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A study of polymorphism and the serum level of Haptoglobin of people suffering from sickle cell disease and/or Hepatitis C

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ABSTRACT

Background. Haptoglobin is a type of alpha2 globulin found in human plasma. Its primary function is to bind to the globin portion of free hemoglobin in the bloodstream .

Objectives. to examine the genetic polymorphism of the Haptoglobin (Hp) gene in patients diagnosed with sickle cell anemia, hepatitis C, and the co-occurrence of sickle cell anemia and hepatitis C .

Patients and Methods. A total of 130 participants were categorized into several groups: 40 patients with sickle cell anemia, 40 patients with hepatitis C, 10 patients with both sickle cell anemia and hepatitis C, and 40 individuals in the control group.The DNA was extracted and the polymerase chain reaction (PCR) was conducted using genotype-specific primers for the three areas of the haptoglobin gene. The genotypes were identified by running electrophoresis on agarose gels and calculating the proportion of each allele that was amplified. After obtaining the sequencing results of the haptoglobin gene and for the studied samples and for the three plots Hp2, Hp 1S and Hp 1F, Sequences for all three segments were registered in the NCBI Genome Bank for the first time locally and some were given independent accession numbers, and it will be considered a database for any future researcher working on the Hp genotypes in Iraq.

Results. In Sickle cell Patient Hp1-1(0.13), Hp2-2(0.55), Hp2-1(0.32), Hepatitis-C Patient Hp1-1(0.13), Hp2-2(0.63), Hp2-1(0.24), Sickle cell with Hepatitis C Patient Hp1-1(0.20), Hp2-2(0.60), Hp2-1(0.20), and Control group Hp1-1 (0.30), Hp2-2 (0.50), Hp2-1 (0.20).
Hp2-2 was the most common phenotype across all categories, The T allele and TT genotype seem more visible in sickle cell anemia and hepatitis diseases than the AA genotypes of Hp-2 643 T>, and no correlation between patients and health control group with Hp1S gene, Also, the present study reports that there is no correlation between patients of sickle cell anemia and health control group with Hp-1F 867 G > A gene, but the G allele and GG genotype more visible in hepatitis diseases than the AA genotypes of Hp-1F 867 G > A gene.

Conclusions. The Hp2-2 haptoglobin phenotype was the most common among patients. Different groups may have an influence on the development of sickle cell anemia and hepatitis C.

Keywords: Polymorphism, Haptoglobin Gene, Sickle Cell Diseases, Hepatitis C

Introduction

With an estimated 20–25 million affected individuals globally, sickle cell disease (SCD) is the most common monogenic disorder and a major public health concern. SCD is a group of related autosomal recessive disorders [1], occurs when there are multiple mutations in the gene that codes for the β-globin subunits of hemoglobin (HBB). This leads to the creation of aberrant molecules of hemoglobin, which can then polymerize and generate sickle-shaped red blood cells [2]. As a result, the body breaks down red blood cells at an accelerated rate, a condition known as hemolytic anemia [3].

In many developed countries, hepatitis C virus (HCV) infections are the leading cause of chronic liver disease. Cirrhosis and hepatocellular carcinoma are two forms of liver cancer that can develop from chronic hepatitis C virus infection; however, some people eliminate the virus on their own [4]. Clinical manifestation of HCV-related liver disease can be influenced by a combination of factors, including environmental factors, viral variables, and an individual's susceptibility to liver damage [5]. When considering HCV infection, the host's genetic makeup is likely to have a major influence. Indeed, there is strong evidence from a number of studies that look at disease relationships that several genes are involved in HCV infection [6]. One kind of alpha2 globulin that is present in human plasma is hemoglobin. The main thing it does is attach to the globin part of free hemoglobin in the blood.[7]. Since the protein is composed of two subunits that are expressed by separate genes, three separate phenotypes can be identified: Hp1–1, Hp2-1, and Hp 2-2. As a positive acute phase protein, haptoglobin can interact with hemoglobin and perform its tasks. Among the Hp phenotypes, there were functional variances [8]. While the Hp 2-1 and Hp 2-2 haptoglobin phenotypes have been linked to antibody-like characteristics, the Hp 1-1 phenotype shows a higher ability to bind to hemoglobin than the other phenotypes. Because of their capacity to suppress prostaglandin synthesis, these traits are also acute phase reactants [9].

Research suggests that there may be an increased risk of disease-related consequences for SCD patients who have HP2-2.[10]. Although there is conflicting evidence on the effects of the HP 1-1 genotype on neurological and cardiovascular outcomes in SCD, some studies have found that this genotype protects against kidney damage and cardiovascular disease, while others have found the opposite to be true [11]. Additionally, there is evidence linking some Hp polymorphisms to hepatitis C; this finding raises the possibility that the Hp phenotype affects how the disease develops clinically [12].

The half-life of serum haptoglobin is about five days under typical conditions. Haptoglobin levels in serum are low or nonexistent when free hemoglobin is present, as in intravascular hemolysis, because the monocyte-macrophage system quickly clears the serum of the hemoglobin-haptoglobin complex [13]. Another mechanism by which the hemoglobin-haptoglobin complex prevents injury to the renal tubules is by blocking hemoglobin escape through the glomerulus [14]. Laboratory markers for the diagnosis of hemolytic anemia include serum haptoglobin testing, which reveals the rate of decline in the amount of free plasma hemoglobin (normal levels range from 36 to 195 mg/dL) [7]. Serum Hp levels was shown to be lower in a healthy control group in relation to sickle cell anemia and hepatitis C virus infection, according to recent investigations [15].

Aim is to examine the genetic polymorphism of the Haptoglobin (Hp) gene in patients diagnosed with sickle cell anemia, hepatitis C, and the co-occurrence of sickle cell anemia and hepatitis C.

Materials and Method

Blood Samples

Each patient and healthy control had a venous blood sample obtained from them, which was five milliliters in volume. In order to extract genome DNA, blood samples were divided into aliquots of two milliliters each and placed in EDTA tubes. Additionally, three milliliters of venous blood samples were collected from each patient who appeared to be in good health. These samples were then placed in clot activator and gel serum separation tubes and allowed to stand at room temperature. The serum was separated by centrifugation at a speed of 300 revolutions per minute for ten minutes. After that, it was transferred into Eppendroff tubes, stored at a temperature of -20 degrees Celsius, and stored for serological analysis.

DNA Extraction and Haptoglobin Genotyping

The extraction of genomic DNA from blood isolates was performed using the Geneaid Genomic DNA extraction Kit from Taiwan.

DNA Template and Polymerase Chain Reaction

The polymerase chain reaction (PCR) technique was employed to amplify the haptoglobin gene, following the methodology described in reference [10]. The PCR technique utilized the specified primers. The primers, both forward and reverse, were provided by Geneaid Genomic DNA extraction Kit firm (Taiwan) as part of their kit (Table 1).

Table (1) The product names, primers used, annealing temperature and the products size for Haptoglobin gene.

Product Name	Product size (bp)	AT	Primer Name	Oligonucleotide sequence (5'-3')
Hp2	935	58	**F3**	CAGGAGTATACACCTTAAATG
			C42	TTACACTGGTAGCGAACCGA
Hp1S	1200	58	**C51**	GCAATGATGTCACGGATATC
			S2	TTATCCACTGCTTCTCATTG
Hp1F	1400	58	**F3**	CAGGAGTATACACCTTAAATG
			C72	AATTTAAAATTGGCATTTCGCC

The primers (F, R), distilled water (D.W), and DNA were combined in a master mix tube, resulting in a total volume of 13μl. The samples underwent three reactions (1, 2, and 3) and the genotypes were determined by analyzing the amplified fragment for each allele using electrophoresis on a 1.5% agarose gel. The results are summarized in table 2

Table (2) PCR condition for amplification of HP gene**.**

Steps		temp	Time	Cycle
Initial denaturation		5.00 min	95	1 time
	Denaturation	0.30 sec.	95	35 cycles
	Annealing	0.45 sec.	58	
	Extension	1.0 min	72	
Final Extension		10.0 min	72	1 time

Sequencing and Alignment of Gene

The PCR products amplified with two primers were sequenced by Macrogen Company (South Korea).

NCBI BLAST tool was used to check the results of this program, and to detect any DNA alterations in the sequences of the gene.

Measurement of Serum Haptoglobin Abundance

The concentration of serum Hp was quantified using an ELISA test (PARS BIOCHEN, China) following the directions provided by the manufacturer. The absorbance readings were measured using a microplate reader at a wavelength of 450 nm.

Statistical analysis

The data analyses were presented as the mean value plus or minus the standard deviation (SD). The comparisons between each patient and healthy control group were conducted using one-way ANOVA, in conjunction with the Least Significant Differences (LSD) method, to determine the significant differences among the means. A value of $P \leq 0.05$ is deemed to be statistically significant. Odds Ratio (online). The statistical analyses were conducted using the SPSS programmed, namely the Statistical Package for Social Sciences (version-20), on a computer. The sequencing was analyzed using the Blast programmed provided by NCBI.

Results

Molecular Analysis

Comparison of Hp Types' Frequencies among Various Groups

Table (3) displays the distribution of the haptoglobin phenotype across the patient groups and the control group. It reveals that the HP (2-2) phenotype is the most common among all groups. The proportions of different phenotypes were as follows: Sickle cell Patient HP1-1 (0.13), HP2-2 (0.55), HP2-1 (0.32); Hepatitis Patient HP1-1 (0.13), HP2-2 (0.63), HP2-1 (0.24); Sickle cell with Hepatitis Patient HP1-1 (0.20), HP2-2 (0.60), HP2-1 (0.20); and healthy control HP1-1 (0.30), HP2-2 (0.50), HP2-1 (0.20). The results indicate that there was no significant difference in the distribution patterns between patients and healthy individuals. The Hp2-2 phenotype was the most prevalent among all groups. The group of individuals with hepatitis C Hp2-2 had the highest prevalence (0.63) compared to other patient categories.

Table (3): Distribution of types of the Haptoglopin gene and Type Frequencies

Groups	Type	Type Frequency
SCA Patient	Hp1-1	0.13
	Hp2-2	0.55
	Hp2-1	0.32
HCV Patient	Hp-1-1	0.13
	Hp2-2	0.63
	Hp2-1	0.24
SCA and HCV Patient	Hp1-1	0.20
	Hp2-2	0.60
	Hp2-1	0.20
Healthy Control	Hp1-1	0.30
	Hp2-2	0.50
	Hp2-1	0.20
p. value		0.61

Analysis sequencing with NCBI Blast

After obtaining the sequencing results of the haptoclobin gene and for the studied samples and for the three plots HP2, HP 1S and Hp 1F, some sequences for all the three segments were recorded in the NCBI Genome Bank for the first time locally under independent accession numbers, and it will be considered a database for any future researcher working on the Hp genotypes in Iraq Table (4).

Table (4): Sequence analysis of amplified product (Hp2 "935 bp", Hp 1S"1200 bp" and Hp 1F "1400 bp"

		Group								References from NCBI (Accession Numbers)
		sickle cell		Hepatitis		sickle cell & Hepatitis		Control		
PCR product	Mutations	Genotypes	N	Genotypes	N	Genotypes	N	Genotypes	N	
Hp-2 Product Size (935 bp)	**643 T>A**	TT	**3**	TT	**7**	TT	**2**	TT	**2**	AH003344, KT923782, KT923758, X00606 , DQ314870, M69197
		AA	**1**	AA	**1**	AA	**0**	AA	**1**	CP034494, AC277990, KT923760, AP023476, NG_012651
Hp-1S Product Size (1200 bp)	**442 G>C**	GG	**4**	GG	**6**	GG	**2**	GG	**2**	AH003142, AH003344, CP068262, X00606, DQ314870, M69197,
		CC	**1**	CC	**1**	CC	**0**	CC	**0**	CP034494, AC277990, AP023476, NG_012651 442. G>C Mutation Find in 1 sample (Hepatitis patient) in this study LC742140
	561 G>C	GG	**3**	GG	**4**	GG	**2**	GG	**2**	AH003142, AH003344, CP068262, X00606, DQ314870, M69197
		CC	**1**	CC	**1**	CC	**0**	CC	**0**	CP034494, AC277990, AP0234fr76, NG_012651
Hp-1F Product Size (1400 bp)	**867 G>A**	GG	**3**	GG	**6**	GG	/	GG	**2**	CP034494, AC277990, AH003344, CP068262, AP023476, NG_030311, NG_012651, DQ314870, M60197
		AA	**1**	AA	**0**	AA	/	AA	**0**	**867. G>A Mutation Find in 1 sample (sickle cell patient) in this study LC742148**

Genomic DNA extraction

Genomic DNA was obtained from blood isolates of patients and controls using the Geneaid Genomic DNA extraction Kit from Taiwan. All samples exhibited bands, which indicated the presence of genomic DNA on the gel electrophoresis.

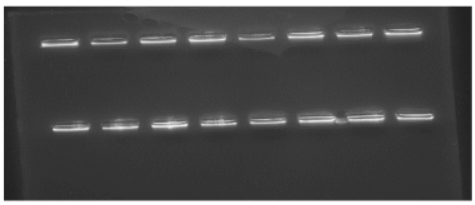

Fig.1 The DNA isolated from the complete blood sample was subjected to gel electrophoresis. The fragments were separated by electrophoresis on a 1% agarose gel using 1X TAE buffer at a voltage of 90V for 1 hour

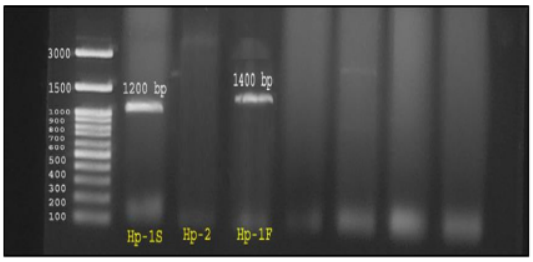

Fig2: Electrophoresis of the amplified HP gene using Hp1-1 agarose gel was performed. After being stained with ethidium bromide, the bands were visualised under ultraviolet light after being fractionated by electrophoresis on a 1.5% agarose gel for one hour at 80V/cm. (M: ladder) ranges from 100 to 3000 bp

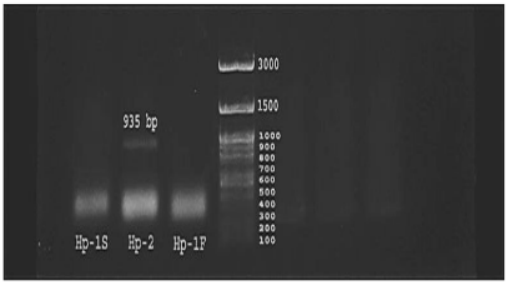

Fig3: An Agarose gel electrophoresis test was used to look at the enlarged HP gene. Electrophoresis was used to separate the bands on a 1.5% agarose gel for one hour at 80V/cm. The bands could then be seen under UV light after being stained with ethidium bromide. Ladder (L: 100 to 3000bp)

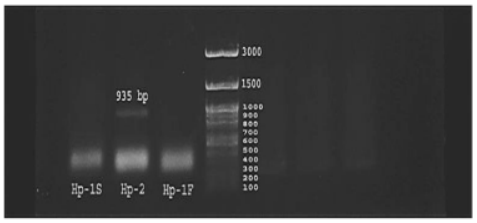

Fig4: Electrophoresis on an HP 2-1 agarose gel for the amplified HP gene. After electrophoresis on a 1.5% agarose gel for 1 hour at 80 V/cm, the bands were stained with ethidium bromide and then visible under ultraviolet light. (M: ladder, L: 100–3000 bps)

Frequency of genotypes for the Haptoglobin (HP2) (643T>A) gene samples of sickle patients and healthy control.

The current study showed that the TT genotype was common in patients and control group (OR=1.5, CI 95% 0.0554 - 40.6353). 0% of patients and control group with TA genotype (OR=0.7778, CI 95% 0.0121 - 49.9059). However, 25% and 33.33% of patients and control group, respectively have AA genotype (OR=0.6667, CI 95% 0.0246 - 18.0601), as the table (5).

Table (5): The genotypes for the HP2 gene (643T>A) were analyses in samples from both SCD patients and healthy controls

Genotypes	Patients No. (%)	Control No. (%)	OR	95 % CI	P-Value

TT	3(75%)	2(66.6%)	1.5	0.0554 - 40.6353	0.8096
TA	0(0%)	0(0%)	0.7778	0.0121 - 49.9059	0.9058
AA	1(25%)	1(33.33)	0.6667	0.0246 - 18.0601	0.8096
Total	4	3			

Frequency of genotypes for the Haptoglobin (HP2) (643 T>A) gene samples of hepatitis C patients and healthy control

The results of this investigation showed that both the control group and patients had the TT genotype as the most common (OR=7.0, CI 95% 0.3972 -123.3538). (87.5%) of patients and the control group had the TA genotype (OR=0.4118, CI 95% 0.0067-25.1686). Nonetheless, the AA genotype is present in (12.5%) of patients and 33.33% of the control group, respectively (OR=0.2857, CI 95% 0.0118 - 6.9142), table (6).

Table (6): The genotypes for the HP2 gene (643 T>A) were analyses in samples from both HCV patients and healthy controls

Genotypes	Patients No. (%)	Control No. (%)	OR	95 % CI	P-Value
TT	7(87.5%)	2(66.66%)	7.0	0.3972 -123.35	0.1837
TA	0(0%)	0(0%)	0.4118	0.0067 - 25.168	0.6724
AA	1(12.5%)	1(33.33)	0.2857	0.0118 - 6.91	0.4409
Total	8	3			

Frequency of genotypes for the Haptoglobin (HP2) (643T>A) gene samples of sickle cell with Hepatitis C patients and healthy control

The results of the table (7) showed that the TT genotype represented 100% in sickle cell anemia with Hepatitis C patients (OR=3.0, CI 95% 0.0780-115.3457), and that the percentage of theTA%0 in patients and control (OR=1.4, CI 95% 0.0201 - 97.4353), AA genotypes was 0% and 25% in patients and the control group, respectively (OR=0.33, CI 95% 0.0087 - 12.8162).

Table (7): Distribution of genotypes for the Hp-2(643 T>A) gene samples of SCD with HCV patients and healthy Control

Genotypes	Patients No. (%)	Control No. (%)	OR	95 % CI	P-Value
TT	2(100%)	2(66.66%)	3.0	0.078 - 115.34	0.55
TA	0(0%)	0(0%)	1.4	0.020 - 97.43	0.87
AA	0(0%)	1(33.33)	0.33	0.008 - 12.81	0.55
Total	2	3			

Frequency of genotypes for the Haptoglobin (Hp-1S) (442 G >C) gene samples of sickle patients and healthy control

According to the current study, both the patients and control group had the GG genotype 80% and%100 respectively (OR=0.6000, 95% CI 0.0172 - 20.9826). 0% of patients and the control group had the GC genotype (OR=0.4545, 95% CI 0.0068 - 30.1733). As for the CC genotype, it is represented in 20% of patients with sickle cell anemia and 0% in control group respectively (OR=1.6667, 95% CI 0.0477 - 58.2849).

Table (8): The genotypes for the Hp-1S gene (442 G > C) were analyses in samples from patients with sickle cell disease (SCD) and healthy control individuals

Genotypes	Patients	Control	OR	95 % CI	P-Value

	No. (%)	No. (%)			
GG	4(80%)	2(100%)	0.60	0.017 - 20.98	0.778
GC	0(0%)	0(0%)	0.45	0.006 - 30.17	0.712
CC	1(20%)	0(0%)	1.66	0.047 - 58.28	0.778
Total	5	2			

Frequency of genotypes for the Haptoglobin (Hp-1S) (44 G >C) gene sample s of Hepatitis C patients and healthy control.

Table (9) indicates that the GG genotypes Hp-1S gene (442 G >C) in HCV patients and control group were 85% ,100% respectively(OR = 0.866795% CI 0.0257 - 29.2031), while GC and CC genotypes were 0% in both patients and control group (OR = 0.333395% CI 0.0051 - 21.6401) and (OR = 1.153895% CI 0.0342 - 38.8798), respectively.

Table (9): The genotypes for the Hp-1S gene (442 G>C) were analyses in samples from both HCV patients and healthy control subjects

Genotypes	Patients No. (%)	Control No. (%)	OR	95 % CI	P-Value
GG	6(85%)	2(100%)	0.8667	0.0257 - 29.20	0.936
GC	0(0%)	0(100%)	0.3333	0.0051 - 21.64	0.605
CC	1(15%)	0(100%)	1.1538	0.0342 - 38.87	0.936
Total	7	2			

Frequency of genotypes for the Haptoglobin Hp-1S (442G>C) gene samples of sickle cell with hepatitis C patients and control.

In the table (10) it was shown that the GG genotype represented 100% in both patients and control group (OR = 1.00, 95% CI 0.0136 - 73.2695), and that the GC and CC genotype represented %0 in patients and control group (OR = 1.00 95% CI 0.0136 - 73.2695) in both.

Table (10): Distribution of genotypes for the Hp-1S (442 G>C) gene samples of SCD with HCV patients and healthy control

Genotypes	Patients No. (%)	Control No. (%)	OR	95 % CI	P-Value
GG	2(100%)	2(100%)	1.0	0.0136 - 73.26	1.0
GC	0(0%)	0(0%)	1.0	0.0136 - 73.26	1.0
CC	0(0%)	0(0%)	1.0	0.0136 - 73.26	1.0
Total	2	2			

Frequency of genotypes for the Haptoglobin (Hp-1S) (561G>C) gene samples of sickle patients and healthy control

According to the current study, both the control group and the patients had the GG genotype of 75%, 100% respectively (OR=0.4667, 95% CI 0.0129 - 16.8867), while, GC genotype in patients and the control group were 0% respectively (OR=0.5556, 95% CI 0.0082 - 37.5655). As for the CC genotype were 25% and 0% of patients and control, respectively (OR=2.1429, CI 95% 0.0592 - 77.5408), Table (11).

Table (11): The genotypes for the Hp-1S gene (561 G > C) were analyses in samples from patients with sickle cell disease (SCD) and healthy control subjects

Genotypes	Patients No. (%)	Control No. (%)	OR	95 % CI	P-Value
GG	3(75%)	2(100%)	0.46	0.0129 - 16.88	0.677
GC	0(0%)	0(0%)	0.55	0.0082 - 37.56	0.784
CC	1(25%)	0(0%)	2.14	0.0592 - 77.54	0.677
Total	4	2			

Frequency of genotypes for the Haptoglobin (Hp-1S) (561 G >C) gene samples of Hepatitis patients C and healthy control

The results of the current study demonstrate the GG Genotypes were common in 80% and 100% in patients and Control group respectively (OR=0.60, 95% CI 0.0172-20.98) and 0% of GC Genotypes in patients and Control group (OR=0.4545,95%CI 0.0068-30.17), while 25% and 0%were CC Genotypes respectively (OR=1.6667,95%0.0477-58.28) according to Table (12).

Table (12): The genotypes for the Hp-1S gene (561 G > C) were analyses in samples from both HCV patients and healthy control subjects

Genotypes	Patients No. (%)	Control No. (%)	OR	95 % CI	P-Value
GG	4(80%)	2(100%)	0.60	0.0172 - 20.98	0.778
GC	0(0%)	0(0%)	0.454	0.0068 - 30.17	0.712
CC	1(20%)	0(0%)	1.666	0.0477 - 58.28	0.778
Total	5	2			

Frequency of genotypes for the Haptoglobin (Hp-1S) (561 G >C) gene samples of sickle cell with Hepatitis C patients and healthy control.

In the table (13) it was shown that the GG genotype represented 100% in both patients and control group (OR = 1.00, 95% CI 0.0136 - 73.2695), and that the GC and CC genotype represented %0 in patients and control group (OR = 1.00 95% CI 0.0136 - 73.2695).

Table (13): Distribution of genotypes for the Hp-1S (561 G>C) gene samples of SCD with HCV patients and healthy Control

Genotypes	Patients No. (%)	Control No. (%)	OR	95 % CI	P-Value
GG	2(100%)	2(100%)	1.0	0.0136 - 73.26	1.0
GC	0(0%)	0(0%)	1.0	0.0136 -73.26	1.0
CC	0(0%)	0(0%)	1.0	0.0136 - 73.26	1.0
Total	2	2			

Frequency of genotypes for the Haptoglobin (Hp-1F) (867 G >A) gene samples of sickle patients and controls.

The GG genotype was the most represented in patients at 75% and 100% in the control group (OR = 0.4667 95% CI 0.0129 -16.8867), while the GA genotype was 0% in both patients and controls (OR = 0.5556 ,95% CI 0.0082 - 37.5655), while the AA genotype was 25% in patients and 0% in the control group (OR = 2.1429,95% CI 0.0592 - 77.5408), table (14).

Table (14): The genotypes for the Hp-1F gene (867 G > A) were distributed among samples of SCD patients and healthy controls

Genotypes	Patients No. (%)	Control No. (%)	OR	95 % CI	P-Value
GG	3(75%)	2(100%)	0.46	0.0129 -16.88	0.67
GA	0(0%)	0(0%)	0.55	0.0082- 37.56	0.78
AA	1(25%)	0(0%)	2.14	0.0592- 77.54	0.67
Total	4	2			

Frequency of genotypes for the Haptoglobin (Hp-1F) (867 G >A) gene samples of hepatitis C patients and controls.

The GG genotype was found to be 100% present in both the control group and the patients (OR = 2.6000,95% CI 0.0397 - 170.3919), while the GA and AA genotypes were found to be 0% present in both the control group and the patients (OR = 0.3846,95% CI 0.0059 - 25.2059) in both, Table (15).

Table (15): Distribution of genotypes for the Hp-1F gene (867 G >A) samples of HCV patients and healthy Control

Genotypes	Patients No. (%)	Control No. (%)	OR	95 % CI	P-Value
GG	6(%100)	2(%100)	2.60	0.0397- 170.39	0.65
GC	0(%0)	0(%0)	0.38	0.0059 - 25.20	0.65
CC	0(%0)	0(%0)	0.38	0.0059 - 25.20	0.65
Total	6	2			

Immunological study

Determination of Hp concentration between patients and controls and Association with haptoglobin genotypes

The findings, as shown in Table (16), demonstrated a statistically significant reduction ($P < 0.05$) in the haptoglobin concentration of patients with three different types of haptoglobin compared to the healthy control group. However, there was no significant difference in the haptoglobin concentration across the patient groups. Based on the different variants of haptoglobin the statistical analysis presented in Table (16) revealed a noteworthy reduction ($P \leq 0.05$) in the serum haptoglobin concentration among Hepatitis C patients with the HP1-1 genotype, as compared to those with the HP2-1 and HP2-2 genotypes. However, no significant differences were observed between the various haptoglobin types in the control group and other patient groups.

Table (16): Examining the correlation between haptoglobin types and serum Hp levels in both patients and controls

Type of Hp Group	HP 1-1 (M±SD)	HP2-2 (M±SD)	HP2-1 (M±SD)	LSD
SCA	5.68 ± 0.46 Ba	5.86 ± 0.41 Ba	5.83 ± 0.45 Ba	0.18
HCV	5.33 ± 0.91 Bb	9.18 ± 2.60 Ba	9.06 ± 2.18 Ba	3.93
SCA and HCV	7.02 ± 1.08 Ba	5.01 ± 1.21 Ba	6.69 ± 1.14 Ba	2.34
Control	22.00 ± 2.82 Aa	24.8 ± 4.08 Aa	24.68 ± 5.75 Aa	3.89
LSD	4.64	.684	5.85	

Discussion

This study aims to investigate the prevalence of Haptoglobin genotypes in Iraqi patients with sickle cell anemia, hepatitis, and co-occurrence of sickle cell anemia and hepatitis C. The sequences for all three segments were recorded in the NCBI Genome Bank for the first time within the local region. Some of these sequences were assigned independent accession codes. This database will serve as a valuable resource for future researchers studying Haptoglopin genotypes in Iraq.

The Hp2-2 genotype was observed to have a higher prevalence in individuals with sickle cell anemia (55%), hepatitis C (63%), and sickle cell anemia with hepatitis C (60%). However, no significant variations were discovered between the three disorders and the healthy control group in relation to this genotype. The results align with the findings published by Fotsing et al [15], who showed a greater occurrence of the Hp2-2 genotype in individuals with sickle cell anemia as compared to the control group. Their investigation revealed a notable disparity in the frequency of the Hp2-2 genotype, with sickle cell anemia patients exhibiting a greater frequency (54%) in comparison to persons with sickle cell trait (42%) and healthy individuals (38%). The findings of Adelike and Haider, [16], in Kuwaiti patients are consistent with these findings. However, the findings of Olatunya et al in Nigeria contradict these results. Olatunya et al [10], found that the distribution of Hp genotypes among the patients and controls were Hp1-1 (42.6%), Hp2-1 (39.6%), and Hp2-2 (17.8%) for patients, and Hp1-1 (54.7%), Hp2-1 (37.5%), and Hp2-2 (7.8%) for controls. There was no difference between the sickle cell anemia patient and control group. Similarly, Ibrahim et al [17], found that 20.2% had Hp1-1 phenotype, 48.8% had Hp2-1 phenotype, and 31.0% had Hp2-2 phenotype in chronic liver disease.

In the current study The T allele and TT genotype seem more visible in sickle cell anemia and hepatitis C diseases than the AA and TA genotypes of Hp-2 643 T>A, while the GG genotype of Hp-1S (442G>C, 561 G>C) was most common in all study groups, as for the Hp-1F 867 G> A gene, the G and GG genotypes were more prominent in sickle cell diseases and hepatitis C compared to the AA and GA genotypes. The T allele might influence protein structure or function of Haptoglobin-2, potentially affecting its ability to bind hemoglobin or scavenge free radicals. This could lead to increased oxidative stress and inflammation, contributing to both sickle cell anemia and hepatitis C pathogenesis.

The TT genotype might be in linkage disequilibrium with other nearby functional variants in the Hp-2 gene, further enhancing its susceptibility effect.

The presence of the GG genotype can result in modified expression or stability of the Hp-1S protein, which can impact its capacity to eliminate damaged red blood cells (such as in sickle cell anemia) or maintain iron balance (which is crucial in hepatitis C).

Similar to Hp-2, Hp-1S GG genotype might be in linkage disequilibrium with other functional variants affecting disease susceptibility.

Or The G and GG genotypes could influence the interaction between Haptoglobin-1F and other proteins involved in immune response or viral entry, potentially promoting these diseases.

Again, linkage disequilibrium with nearby functional variants could be contributing to the observed association. Additional factors as, sample size and potential for population-specific effects, environmental factors interacting with genetic susceptibility.

Haptoglobin allele frequencies exhibit notable variations across different geographical regions and ethnic groups. The Hp-1 allele frequency is found to be the lowest in Southeast Asia and the highest in Africa and South America, as reported in previous studies Langlois and Delanghe, [18]. Diverse and inconsistent findings regarding the distribution of Hp polymorphisms in sickle cell patients and hepatitis C patients underscore the need for further research in diverse populations. This will deepen our understanding of this genetic variation and its potential role in disease complications. Given the strong evidence linking the Hp2 allele to increased susceptibility to vascular disease, oxidative stress, and inflammation, patients with the Hp2-2 genotype deserve close monitoring and personalized health care strategies [19].

The current findings indicate a substantial drop ($P<0.05$) in the concentration of serum haptoglobin in individuals with sickle cell anemia and hepatitis C, compared to the control group. The current findings align with those of previous researchers who similarly observed a statistically significant decrease ($P < 0.05$) in Hp levels in individuals with sickle cell anemia compared to the control group [20-22].

The fluctuation in the Hp level is contingent upon the extent of hemolysis. As the degree of hemolysis increases, more haemoglobin (Hb) is released into the bloodstream. This leads to a higher binding ratio of Hb to haptoglobin (Hp) and a decrease in the level of free Hp in the blood [22] Haptoglobin (Hp) is a glycoprotein that prevents oxidative damage by attaching to free haemoglobin (Hb). This is achieved by inhibiting the redox interaction between the heme iron of Hb and proteins and lipids. In the case of sickle cell anemia, the release of free Hb and the increased oxidative stress throughout the body caused by continuous hemolysis make it particularly important for Hp to counteract any possible oxidation caused by the heme in Hb [15]. Serum Hp levels are lower in people with hepatitis C than in the healthy group used as a comparison [9,23,24].

Recent research indicates that blood haptoglobin levels are lower in hepatitis C patients compared to healthy controls. I attribute this decline to the liver's impaired generation of haptoglobin, caused by the virus-induced damage it has suffered. The decrease in blood haptoglobin concentrations in patients with Hepatitis C has significant clinical implications [12].

A significant consequence of this reduction in haptoglobin is the heightened susceptibility to oxidative stress. The reason for this is that unbound haemoglobin, which is typically bound by haptoglobin, can now inflict harm on cells by producing oxygen radicals or free radicals. Moreover, a decline in haptoglobin can result in an elevated susceptibility to infections as a consequence of compromised immunological function [25]. The current results support the concept that individuals with specific variations of the haptoglobin gene, such as Hp2-2, which have very low biological activity, are more prone to experiencing the effects of disease or injury. These findings align with previous research conducted by [26-28].

The three Hp genotypes have identical affinity for binding free Hb, but they vary in the pace at which they release their heme. Due to its complex structure, Hp 2-2 polypeptide exhibits a slower removal of iron from the extravascular space. This leads to a prolonged presence of free Hb in circulation, resulting in an increased level of oxidative stress. Nevertheless, among these molecules, Hp 1-1 exhibits the highest level of biological activity and effectively shields the endothelium from oxidative stress [29]. In chronic hepatitis C, a change in the

distribution of Hp phenotypes has been found, which may indicate a function of Hp in the development of the disease [30]. When assessing the development of hepatitis C infection, it is important to consider data on the prevalence of the Hp phenotype in chronic HCV.

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CONFLICT OF INTEREST

None

NOVELTY STATEMENT

This study aims to investigate the prevalence of Haptoglobin genotypes in Iraqi patients who have sickle cell anemia, hepatitis, and sickle cell anemia with hepatitis C. The sequences for all three segments were recorded in the local NCBI Genome Bank for the first time, with some being assigned distinct accession numbers. This database will serve as a valuable resource for future researchers studying Haptoglopin genotypes in Iraq

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