducted by (Kikodze *et al.,* 2013) for obtaining results with significant differences in the level of concentration with IL-17 in patients with diabetes type 1(Kikodz *et al.,* 2013).

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