RESEARCH ARTICLE

Hepatitis C and IL-6 with 174G/C Gene Polymorphism in β-Thalassemia

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ABSTRACT

Polymorphisms have been shown to affect the progression of disease in patients with Iraqi thalassemia in the 174 G\C area of Interleukin-6 (IL-6). The objective of this research was, therefore, to determine the connection of IL-6 gene polymorphisms and thalassemia severity in Iraqi patients. Case-control research of 60 patients diagnosed with β-TM at the thalassemia center in AL-Zahra hospital in AL-Najaf City, Iraq, with a group of 40 healthy patients, was used as control; the patients (26 males and 34 females) were 3–49 years of era.

Blood samples were gathered and controlled from all patients. Blood used to extract DNA to detect IL-6 G\C polymorphism using SSP-PCR.

The outcome showed that the male age range (56.6%) was higher than the woman (42.6%), and the age range (10–19) was higher than the other. The outcome shows that GG genotype and G allele in thalassemia patients are a risk factor of severity, whereas CC genotype and C allele are a protective factor of severity.

Conclusion: The polymorphism in IL-6 at a place (174 G\C) is associated with thalassemia pathogenesis in the GG genotype and IL-6 considers the seriousness of thalassemia as a predictive factor

Keywords: HCV, IL-6 polymorphism, Splenectomies, Thalassemia.

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INTRODUCTION

Beta-thalassemia is a group of hereditary blood disorders that will be determined with reduced erythrocyte hemoglobin, reduced erythrocyte output, and anemia due to the reduction or lack of beta-globin chain synthesis. In this patient, iron overload, cardiovascular arrhythmia, hepatitis, osteoporosis, and endocrine disease are the most significant problem.[1]

In a spleen, damaged or faulty red blood cells are usually removed. There is a big amount of faulty red blood cells in individuals with thalassemia, which leads to an enlarged hyper-functioning spleen (splenomegaly). Removal of the spleen can thus prolong the survival of red blood cells by decreasing the quantity of red blood cells taken from circulation and may eventually lead to decreased blood transfusion needs.[2]

The main cause of post-transfusion hepatitis infection (PTH) is the hepatitis C virus (HCV). The virus infects liver cells and creates serious hepatic inflammation with long-term issues.[3]

Cytokine and other immune response regulators may play a significant role in thalassemia pathogenesis.[4] IL-6 is a pro-inflammatory cytokine, a pleiotropic 26 kDa protein generated by a variety of cell kinds such as fibroblasts, monocytes, endothelial cells, B cells, and T cells, affecting the function of a wide spectrum of cell kinds involving B cell differentiation and increased antibody manufacturing. In addition, IL-6 enhances T-helper differentiation of 17 cells and suppresses regulatory T-cells differentiation.[5,6]

Polymorphism can function as a modulating factor by causing modifications that may interfere with significant processes in the thalassemia disease pathophysiology.[7] Polymorphism in the cytokine gene may affect immune response, inflammation, and tissue injury, disease predisposition, predict treatment outcome and may affect thalassemia outcome.[7]

The gene IL-6 is a single copy of the human chromosome 7p15- p21.[8] The gene is made up of 5 exons and 4 introns. The IL-6 gene is polymorphic in both 5 and 3 flanking regions, recently biological function has been demonstrated in the promoter region-174 single G\C base swap polymorphism.[9]

MATERIALS AND METHODS

The research was conducted at the Department of Medical Laboratory Techniques Bacteriology and Molecular Laboratories, Medical Technology Collage, Islamic University, Najaf, Iraq. It included patients diagnosed with beta-thalassemia while excluding patients with alpha-thalassemia or other inherited disease.

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Patients and Control Group

Beta-thalassemia patients diagnosed with an inherited blood disease center at Al-Zahra Teaching Hospital in AL-Najaf from December 2016 to the end of December 2016 ; 60 patients aged between (3–49) years; in relation to the control group, 40 randomly healthy (male and female) patients aged between (5–47) years were comprised. For each subject, both physical and clinical exams were performed, and the information was recorded in a data sheet. This research agreed with the ethics of the Ministry of Health and the Al-Zahra Teaching Hospital. All respondents received verbal informed consent. Blood specimens were gathered for IL-6 174 G\C gene polymorphism PCR amplification.

Deoxyribonucleic acid (DNA) extraction and polymerase chain reaction (PCR)

Genomic DNA was harvested from fresh peripheral blood (3 mL in EDTA) using a kit that was available commercially under the GeneiusTM Micro DNA Extraction Kit (Geneaid, USA) protocol and then stored at -20 C until use. Single nucleotide polymorphisms (SNPs) linked to IL-6 (-174 G\C) were defined utilizing Polymerase chain reaction with site-specific primers (PCR – SSP) in 2 responses utilizing one widespread forward F: GAG CTT CTC TTT CGT TCC and two inverse primers R1: CCT AGT TGT GTC TTG CC and R2: CCT AGT TGT GTC TTG CG[10] with amplicon product 234 bp. The reaction mixes (25 µL) included 5 µL DNA template, 12.5 µL master mix (Promega, USA) and 4 µL prim (2 µL foreword and 2 µL reverse) and 3.5 µL Nuclease Free Water (USA Applied PCR System). Table 1 shows PCR circumstances for the IL-6 gene. PCR products were electrophoresized with 1,5% agarose gels, stained with ethidium bromide (Biobasic, Canada) and visualized with UV lighting and photographed using a Cleaver gel documentation scheme (Biometer/Germany) (Table 1).

Statistical analysis

Statistical analysis was carried out using the system/version 17 of the Statistical Package for Social Science (SPSS) and Microsoft Office Excel 2007. Results were expressed as mean ± S.D. When it is less than 0.05, p-value was regarded important. Variance assessment (ANOVA) has also been implemented. To assess the power of the connection between two categorical factors, such as the presence of certain genotype, and the status of the disease, the odds ratio (OR) was used. The statistical significance of the measured OR is evaluated using a unique formula of 2.

RESULTS AND DISCUSSION

Demographical Distribution of Thalassemia patients

Demographic features by case-control research disclosed the following outcomes for 60 patients attending the inherited blood disease center in AL-Najaf province:

Table 1: PCR conditions for IL-6 174 G\C gene

Step	*Temperature*	*Time*
Initial Denaturation	94˚C	5 Minutes
Denaturation	94˚C	30 Seconds
Annealing	54˚C	60 Seconds
Extension	72˚C	60 Seconds
Final Extension	72˚C	7 Minutes

Gender Distribution

The research appears to be 56.67 % male and 43.33 percent female, and, as shown in Figure 1, the static analysis revealed non- significant differences in ($p > 0.05$) among patients (Table 2).

This research shows a high level of male patients compared to female patients, the rise in male numbers was discussed by Talsania *et al*. (2011), who stated that rural patients and traditional rural customs used to take care of male patients rather than female patients and the most patients suffering from thalassemia in this research were rural patients or low-income patients.[11]

Age Distribution

Their age split patients ranges into five classifications. The strongest patient age ratio was in 10-19, followed by 1–9, 20–19, 30–39, and 40–49, being 31, 14, 9,4, and 2, respectively. The results showed a smaller number of older patients compared to the high number of younger patients, as shown in Figure 2.

This outcome shows that the age range (10–19) is greater than the other age range; this may be because patients with β-TM showed a lack of life expectancy in the first or second centuries of life.[12] In Babylon, Muhsin and Abdul-Husin discovered that 34.2% of patients were within the 10–20 age range.[13] A comparable founded in the province of Karbala was also recorded by Ali, the age range (10–20) being 62.5 % of patients.[14] Medical therapy converted people with thalassemia from a quickly deadly disease in early adolescence to a chronic disease compatible with extended lives up to fourth or five decades, but complication issues such as growth retardation, iron-overload-related heart, endocrine and liver disorders, The primary cause of death among these patients is the toxicity of iron chelators, severe bacterial and viral infections.[15]

Distribution of splenectomy with Hepatitis C Virus (HCV)

The distribution of HCV and splenectomy-infected thalassemia patients is 13.33% higher than non-HCV-infected splenectomies,

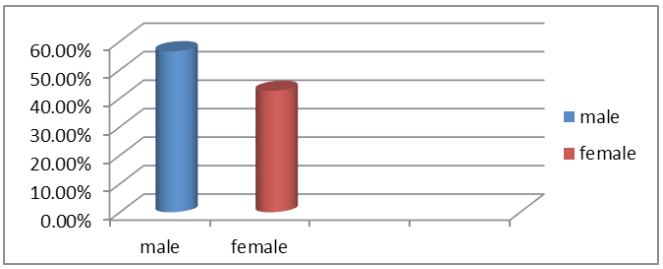

Figure 1: Distribution of the β-Thalassemia major patients according to gender

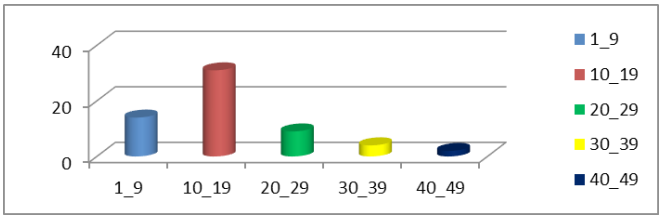

Figure 2: β-Thalassemia distribution according to age

resulting in a significant p-value of 0.0202* as shown in Table 3.

This result shows HCV-infected patients with thalassemia and splenectomies is 13.33 % higher than non-HCV-infected splenectomies, resulting in a significant P-value of 0.0202*. This research was comparable to the Hussain (2008) study, which found that blood transfusion figures were considerably greater in anti-HCV positive patients and splenectomy than in anti-HCV negative patients.[16] This research arrangement with Saeed *et al*. (2015) shows that HCV-infected thalassemia patients had a greater likelihood of splenomegaly compared to HBV-infected patients, and splenomegaly was present in most HCV-infected patients. The maximum rise in spleen size was noted in people infected with HCV, *i.e.,* 12 cm.[17]

Distribution of splenectomy with gender

This research appears to be more than female than male splenectomies as shown in Table 4, in which male is 15%, female is 5%.

This research disclosed that male patients with splenectomies are more than female as shown in Table 4, where male is 15%, female is 5%. Memon *et al*., (2017) discovered that male was more likely to have splenectomy than female (19 and 8) in this respect.[18] Saha *et al*. (2015) also recorded an enhancement in quality of life as a result of a substantial decrease in blood transfusion following the procedure.[19] Similarly, in terms of physical activity with improved attendance at college and involvement in outdoor sports, Hussain *et al*. (2011) also noted improvement in the quality of life in its 21(75 %) patients.[20]

Distribution splenectomy with age

The research discovered that β-thalassemia patients who create splenectomies are the largest proportion in the age range of (10–19) is 11.67%, then (20–39) is 5%; lastly, the age range of (30-39) is 3.33%, while the age range of (1–9) and (40–49) is not discovered to be any splenectomies (0%) (Table 5).

The patients who create splenectomy were between 10 and 39 years of age in our series. Probably the reasons for our patients ' elderly era include delayed splenectomy referral and patients ' unwillingness to undergo significant surgery.

In thalassemia, splenectomy is reserved for patients with marked splenomegaly-related diseases, enhanced demands for transfusion, and complications such as pancytopenia. Splenectomy should not be carried out slightly, and the hazards in each case should be weighed against the potential advantages. Some writers think that splenectomy should be done as quickly as there is an enhanced need for transfusion before hypersplenism or hemosiderosis becomes evident.[21]

Molecular study

PCR-SSP method identified the distribution of IL-6 174G\C polymorphism, there are three genotypes at this locus; GG, GC, and CC with 234bp band dimensions as shown in Figure 3

The genotypes frequency in thalassemia patients were as follow; GG(55%), GC(40%) and CC(5%); while in the healthy subjects ;GG (10%) ,GC (60%), CC (30 %), Table 6

Table 2: Correlation between age and gender

Gender \ *Age*	*1-9*	*10-19*	*20-29*	*30-39*	*40-49*	*total*
Male	5 (8.33%)	21 (35%)	5 (8.33%)	1 (1.67%)	2 (3.33%)	34 (56.67%)
Female	10 (16.67%)	10 (16.67%)	3 (5%)	3 (5%)	0 (0%)	26 (43.33%)
Total	15 (25%)	31 (51.67%)	8 (13.33%)	4 (6.67%)	2 (3.33%)	60 (100%)

Table 3: Distribution of splenectomy with HCV

	Infected with HCV	*Non-infected with HCV*	*OR (95% CI)*	*P-value*
Splenectomies	8 (13.33%)	4 (6.67%)	4.857 (1.256 -18.78)	0.0202*
Non-splenectomies	14 (23.33%)	34 (56.67%)	0.2059 (0.05325-0.7960)	0.0202*

Chi-square, df = 5.813, 1 p value = 0.0159 (0.05%) (significant)

Table 4: Distribution of splenectomy with gender

	Male	*Female*	*Total*
Splenectomies	9 (15%)	3 (5%)	12 (20%)
Non-splenectomies	25 (41.67%)	23 (38.33%)	48 (80%)
Total	34 (56.67%)	26 (43.33%)	60 (100%)

Table 5: Distribution splenectomy with age

	1- 9	*10-19*	*20-29*	*30-39*	*40-49*	*Total*
Splenectomies	0 (0%)	7 (11.67%)	3 (5%)	2 (3.33%)	0 (0%)	12 (20%)
Non-splenectomies	15 (25%)	24 (40%)	5 (8.33%)	2 (3.33%)	2 (3.33%)	48 (80%)
Total	15 (25%)	31 (51.67%)	8 (13.33%)	4 (6.67%)	2 (3.33%)	60 (100%)

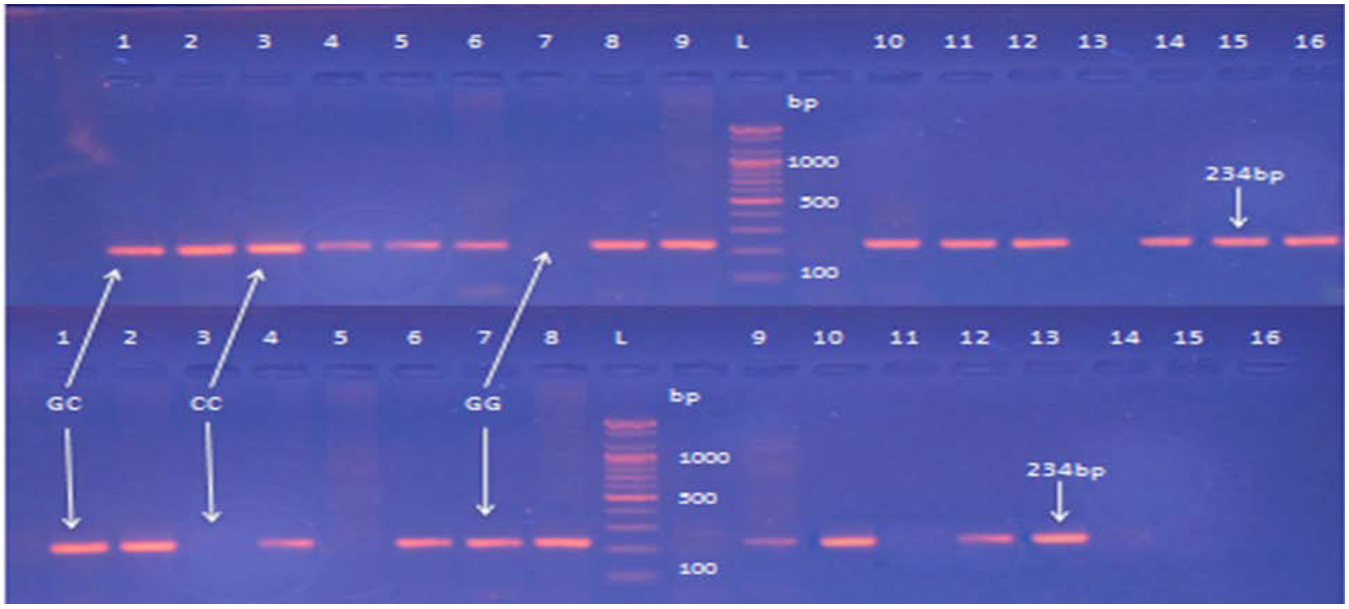

Figure 3: In thalassemia patients, PCR's ethidium bromide-stained agarose gel enhanced 234bp of the IL-6 gene.

Table 6: Genotype and Allele frequency of the IL-6 174 G\C promoter variant among thalassemia patients and control .

		Patients	*Control*	*OR (95% CI)*	*p-value*
Genotype	GG	22 (55%)	4 (10%)	11.0 (3.291-36.76)	0.0001***
	GC	16 (40%)	24 (60%)	0.4 (0.18-1.08)	N.S
	CC	2 (5%)	12(30%)	0.12 (0.02-0.59)	0.0032**
Allele frequency	G	60 (75%)	32 (40%)	4.5 (2.29-8.84)	<0.0001***
	C	20 (25%)	48 (60%)	0.2 (0.11-0.43)	<0.0001***

p ≤0,05, OR: Odds Ratio, CI: Confidence Interval

This is consistent with Bagheri *et al*., a previous study (2005), which discovered that GG more frequency in Iranian thalassemia patients and explained that GG, GC genotype yields elevated IL-6 production leading to disease severity while CC genotype yields low IL-6 genotype production.[22] This observed is comparable to the consequence proposed by Vicari *et al*., (2015) they explain an important connection in 174 G/C between IL-6 SNP and Sickle cell anemia and GG genotype were more common in patients than in healthy groups.[23] Du *et al*. (2015) showed a significant link between IL-6 rs1800796 and cancer risk, with G allele as a danger allele.[24] Duch *et al*. (2007) discovered that the genotype of GG in Brazilian people is associated with the growth of multiple myeloma.[25] Chua *et al*. (2009) found that the homozygous G genotype was significant in SLE patients. In contrast, the heterozygous G/C genotype was important in the controls, suggesting that the C allele could mask the G allele when both alleles are present in heterozygous patients.[26]

The genotypes frequency in thalassemia patients that infected with HCV were as follow; GG(22.5%), GC(27.5%) & CC(5%) ; while in the thalassemia patients non-infected with HCV is ;GG (32.5%) ,GC (12.5%), CC (0 %), Table 7.

Table 7: Genotype frequency in thala's a emma patient

		Infected with HCV	*Non-infected with HCV*	*OR (95% CI)*	*p-value*
Genotype	GG	9 (22.5%)	13 (32.5%)	0.2663 (0.06995- 1.014)	0.0476*
	GC	11 (27.5%)	5 (12.5%)	2.600 (0.6891 -9.809)	0.1349 N.S
	CC	2 (5%)	0 (0%)	4.512 (0.2030 - 100.3)	0.2962 N.S
Allele frequency	G	29 (72.5%)	31 (77.5%)	0.3118 (0.1005 -0.9672)	0.0330*
	C	15 (37.5%)	5 (12.5%)	3.207 (1.034 - 9.947)	0.0330*

Distribution of genotypes and alleles of in IL-6 -174 G\C of Thalassemia patients with HCV.

IL-6 is a pleiotropic cytokine that uses various processes to exercise its complicated biological activities.[27] In a variety of chronic diseases[28] as well as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma,[29] elevated concentrations of IL-6 were correlated with morbidity and disease activity. IL-6 is a well-known pro-inflammatory cytokine with pro-tumorigenic potential[30] and emerges as a main regulatory signal in the growth of the newly defined pro-inflammatory T-cell subset of T-cells, established as Th17 cells.[31] Also correlated with greater levels of liver necroinflammation[32] and fibrosis[33] was the IL-6 rs1800795 G allele.

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