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Relationship between rs1800796 Polymorphism of *IL-6* Gene and IL-6 Serum Level with Thyroid Hormones in a Sample of Iraqi Celiac Disease Patients

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Abstract: Celiac disease (CD) is autoimmune human leukocyte antigen HLA– linked enteropathy that develop upon ingestion of gluten containing diet, with diarrhea, malabsorption and weight loss as a major presentation. The disease is closely linked to a number of extra intestinal disorder especially endocrine diseases. This study aimed to find Relationship between IL-6 gene polymorphism rs1800796 and IL-6 serum level with thyroid Hormones in a sample of Iraqi celiac disease patients. The CC genotype showed a higher frequency in the control while the GG genotype showed a higher frequency in the patients.

Keywords: celiac disease (CD), genetic polymorphism *IL-6* gene, thyroid stimulation hormone.

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Introduction

Celiac 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 (1). Celiac disease is an autoimmune chronic inflammatory disease of the upper small intestine triggered by gluten protein intolerance, (2) which is prevalent in "genetically predisposed individuals." Gluten is the wheat grain protein richly consumed (3) it is comprised of prolamin and glutelin proteins. Both proteins abundantly possess glutamine and proline residues, which defy gastrointestinal digestion and promote the deamination process through the tissue transglutaminase (tTG) enzyme. It may lead to mucosal inflammation and villous atrophy, thus causing malabsorption. (4). Clinical spectrum of CD includes, the following, typical or classical, atypical or non-classical, and silent (5). When a person consumes gluten, the small intestine is harmed, actually results in gastrointestinal complaints, malnutrition, small bowel mucosal damage, and malignancies (5). The disease can occur at any age, with a variety of symptoms (6). 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 (7, 8). The thyroid gland its hormones play multifaceted roles in organ development and in the homeostatic control of fundamental physiological mechanisms such as body growth and energy expenditure (9). It is these cells that produce the thyroid hormones triiodothyronine and thyroxine (T3 and T4), which are iodinated dipeptides that are synthesized, stored and secreted in a complex series of reactions involving bidirectional transport to and from the lumen (10) Thyroid gland produces thyroid hormone, which has clinically important actions practically in every system in the human body; it is synthesized through iodination of tyrosine residues in the glycoprotein thyroglobulin. There are two major thyroid hormones secreted by the endocrine thyroid gland, these are thyroxine (T4) and tri-iodothyronine (T3), which are, uniquely, hormones that contain iodine atoms which are essential for the endocrine activity of these hormones.). The major regulator of the thyroid functional is t (TSH) also called thyrotropin, secreted by the anterior pituitary. TSH stimulates production and release of thyroid hormones. (12,13)
Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells; a given cytokine may be produced by more than one type of cell (18,19) cytokine leads to inflammation and is associated with inflammatory autoimmune diseases (20). play a major role in response to the inflammatory stimuli and tissue damages. It is produced by various cells, including T and B cells, monocytes, fibroblasts, endothelial cells, IL-6 regulates the growth and differentiation of various cell types with major activities on the immune system, hematopoiesis and inflammation. The elevation of serum IL-6 precedes that of acute phase proteins (21). The chromosomal location of IL-6 and its receptor is 7p21. The fact that a vast majority of IL-6 is expressed from active macrophages, the differentiation in the capacity of B lymphocytes to produce immunoglobulin and the fact that T cells are active are all important factors in proliferation and differentiation Signiant high serum IL-6 levels have been observed in patients with CD compared with healthy controls (22). Genetic polymorphisms modifying IL-6 levels may therefore potentially be involved in susceptibility to CD. (25).

Materials and methods

The present study included 120 subjects within two groups (patients and control). Patients group comprise of 60-positive Iraqi celiac disease patients who were diagnosed by specialists in Gastro Intestine Track Center in Baghdad depending on the basis of medical signs and symptoms in addition to the results of serological test for the period from1st of December 2021 to the last of April 2022. A questionnaire has been taken from the patients, and the case sheet included age, sex, in this study,120 volunteers were use and divided to two groups, The first group was included Patients while second group was included apparently healthy. Five milliliters of venous blood samples were withdrawn from all subject under aseptic precautions. The collected blood samples were divided into two parts, 2 ml of peripheral blood placed into sterile plain tube that contained EDTA and 3 ml of serum were collected and placed. The

blood and serum were placed in a cool - box under aseptic conditions and transfer to the laboratory, CD patients the Elisa test was carried out to detect the Anti-tissue Transglutaminase antibodies, IgA and IgG. Serum hormones were measured by (COBAS)e411, (rs1800796) of the *IL-6* gene was done, by using Taq man SNP Genotyping Assays. the DNA was extracted, using DNA extraction kit Easy Pure® Genomic (Trans Gen, biotech. EE101-01). primer sequences were designed according to their reference sequence (rs) in the database of National Center for Biotechnology Information (NCBI). The forward primer5'-TGGCAAAAAGGAGTCACACA and the Reverse primer 5' - CCAAGCCTGGGATTATGAAG, the thermal cycling program was as follows: enzyme activation in 95 C° for 10 min, followed by 40 cycles of two steps (first one was denaturation 95 C° for 20 sec and second step of annealing for 1 min (60 C°) and extension (72 C°) for 20 sec.

Statistical analysis

Difference between groups was tested using The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. The allelic and genotype association of SNP were evaluated by Pearson's Chi-square test; and odds ratio (OR) and 95 per cent confidence intervals were determined. For comparison of more than two groups, one way ANOVA was used.

Results and discussion

Comparison between celiac disease patients and apparently healthy control in levels of thyroid hormone

The comparison of the mean value of the selected hormonal profile between celiac patients' groups and controls group-containing (T3, T4, TSH,) as shown in table (1).

Table (1): A comparison between Celiac disease patients and apparently control groups in thyroid hormone

Characters	Mean ± S.E.		T-test	P-value
	Patients (no.60)	Control (no.60)		
T3	5.10 ± 3.15	1.50 ± 0.56	1.14^{ns}	0.25
T4	10.35± 8.11	9.94 ± 0.25	0.98^{ns}	0.32
TSH	0.789 ± 0.10	0.633 ± 0.09	0.128 *	0.039
** =P-value <0.01, N.s.= not significant				

The results of thyroid hormone in celiac disease patients and control were listed in table (1) patients with CD showed not significant difference in T3 compared to apparently healthy control (5.10 ± 3.15 pg./mL, 1.50 ± 0.56 pg./mL, P <0.25), total thyroxin (T4) level (10.35 ±8.11vs 9.94 ± 0.25 respectively, non-significant) while significant results were found in TSH level compared to apparently healthy control (0.789 ± 0.10 vs 0.633 ± 0.09 respectively <0.01). There is a relationship between autoimmune thyroid disease (AITD) and celiac disease. Celiac disease is an autoimmune disorder that causes inflammation and damage in the lining of the intestine after eating gluten, a protein found in wheat, rye, and barley. And AITD includes thyroid conditions that are caused by the immune attack against the thyroid gland, most commonly

Hashimoto's thyroiditis or Graves' disease (28). However, there was significant association between hyperthyroidism and CD. Overall, the heterogeneity in our meta-analysis was low, particularly in that of euthyroid autoimmune thyroid disease and Results of the present study were agreed with (29) who noticed significant correlation exist between anti-gliadin level and the level of two hormones included in this study in CD patients. (30) referred that patient with mild serum TSH elevation, thyroid function should be more frequently tested because of increasing risk for developing overt thyroids Individuals with an overactive thyroid often experience symptoms that may include anxiety, heat sensitivity, vision issues, insomnia (and other sleep complications), tremors, weight loss, lighter menstrual periods, weak muscles, mood swings, high blood pressure. Graves' disease is the most common thyroid condition and an autoimmune disorder that causes hyperthyroid an underactive thyroid, on the other hand, secretes too little hormone, which means the body consumes less energy than it should. this is medically known as hypothyroidism**.** In hypothyroidism the symptoms that include dry skin, weight gain, depression, fatigue, memory problems, bloating, constipation, difficulty processing information, hoarse voice, and slow heart rate. In many cases, people with hypothyroidism may have an enlarged thyroid gland, or what's called goiter in medical terms. On rare occasions, it may lead to coma, although most cases are mild. Damage from chemotherapy surgical removal of the thyroid gland may cause hypothyroidism. However, Hashimoto's thyroiditis (better known as Hashimoto's disease) is the leading cause of hypothyroidism (31).

Estimation of serum level IL-6 in CD patients and control

The results of IL-6 serum level were listed in table (2), As shown in the table patients with CD showed highly significant increase in IL-6 level compared to apparently healthy control (3.92 $\pm$ 0.13pg\mL, 1.32 $\pm$ 0.9713pg\mL) respectively (P <0.01).

Table (2): Serum level mean of IL-6 in CD patients with celiac and control groups

Characters	Mean $\pm$ S.E.		T-test	P-value
	Patients (no.60)	Control (no.60)		
Interleukine6	3.92 $\pm$ 0.13	1.32 $\pm$ 0.97	15.98**	0.000
** =P-value <0.01, N.s.= not significant				

IL-6, a pleiotropic cytokine, is mainly produced in lamina propria myeloid cells in response to intestinal damage and has a significant function in inflammation, as well as in mediating the innate and adaptive immune responses, making IL-6 an important factor in CD pathogenesis Interleukin 6 (IL-6) stimulates the production of acute phase reactant proteins that cause inflammation or tissue injury (32) On the other hand, studies have shown that serum levels of IL-6 in CD patients increase after consumption of gluten-containing foods in untreated patients and decrease a year after commencement of a gluten-free diet (33).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 CD (34). Results of the present study were agreed with (35) who noticed that there was a significant difference between serum IL-6 levels in CD and healthy subjects (p = 0.0001).

Genotype and allele frequency genes (rs1800796) polymorphism (-572 G/C)

The genotypes and allele frequency distributions of IL-6 (rs1800796) for both celiac disease and control were presented in table (3). polymorphism of IL-6 (rs1800796) occurred in their genotype (CC, CG and GG) in both a CD patients and control groups that cores to two allele (C and G). Compared GC genotype between control and patients heterozygous GC genotype was associated with significantly increased risk for celiac disease (X^2= 53.55, P-value= 0.0001) Compared GG genotype between control and patients, homozygous wild GG genotype was associated with significantly increased risk for celiac disease (X^2=18.18, p-value =. 0.0001). In addition, allele frequency for the G allele is associated with significantly increased risk for celiac disease) they investigate the prevalence of homozygous mutant (CC) of the rs1800796 polymorphism was high protective factor Additionally, the frequency of the mutant allele (C) was even higher in control compared to patient's (X^2=88.36,p=0.0001)showing a crucial genetic susceptibility factor on predisposition to the development The results indicate that there was a significant difference in genotype Frequency between CD patients and control group for the three genotypeCC(3.33%vs94.66%p=<0.0001,O.R =.0.001,C.I(0.00010.008),GC(61.66%vs,3.33 %)respectively,P= <0.0001, O.R=46.65) C.I (10.38-209.63) and GG (35%VS 2%)respectively= <0.0001, O.R=65.86 C.I (3.87-1118.75).In term of allele frequency the allele G increase in patients (66%vs 1.6%) while allele C was decreased in patients (34%vs 98.4%) CD, this may suggest that this allele may have a protective effect against celiac disease initiation.

Table (3): Genotypes distribution and Allele frequency of rs1800976 genotype in celiac disease patients and control group

Genotype (rs1800796) gene (-572 G/C)	Patient NO=60	Control NO=60	Chi-square	P-value	O.R.(C.I.)
CC	2 (3.33%)	55 (94.66%)	88.36	<0.0001	0.001**(0.0001- 0.008)
GC	37 (61.66%)	2 (3.33%)	53.55	<0.0001	46.65**(10.38-209.63)
GG	21 (35%)	3 (2%)	18.18	<0.0001	65.86*(3.87-1118.75)
Allele frequency					
C	34%	98.4%	-	-	-
G	66%	1.6%	-	-	-

Interlukin 6 is considered as an important cytokine that associated with the progression of different type of out immunie disease because it is considerd as a key anti inflammatory cytokin that can regulate the extression of different molecules that are involved in immune respones (36).The results are in agree ment with Barartabar *et al.*, (2018) who show a significant relation between IL-6 (-572G/C) (rs1800796) and CD.This suggests that the *IL-6* (-572G/C) polymorphism should be evaluated as a risk factor in the development of

difference disease including CD.The rs1800796 polymorphism showed parallel links between serum IL-6 and the polymorphism of this cytokine in CD patients. The -572G/C polymorphism is a functional variant and promoter region directly responsible for serum levels of IL-6. The IL6 gene is mainly regulated at the transcriptional level and several polymorphisms affecting transcription have been found relevant to final cytokine levels(38).Little is know about the mechanisms by which IL-6 contributes to the development of various disease including CD.IN CD the pathological featuresof glutian sensitivity are associated with local and systimitic increase in IL-6 in addition to other proinflammatory cytokines (39).there is some evidence that the glutian spesific T-cell clones secrete predominantly THO profile cytokines.Similar THO cytokine responesto gliadin have been observed in CD subjects .on the basis of these data it is possible that high IL-6 concentrations observed are caused by increasedTHO cytokine production (39).IL-6 (rs1800796), a function variant located in the promoter region of IL-6, has been evaluated for its association with many kinds of diseases, including cancers, celiac disease, chronic HBV infection, acute coronary syndrome, ischemic stroke, periodontitis, IgA nephropathy, hip fracture, osteoarthritis, acute chorioamnionitis, etc., (40).The elevated serum levels of IFN-γ, IL-6, and IL-8, which have been shown to be high in the gut mucosa, suggest that CD induces secretion and systemic activation of these cytokines(41).

Impact of rs1800796 on IL-6 gene serum level

The IL-6 serum level polymorphism and its association with (rs1800796) genotypes between the studied groups (patients and control) were illustrated in table (4). when CD patients comparison within these genotypes according to IL6 there was a significant increase of CC genotype in CD patients compared with control group (2.66±0.3 pg/mL, 1.14±0.06 pg/mL respectively T-test=4.17), At the same time the frequency of GC genotype was significantly $p \leq 0.003$ higher in CD patients than in control group (4.21±0.22vs1.17±0.005, respectively T-test=3.13), while the frequency of GG genotype was significantly $p \leq 0.01$ higher in CD patients than in control group (3.50±0.14 vs1.2±0.03, respectively T-test=7.15).

Table (4): Impact of rs1800796 on IL-6 Gene Serum Level

Genotype (rs1800796) gene (-572 G/C)	Means of IL-6 Gene Concentration Patient	control	T-test	P-value
CC	2.66± 0.3	1.14± 0.06	4.17	<0.0001*
GC	4.21± 0.22	1.17±0.005	3.13	0.003*
GG	3.50± 0.14	1.2 ± 0.03	7.15	<0.0001*
	*** =P-value <0.01, N.s.= not significant**			

Celiac patients had high levels of IL-lB, IL-6, and IL-l-RA. Treatment with a gluten-free diet improved BMD and induced a nonsignificant diminution in

IL-lB and a significant decrease in IL-6 serum levels. In addition, patients with normal bone densities or with milder or minimal bone loss had a significantly greater IL-l-RA than patients with more severe bone loss (42) .On other hand ,Dema *et al* .in 2009 find of a significant association of IL6 with female CD patients increases the list of relevant cytokines, from a genetic point of view, in this pathology. IL-6 exhibits important and diverse functions in immune and inflammatory. In CD, mucosal damage occurs with both a natural and an acquired immune response, previous studies have shown that intestinal inflammation in CD is due to difference cytokine production that are responsible for the pathogenesis of the disease. In addition to that, IL-6 has been determined to increase and play a role in intestinal inflammation in CD patient. studies have reported a relation between IL-6 polymorphism and high serum level (43). In comparison with the CC genotype, the IL-6 (rs1800796G\C) G allele has been determined to be responsible for greater IL-6 production and higher serum level. The -174 IL-6 (G > C) rs1800795 polymorphism regulates IL-6 expression which may be associated with the clinical outcome in patients with CD.

However, previas studies on this genetic polymorphism have presented contradictory results regarding the genotype associated with the progression or development of various disease and type of cancer (43). Fermandes *et al* (2020) reported that patients with the GC genotype had higher IL-6 level than patients, with CCand GG genotype, and G allele was identified as a risk factor for the development of CD. the newly discovered subset of T helper 17 (Th17) cells has expanded the IL-6 function. Our findings indicate that the inflammatory responses in CD may be characterized by the elevated levels IL-6, which can be considered as Th1- and Th2-derived cytokines, respectively .Dienz and Rincon showed that IL-6 can modulate the Th1/Th2 balance toward Th2. The important aspects of the proliferation and variation of IL-6 belong to the differentiation capacity of B lymphocytes to produce immunoglobulin and activate T cells (41,44).

Conclusion

Present study was noted that the peak of occurrence of celiac disease was at age (20-31) years with significant differences compared with other ages.

Celiac disease was found in female of Iraqi patients significantly more than male,

TSH thyroid hormone showed significant elevation in patinas level when compared to control, The G allele of the SNP (rs1800796) of *IL-6* may be considered as a risk factor for Celiac disease Progression, mutant CC is protective factor genotype in a sample of Iraqi patients.

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