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Genetic Polymorphism of TLR5 and TLR6 in Iraqi Patients with Heart Failure Disease

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Abstract

In spite of the high rate of morbidity and mortality heart failure (HF) is common, and none of the medications are now entirely available for HF treatment. In addition to many environmental influences and clinical diseases, genetic factors may also contribute to the progression and development of HF. In the current study, samples of blood were collected from 150 heart failure patients and 130 healthy controls. We evaluated the association of four single nucleotide polymorphisms (snps) of Toll-like receptors (TLR6 and TLR5) with (HF) susceptibility in the Iraqi population. In this work, (SNP) called Toll-like receptor 5 (rs5744168, rs2072493) and Toll-like receptor 6 (rs1039559, rs5743810) were employed. (PCR-RFLP) for snps (rs5744168, rs2072493, and re5743810), and sequencing for snps were used to assess the allele and genotype frequencies in both the patient and control groups (rs1039559). In patients with heart failure and healthy controls, a significant difference was discovered in the genotypic and allelic frequencies of snps. From our results, we suggest that these snps act as a potential predisposing factor for HF development.

Keywords: Heart failure, toll-like receptors, snps, polymorphism

تعدد الأشكال الوراثي لـ TLR5 و TLR6 في المرضى العراقيين المصابين بمرض قصور القلب

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الخلاصه

على الرغم من الارتفاع الشائع جدا لوفيات مرضى قصور القلب شائع جدا ، الا انه لا يتوفر أي من الأدوية الآن لعلاجه بالكامل .اضافة إلى العديد من التأثيرات البيئية والأمراض السريرية ، قد تساهم العوامل الوراثية أيضًا في تقدم وتطور قصور القلب. في الدراسة الحالية ، تم جمع عينات من الدم من 150 مريضا بقصور القلب و 130 من الاصحاء. قمنا بتقييم ارتباط أربعة أشكال متعددة من النوكليوتيدات المفردة (SNPs) من المستقبلات الشبيهة بالرصد (TLR6 و TLR5) مع قابلية الإصابة بأمراض قصور القلب (HF) في السكان العراقيين. في هذا العمل ، تم استخدام أشكال متعددة من النوكليوتيدات المفردة (SNPs)

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تسمى مستقبلات شبيهة بالتول رقم 5 (rs5744168، rs2072493) ورقم 6 (rs1039559، rs5743810). تم استخدام تعدد الأشكال (PCR–RFLP) من أجل rs5744168) SNPs و rs2072493 و re5743810) وتسلسل SNPs لتقييم ترددات الأليل والنمط الجيني في كل من مجموعات المرضى والسيطرة (rs1039559). في المرضى الذين يعانون من قصور القلب والضوابط الصحية ، تم اكتشاف فرق كبير في التواتر الوراثي والأليلي للنيوكلوتايد. من النتائج نقترح أن هذه النيوكلوتايد قد تعمل كعامل مساعد محتمل لتطوير مرض قصور القلب.

1. Introduction

Heart failure (HF) is a complicated clinical condition that occurs when the heart is unable to pump enough blood to sustain blood flow, either owing to structural or functional ventricular filling or blood ejection dysfunction. Globally, HF affects millions of patients and is a leading cause of morbidity and death [1, 2].

Heart failure is classified by the American Heart Association into three types: congestive, right-sided, and left-sided. The first form is diastolic HF, which happens when the left ventricle is unable to relax correctly, preventing the heart from getting the necessary quantity of blood during the break between beats. The second kind is systolic HF, which occurs when the left ventricle loses its ability to contract consistently, decreasing the heart's ability to aggressively pump blood into circulation. While right-sided HF is primarily caused by the right ventricle's inability to adequately fill with or inject blood. Congestive heart failure (HF) is a disorder in which the heart beats more slowly than normal, causing blood to back up in the veins as it returns to the heart, creating congestion in the body's tissues such as the arms, legs, ankles, feet, and lungs. A multitude of disorders that affect the heart muscle may cause HF. According to epidemiological research, ischemic heart disease (IHD) is the most common illness [3].

Other factors that contribute to the progression of HF include infection, excessive alcohol consumption, metabolic syndrome, atherosclerotic disease, myocarditis, dilated cardiomyopathy (DCM), cardiomyopathy of unknown cause, hypertension, atrial fibrillation, and cardiomyopathy due to inflammation [1, 4].

Inflammation has a significant role in the development of HF and myocardial ischemia. More study is being conducted to investigate the involvement of Toll-like receptors (TLRs) in HF caused by inflammation. Toll-like receptors (TLRs), which belong to PRRs, are subjected to the release of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) to generate innate immune responses. More and more emerging data indicate that TLR signalling pathway molecules are involved in the progression of HF.. [5].

TLRs are extracellular, transmembrane, and intracellular transmembrane glycoproteins of type I. Too far, 13 TLRs have been identified in mammals, including 10 in humans and 12 in mice [6, 7]. TLRs are classified into two categories depending on their location inside the cell: TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11 are located on the plasma membrane, while TLR3, TLR7, TLR8, and TLR9 are found in endosomes [8, 9]. There is evidence that TLR-mediated innate immune activation in the heart contributes to HF [10, 11]. TLRs are found in many different kinds of cardiac cells, including cardiomyocytes, smooth muscle cells, and endothelial cells [12, 13]. In addition to exhibiting varied symptoms, these TLRs play various functions in the development of HF. According to researchs, TLR2, 4, 5, 6, 7, 8,

and 9 gene polymorphisms have been connected to the onset of infections, malignancies, and autoimmune inflammatory disorders [14-16].

Two pathways have been identified for TLR4 signaling. The first pathway is dependent on myeloid differentiation primary response gene 88 (MyD88). Activation of this pathway leads to the activation of the mitogen-activated protein kinase cascade and nuclear factor kappa B, which induces the expression of proinflammatory cytokines [17].

The TLR5 gene, which includes six exons, is found on human chromosome 1's long arm (hCh1q). There are currently nine known polymorphisms in the promoter and coding regions of the gene. These mutations have been shown to disrupt TLR5 expression and flagellin binding, rendering patients more vulnerable to infections and inflammatory disorders [18]. TLR-6, on the other hand, has been shown to collaborate with TLR-4 to mediate inflammatory responses in response to oxidized low-density lipoprotein (oxLDL) activation [19]. As a result, TLR-4- and TLR-6-knockout mice both had reduced amounts of tissue factor created by a hypercholesterolemic diet as compared to control mice [20]. In hypertensive women, the TLR-6 SNP Pro249Ser has also been associated with decreased left ventricular wall thickness and inflammatory response [21]. Furthermore, it has been proven that the TLR-6 Ser allele reduces IL-6 production in response to TLR-6 agonist activation [22].

In this study, we will focus on the roles of TLR5 and TLR6 SNPs in heart failure illness and their effect as either a protection or risk factor for HF patients.

2. Materials and Methods

2.1 Study Subjects

Research was carried out on 150 HF patients and 130 controls of Iraqi population, patient samples were obtained from the Department of Cardiology of Ibn Sina hospital in Baghdad, Iraq, from March to June/ 2022. Age and sex were matched between samples. Electrocardiograms (ECG), echocardiograms, cardiac CT scans, and blood tests were used to make the diagnosis. Patients with other types of diseases were excluded.

2.2 Genomic DNA Isolation

In vacutainer (BD, NJ, USA) containing anticoagulant K2EDTA solution blood samples were collected by using venipuncture in vein. From peripheral blood leucocytes total genomic DNA was isolated following the manufacturer’s protocol (Sigma/ USA).

2.3 Primer Sequence

References [23,24] were used. Table 1 lists the sequence of the primer set, the gene names, the SNP names, the sizes of the PCR products, and the matching restriction enzymes employed for RFLP. All of the primers were commercially produced by (Macrogen Corporation - Korea).

Table 1: Detail of PCR primers of TLR5 and TLR6 used in this study.

Gene	SNP name	alleles	Forward/Reverse primer sequence (5'-3'	PCR product size bp	Restriction enzyme
TLR5	R392X rs 5744168	C>T	F-GGTAGCCTACATTGATTTGC R-GGATTCTCTGAAGGGGTTTAT	346	Dde1
TLR5	N592S rs2072493	A>G	F-GACTAAGCCTCAACTCCAACA R-GACTTCCTCTTCATCACAACC	314	Mun1
TLR6	S249P rs5743810	C>T	F-CTAGTTTATTCGCTATCCAAG R-TTGTCAATGCTTTCAATGTCG	312	Eco47I
TLR6	rs1039559* Intron	T>C	F-GTGGTTGTGTGTTTTGACCTGT R-TGTTGGATCACTTTCTCAATGC	452	-------

PCR = Polymerase chain reaction, TLR = Toll-like receptor, bp = Base pairs, SNP = Single nucleotide polymorphism.* sequencing was used for the SNP rs1039559 of TLR.

2.4 Polymerase Chain Reaction (PCR)

The PCR reaction was performed in a total volume of (20 µl), and the PCR cycle parameters are indicated in Table 2. Electrophoresis on 1.2% agarose gel was used to separate the PCR products and the ladder marker. After staining with ethidium bromide, the gel was seen under Gel documentation - UV trans-illuminator after 1.5 hours of electrophoresis at 70 V. The molecular size of the bands was estimated using a DNA ladder (100bp DNA ladder) (Sigma/ USA).

Table 2: PCR Program for *TLR5* and *TLR6* genes

Steps	Temperature (°C)	Time	
Initial Denaturation	94	5 min	
Denaturation	94	30sec.	(35 cycle)
Annealing	*	40 -60 sec.	
Extension	72	40sec	
Final Extension Step	72	5-8min	

* The annealing temperature was chosen based on the melting temperature of each primer.

2.5 Restriction Fragment Length Polymorphism (RFLP)

PCR product was digested overnight with a suitable restriction enzyme. After digestion, electrophoresis was carried out using 1X gel loading buffer (GLB). The agarose gel concentration and electrophoresis time were adjusted to correspond to the sizes of the PCR products.

2.6 Sequencing

Ten samples were assessed by the RFLP for conformation in each set of SNPs, and all samples of TLR6 SNP (rs1039559) were sequenced together with their appropriate controls. Sanger sequencing was performed on the amplified PCR fragments using an ABI3730XL automated DNA sequencer (Macrogen Corporation – Korea). After aligning with a reference sequence in the Gene Bank, the findings were provided through email and examined using Geneious software (http://www.geneious.com).

3. Statistical analysis

SPSS software version 17 was used to do statistical comparisons between patients and controls. For allelic frequencies, the Hardy-Weinberg equilibrium was investigated. Furthermore, identified genotypes in both control and patient samples were assessed using a

number of methodologies such as Pearson Chi-Square, Fisher's exact test, Student's t-test, and genotype-phenotype association.

4. Results and Discussion

A total of 150 HF patients and 130 controls enrolled in this study. The PCR-RFLP approach was used to examine single nucleotide polymorphisms in TLR genes. Sequencing was carried out to corroborate RFLP results and to find a nucleotide alteration in the TLR6 SNP (rs1039559).

4.1 Association of *TLR5* gene SNPs

We studied the distribution of alleles and genotypes frequency of TLR5 SNPs genotypes (rs 5744168 and rs2072493) among Iraqi patients with HF and healthy controls in this case-control research. More heterozygotes have been discovered than expected. This shows that (rs 5744168 and rs2072493) SNPs of TLR5 may have some importance for the development of HF.

There are many increased heterozygotes mutant type (CT and AG) of (rs 5744168 and rs2072493) SNPs respectively in HF compared with healthy subjects. While homozygote mutant type (TT and GG) of these SNPs were decreased in HF patients. The distributions of allele and genotype frequencies of (rs 5744168 and rs2072493) SNPs are reported in Table (3). In the case of (rs5744168) SNPs we found there were ~~a~~ highly significant differences between genotype TT in HF patients in comparison with control groups $p< 0.001$. While no significant differences were shown with heterozygote CT $p\geq 0.05$. The frequency of CT genotype in HF was 28.0% and the odd ratio was 1.65 (95%CI=0.92 -2.97) while the frequency of CT genotype in control group was (25.40%). The estimated frequencies TT genotype in HF was (24.67%), while the frequency of TT genotype in control group was (3.84%), and the odd ratio was 9.59 (95%CI=3.48 to 32.54).

The significance analysis was higher in TT genotypes which were ($p< 0.0001$). The significance of such an association was assessed by Fisher`s Exact Probability. Such assessment is ~~more~~ preferred because it allows for correction of probability and ~~it~~ is not affected by small numbers (less than 5). It seems that the disease can occur when the patients have homozygote of mutant TT (odd ratio of (TT) was 9.59) and less than when patients have heterozygote CT (odd ratio was (1.65). This could be reflecting the link between the (T) allele sequence and altered balance in the isoforms and isoforms of TLR5 mRNA (probably with protein). Table 2 shows that the genotype distribution of the rs5744168 polymorphism in HF was not in Hardy Weinberg equilibrium (p 0.05). The (CT+TT) genotype was substantially related to the probability of HF in patients compared to controls in a logistic regression analysis of the rs5744168 SNP (52.66 vs. 26.16%; OR = 3.14; 95% CI = 1.84-5.39; $p =0.001$). Under the recessive model, the TT genotype was also associated with a greater risk (24.66 vs. 3.08%; OR = 5.59; 95% CI = 2.88-10.85; $p= 0.001$). In the overdominant model, no significant changes were discovered (Table 3).

Table 3 shows the prevalence of genotypes and alleles of the TLR5 SNP (rs2072493). Heterozygote mutant type AG and allele G, as well as mutant type GG, were shown to have a significantly significant connection with HF patients (p>0.001). The genotype findings were (AG genotype: OR 2.22, 95%CI 1.18 - 4.18, and p=0.009) and (GG genotype: OR 1.97, 95%CI 1.04 - 3.75, and $p=0.036$) (2).

It seems that the illness may develop when patients are heterozygotes of mutant AG (odd ratio of (AG) was 2.22) and less when patients are homozygotes of mutant GG (odd ratio was (1.97), suggesting that the presence of G allele acts as a risk factor for HF disease. Table 2 shows that the genotype distribution of TLR5 SNPs (rs2072493) in HF patients was not in Hardy-Weinberg equilibrium (p 0.05). The (AG+GG) genotype was significantly linked with the incidence of HF in patients compared to controls in a logistic regression analysis of the rs2072493 SNP (56.66 vs. 38.46%; OR = 2.09; 95% CI = 1.00-3.29; *p= 0.05*). While there were no significant differences between the overdominant and recessive models, as demonstrated in (Table 4).

Table 3: Numbers and percentage frequencies of *TLR5* gene SNPs (rs 5744168 and rs2072493) genotypes and their Hardy-Weinberg equilibrium (HWE) in HF patients compared with control groups

genes	SNPs			patients N=150	HWE p-value	controls N=130	HWE p-value	P- value	OR(95%CI)
TLR5	rs5744168 C > T (Arg392X)	Genotypes	CC CT TT	71(47.33%) 42(28.00%) 37(24.67%)	0.001**	92(70.67%) 33(25.40%) 5(3.84%)	0.358 NS	- 0.09 NS 0.0001**	Ref. 1.65(0.92-2.97) 9.59(3.48-32.54)
		Alleles	C T	184(61.33%) 116(38.16%)		217(83.46%) 43(16.54%)		- 0.0001**	- 3.18(2.10-4.87)
	Rs2072493 A > G	Genotypes	AA AG GG	65(43.33%) 45(30.00%) 40(26.67%)	0.001	80(61.54%) 25(19.23%) 25(19.23%)	0.001	- 0.009** 0.036NS	Ref. 2.22(1.18-4.18) 1.97(1.04-3.75)
		Alleles	A G	175(58.33%) 125(41.67%)		185(71.15%) 75(28.85%)		- 0.002**	- 1.76(1.24-2.51)

NS=Non-significant, * significant at p-value ≤ 0.05, ** significant at p-value ≤ 0.001, 95% CI: 95% confidence interval. Ref. = reference. The genotype-phenotype association was used.

Table 4: logistic regression of TLR5 SNPs in HF patients compared to control

gene	model	genotypes	Patients (n= 150)		Control (n=130)		OR (95%CI)	p-value
			N	%	N	%		
TLR5 rs5744168	Codominant	CC	71	47.33	96	73.84	Reference	-
		CT	42	28.00	30	23.08	6.00(3.30-10.95)	0.09
		TT	37	24.67	4	3.08	21.00(6.75-8505)	**0.0001**
	Dominant	CC	71	47.33	96	73.84	Reference	-
		CT+TT	79	52.66	34	26.16	3.14(1.84- 5.39	**0.000**
	Overdoinant	CC+TT	108	72.00	100	76.92	Reference	-
		CT	42	28.00	30	23.08	1.30(0.73 - 2.32)	0.411
	Recessive	CC+CT	113	75.33	126	96.92	Ref.	-
		TT	37	24.66	4	3.08	5.59(2.88 - 10.85)	**0.000**
	Codominant	AA	65	43.33	80	61.54	Reference	-
		AG	45	30.00	25	19.23	2.22(1.18-4.18)	**0.009**

TLR5 rs2072493		GG	40	26.67	25	19.23	1.97(1.04-3.75)	0.036
	Dominant	AA AG+GG	65 85	43.33 56.66	80 50	61.54 38.46	Reference 2.09(1.2 - 3.48)	- **0.003**
	Overdoinant	AA+GG AG	105 45	70.00 30.00	105 25	80.67 19.23	Reference 1.80(1.00- 3.29)	- 0.039
	Recessive	AA+AG GG	110 40	73.33 26.66	105 25	80.67 19.23	Reference 1.53(0.84 - 2.82)	- 0.157

The analysis was adjusted for age and gender; OR: Odds ratio; CI: Confidence interval; p: Two-tailed Fisher exact probability; Significant p-value is indicated in bold.

4.2 TLR5 linkage disequilibrium and haplotype analysis

A pairwise linkage disequilibrium (LD) analysis was conducted on two TLR 5 gene variations (rs5744168 and rs2072493). The SNP (rs5744168) had a strong LD with (rs2072493) (D'=1, r^2 =0.7) in HF patients, and the haplotype T-G had a higher frequency in HF patients than in controls (0.383 vs. 0.165), which was significant (*p =0.008*). The OR was 3.137, indicating that the T-G haplotype has an impact on increasing the risk of HF. (Table 5) (Figure1).

Table 5: Estimated numbers and frequencies of haplotypes of TLR5 in HF patients and controls. Haplotype rs5744168 and rs2072493 HF patients (n=150) Control (n=130).

Haplotype rs5744168 and rs2072493	HF patients (n=150)		Controls (n=130)		OR	95% CI	p-value
	N	Frequency	N	Frequency			
C-A	175	0.583	185	0.712	0.568	0.399-0.808	0.001
C-G	10	0.033	32	0.123	0.246	0.118-0.510	5.88e*-005
T-G	115	0.383	43	0.165	3.137	2.100-4.687	1.16e*-008

Significant at p-value ≤ 0.001, 95% CI: 95% confidence interval. *exponent.HF: heart failure.

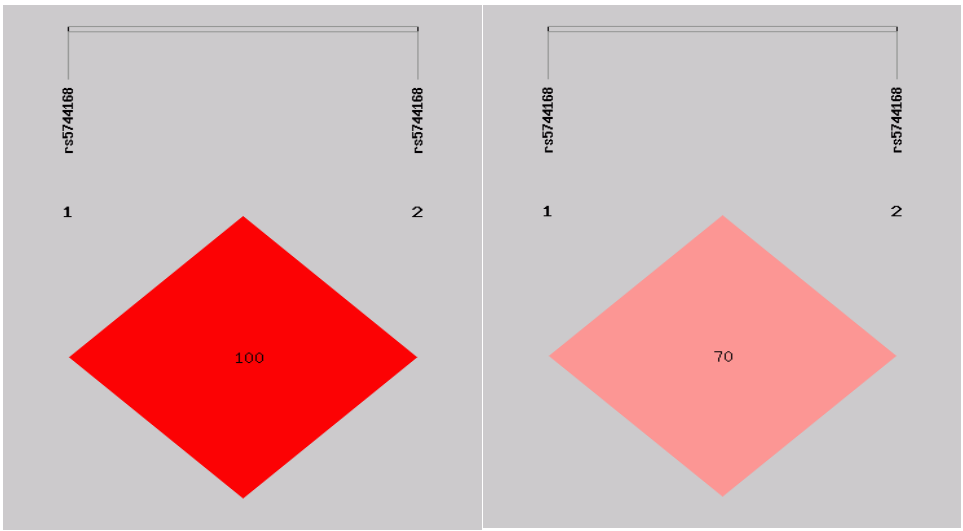

Figure 1: Pairwise linkage disequilibrium coefficient (D') and correlation coefficient (r^2) between TLR5 SNPs (rs5744168 and rs2072493) in HF patients and controls.

Association of *TLR6* gene SNPs

The genotype and allele frequency of the two TLR6 SNPs (rs1039559 and rs5743810) was studied in this investigation. Table 6 shows that the distributions of (rs1039559) SNP did not depart substantially from HWE (P > 0.05), however, rs5743810 SNP did diverge considerably from HWE (P > 0.05). Association analysis indicated significant changes in genotypic and allelic frequencies between the HF and control groups for two TLR6 SNPs (rs1039559 and rs5743810) (Table 4). However, the genotypic frequencies of rs1039559 T/C in the HF group were 26.67%, 50.0%, and 23.33% for TT, CT, and CC, respectively, while in the control group they were 73.84%, 23,08%, and 3.08%, respectively. When compared to healthy controls, the heterozygous CT and homozygote CC genotypes of rs1039559 show a strong correlation with HB (OR = 6.00, 95% CI = 3.30 - 10.95, *P = 0.000* and OR = 21.00, CI = 6.75 - 85.05, *P = 0.000*; see Table 3). Logistic regression analysis of the rs1039559 SNP revealed that (CT and CC) genotypes were significantly associated with the risk of HF in patients compared to controls in overdominant and recessive models (50.00 vs. 23.07%; OR = 3.33; 95% CI = 1.93-5.81; *p= 0.001*) and (23.33 vs. 3.07%; OR = 9.59; 95% CI = 3.26-38.03; While there were no significant changes observed in the dominant model, as demonstrated in (Table 7).

The genotype for rs5743810 was discovered in both groups, with 11.53% of the control group and 31.33% of HF patients having it. Neither the control group nor the sick have the genotype TT. This SNP's T allele was detected in 15.67% of HF patients and 5.77% of healthy controls, with no discernible differences in frequency between the two groups (OR = 1.71, 95%CI = 0.98 — 3.00, *P = 0.063*; Table 3). As indicated in logistic regression analysis Table 4, both genotypes (CT+TT and CT) under dominant and over dominant models of rs5743810 SNPs exhibited the same significant differences compared to controls (31.33 vs. 11.53%; OR = 3.50; 95% CI = 1.97-731; *p= 0.001*). The recessive model was not present in either the patients or the controls in this investigation.

Table 6: Numbers and percentage frequencies of *TLR6* gene SNPs (rs1039559and rs5743810) genotypes and their Hardy-Weinberg equilibrium (HWE) in HF patients compared with control groups

genes	SNPs			patients N=150	HWE p-value	controls N=130	HWE p-value	P-value	OR(95%CI)
TLR6	Rs1039559 T > C	Genotypes	TT CT CC	40(26.67%) 75(50.0%) 35(23.33%)	0.989NS	96(73.84%) 30(23.08%) 4(3.08)	0.39NS	- 0.0001** 0.0001**	Ref. 6.00(3.30-10.95) 21.00(6.75-8505)
		Alleles	T C	155(51.67%) 145(48.33%)		222(85.38%) 38(14.62%)		- 0.0001**	- 5.47(3.56-8.48)
	Rs5743810 C > T	Genotypes	CC CT TT	103(68.67%) 47(31.33%) 0	0.022*	115(88.47%) 15(11.53%) 0.00	0.485NS	- 0.0001** -	Ref. 3.50(1.79-7.13) -
		Alleles	C T	253(84.33%) 47(15.67%)		245(94.23%) 15(5.77%)		- 0.063NS	- 1.71(0.98-3.00)

NS=Non-significant, * significant at p-value ≤ 0.05, ** significant at p-value ≤ 0.001, 95% CI: 95% confidence interval. Ref. = reference

Table 7: logistic regression of TLR6 SNPs in HF patients compared to control

Gene	model	genotypes	Patients (n= 150)		Control (n=130)		OR (95%CI)	*p*-value
			N	%	N	%		
TLR6 rs1039559	Codominant	TT CT CC	40 75 35	26.67% 50.0% 23.33%	96 30 4	73.84% 23.08% 3.08%	Reference 6.00(3.30-10.95) 21.00(6.75-8505)	- **0.0001** **0.0001**
	Dominant	TT CT+CC	40 110	26.66 73.33	96 34	73.84 26.15	Reference 0.97(0.55 - 1.72)	- 1.000
	Overdoinant	TT+CC CT	75 75	50.00 50.00	100 30	76.92 23.07	Reference 3.33(1.93- 5.81)	- **0.000**
	Recessive	TT+CT CC	115 35	76.66 23.33	126 4	96.92 3.07	Reference 9.59(3.26 - 38.03)	- **0.000**
TLR6 rs5743810	Codominant	CC CT TT	103 47 0	68.67% 31.33% 0.00	115 15 0	88.47% 11.53% 0.00	Reference 3.50(1.79-7.13) -	- **0.0001** -
	Dominant	CC CT+TT	103 47	68.66 31.33	115 15	88.46 11.53	Reference 3.50(1.79 - 7.13)	- **0.000**
	Overdoinant	CC+TT CT	103 47	68.66 31.33	115 15	88.46 11.53	Reference 3.50(1.79 - 7.13)	- **0.000**
	Recessive	CC+CT TT	150 0	100	130 0	100	Reference -	-

The analysis was adjusted for age and gender; OR: Odds ratio; CI: Confidence interval; p: Two-tailed Fisher exact probability; Significant p-value is indicated in bold

4.3 TLR6 linkage disequilibrium and haplotype analysis

A pairwise linkage disequilibrium (LD) analysis was conducted on two TLR5 gene variations (rs1039559 and rs5743810). The SNP (rs rs1039559) had weak LD with (rs5743810) (D'=0.9, r^2 =0.25) in HF patients, however, the haplotype T-C was more common in controls than in HF patients (0.845 vs. 0.517), and the difference was significant (p-value=7.55e-015). As seen in Table 8, (Figure2).

Table 8: Estimated numbers and frequencies of haplotypes of TLR6 in HF patients and controls. Haplotype rs1039559 and rs5743810 HF patients (n=150) Control (n=130).

Haplotype rs1039559 and rs5743810	HF patients (n=150)		Controls (n=130)		OR	95% CI	p-value
	N	Frequency	N	Frequency			
C-C	98	0.327	23	0.088	4.999	3.058-8.171	9.46e-012
C-T	47	0.157	15	0.058	3.034	1.653-5.569	0.0001
T-C	155	0.517	222	0.845	0.183	0.121-0.276	7.55e-015

Significant at p-value ≤ 0.001, 95% CI: 95% confidence interval. *exponent. HF: heart failure.

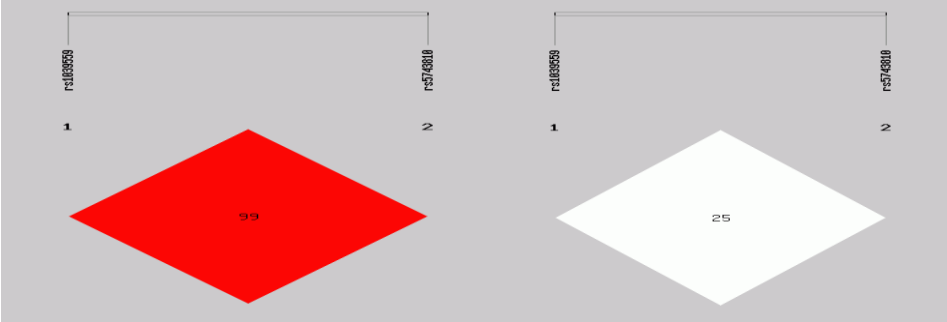

Figure 2: Pairwise linkage disequilibrium coefficient (D') and correlation coefficient (r^2) between TLR6 SNPs (rs1039559 and rs5743810) in HF patients and controls

Discussion

Polymorphisms in multiple genes are thought to be caused by genetic changes in DNA composition [25]. SNPs may result in DNA genetic polymorphisms that change how a gene-encoded protein performs and/or behaves by changing amino acids [26]. For the first time, we provide data on the frequency of the polymorphisms R392X rs 5744168 (28.0%) and rs2072493 N592S (30.0%) in the TLR5 gene, as well as their association with HF patients in the Iraqi population. There hasn't been any previous study on these SNPs in HF patients, however, there have been studies on these SNPs and other illnesses. A study of the Jewish community [27] found that R392X rs 5744168 (TLR5-stop) exhibited 6% and 0.9% heterozygosity in UC and CD patients, respectively, compared to unaffected controls (5.4%). Furthermore, this SNP was shown to be unrelated to cardiac disease (CD) and urinary disease (UC) in non-Jewish groups.

A lower quantity of flagellin (the TLR5 ligand) was identified in R392X heterozygous CD patients using a specific antibody [27]. SNP has previously been connected to an increased prevalence of urinary tract infections in adult women [28]. It can also have an effect on lung function in persons with cystic fibrosis [29]. The N592S rs2072493 SNP in the TLR5 gene was first discovered in the whole Caucasian population [30]. According to a different study, N592S rs2072493 was linked to a lower survival rate for those with colorectal cancer in the German population [31]. We are describing the connection of these SNPs in HF patients from the Iraqi community here for the first time, in accordance with the literature that is currently available.

Lipoproteins are a crucial surface antigen for Gram-negative bacteria that TLR6 can recognize and bind to [32,33]. We postulated that deregulation of the TLR6 gene function may contribute to the emergence of metastatic HF and that the TLR6 gene may be essential for the activation of the innate immune system. The investigation of the relationship between TLR6-associated SNPs and elevated risk of HF development in the Iraqi population was the main goal of the current study. The SNPs investigated, specifically, rs5743810 (C>T), were found in exonic areas of chromosome 4. While chromosome 4's intronic regions include the variant rs1039559 (T>C).

Examining rs5743810 and rs1039559 indicated a correlation between HF risk in this population and the genotypic and allelic frequency distributions of these loci. There hasn't been any conclusive research so far linking these polymorphisms to the onset of HF. As a result, the rs1039559 polymorphism may have an impact on TLR6 expression in the HF

population. However, the minor Pro allele of rs5743810 had a much greater frequency, indicating that it had a strong relationship with the development of HF.

As a downstream gene variant that is found in an exonic region of the gene, the rs5743810 SNP is a downstream gene variant. Due to this localisation, TLR6 expression and function may be impacted by rs5743810. The transition from Serine to Proline in the amino acid causes TLR6 to lose some of its functional capacity and puts people at risk for innate immune system dysregulation, which may lead to disease development. Due to their functions in controlling gene expression, polymorphisms in coding areas are thought to now be relevant to the development of disease. To mediate the cellular response to bacterial lipoproteins and to activate the NF-B pathway and inflammatory processes, TLR6 functionally interacts with TLR2. and consequently, may contribute to disease development and progression [34–35]. However, in silico predictions showed that rs5743810 (Ser249- Pro) had no negative effects, demonstrating that this SNP contains protective mutations [36]. These findings might not match our findings because they suggest that the rs5743810 (Ser249Pro) SNP of the TLR6 gene may have a role in the development of HF.

5. Conclusions

The current research demonstrates that TLR5 and TLR6 polymorphism variations play an important role in crucial HF susceptibility risk in the Iraqi population. All of the SNPs studied in this research may work as a risk factor for the development of HF in the Iraqi population.

6. References

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