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EFFECT OF GENETIC POLYMORPHISMS OF THE HBB GENE ON THE BIOCHEMICAL PARAMETERS OF BETA THALASSEMIA PATIENTS IN MAYSAN GOVERNORATE - IRAQ

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Abstract

Thalassemia is a recessive genetic disease resulting from a disorder and defect in the production of hemoglobin. The current study was designed to evaluate the effect of mutations and their genetic forms on some biochemical parameters of thalassemia patients in Maysan Governorate. This study was conducted during the period from December 2020 until the end of October. The mutations and their genetic structures were identified through genetic sequencing technology and the level of concentrations of biochemical parameters was determined using Cobas Integra 400 plus Analyzer and Cobas e 411 devices. The study included (100) patients and (70) individuals as a control group. The results of the study of the effect of mutations (205.C>T, 294.T>C, 389.G>A, 49.T>C and 624.C>A) showed a significant relationship with each of ferritin, inorganic phosphorous, urea and ALT, ALP, AST, and insignificant relationship with creatinine and calcium. The detected mutations are not found in other parts of Iraq and neighboring countries. the study confirms the heterogeneity of the effect of the diagnosed mutations on the chemical parameters in different regions of Maysan that were studied for the first time in this province, and that needs more future studies to detect the mutations that were not identified in this study.

Key words: Beta-Thalassemia, Biochemical, Genetic Polymorphism and HBB Gene.

1. Introduction

β-thalassemia is one of the most common epidemic single-gene diseases in the world. It has several hemoglobin synthesis defects, including one caused by a lower yield of β-globin protein. Thalassemia is classified clinically into three types: thalassemia major, thalassemia minor, and thalassemia intermediate (Rund and Rachmilewitz, 2005). β-thalassemia is widespread throughout the world. The majority of cases ofβ-thalassemia are caused by mutations in theβ-globin gene, which include deletion, insertion, and conversion of one or more nucleotides within the gene sequence.

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More than 200 mutations associated with -thalassemia disease have been identified worldwide (Anchalee *et al*., 2012; Boonchai *et al*., 2014; Qiuying *et al*., 2017). Because of the wide range of mutations in the β-globin gene, they differ from one population to the next; in every affected population, there are numerous changes in -globin nucleotides that result in unusual genetic mutations (Traeger Synodinos *et al*., 2012; Harteveld and Higgs, 2010).

More than 90 % of globin mutations have been determined using the PCR technique, which is widely used in laboratories due to the ease with which such mutations can be detected (Baig *et al*., 2006; Owayes, 2020). However, PCR sequencing typically detects one mutation per reaction and can be time-consuming and costly (Thein, 2004; Azhar *et al*., 2020). All living organisms' biological processes are chemical reactions that are mostly regulated by

enzymes, which are biological catalysts. Many of these reactions would not occur in the absence of enzymes. Enzymes catalyze all cell metabolism processes. Enzymes have numerous medical applications, including the diagnosis of specific diseases. All tissues and organs of a living organism's body contain thousands of different types of enzymes, each specific to a specific chemical reaction. The concentration of a specific enzyme varies from tissue to tissue, but it is generally higher than in blood serum. As a result, any damage to these tissues and organs causes leakage of their components into the circulatory system. Increases their blood serum concentrations The magnitude of this increase reflects the extent of damage to these tissues and organs. This emphasizes the significance of determining enzyme activity in various disease states (Murry *et al*., 2012; Nelson and Cox, 2013). Organ damage is usually diagnosed by measuring the activities of certain enzymes in the blood. As a result, the current study focused on detecting the levels of several enzymes in the blood of patients with beta-thalassemia. As they are indicators of liver function, they included ALT, which is mostly found in the cytoplasm, and AST, which is mostly found in mitochondria. The researchers also measured ALP, which is present in osteoblasts as well as liver and kidney cells, as it is an indicator of bone and liver function (Owayes, 2020). As well as detecting levels of creatinine, urea, calcium, and inorganic phosphorous. The liver is also the primary iron storage organ and the only site. Iron is normally bound to protein in the liver, and free iron is extremely toxic Iron in its unbound form, stimulates the production of free radicals which cause toxicity and liver and Renal damage (Sobhani *et al*., 2009; Shaalan *et al*., 2022; Abdul Zahra *et al*., 2016).

2. Materials and Methods

Samples Collection

This study was conducted in the Maysan Governorate Center for patients with beta-thalassemia registered in the Center for Genetic Blood Diseases. The patients were on continuous treatment after they were diagnosed by specialist doctors based on laboratory tests of blood film, hemoglobin-electrophoresis, and iron levels. Where (100) patients of both sexes and (70) patients were included as a control group, their ages ranged between (1-50 years). Venous blood was collected and separated into two types of tubes. EDTA tubes for DNA extraction and anticoagulant-free gel tubes for serum were kept at -20 °C until analysis.

Determination of Biochemical Parameters

After obtaining parental consent, five milliliters of intravenous blood were collected from patients, and the control regimen was used on healthy subjects. Before each transfusion, the patient's blood was drawn. The collected blood was roughly evenly divided (2.5 ml) into two types of tubes, an EDTA tube for anticoagulation and a gel tube to obtain serum after leaving the blood in this tube for (30) minutes until the blood clotted and then centrifuged for 5 minutes to isolate the serum. Taking into account the lack of hemolysis at 4000 rpm, serum was collected in Eppendorf tubes, then used in biochemical tests, and blood was studied in EDTA tubes. RBC serum (hemolysis product) was used to estimate the number of enzymes' activities The ALT, AST, and ALP levels were calculated. A Cobas Integra 400 plus Analyzer was used to measure urea, creatinine, calcium, and inorganic phosphorous, and a Cobas e 411 device was used to measure ferritin in the serum using well-established spectrophotometric methods, according to the protocols of these devices. Following that, the results were statistically analyzed.

DNA extraction and Amplification

The presence of isolated genomic material was detected in our study after performing DNA electrophoresis before amplification (DNA bundles for chromosomes), where the extraction results showed that the amount of DNA ranged from 19 to 89.7 ng/l and the degree of purity (260/280) ranged from 1.7 to 2.05, which is within the normal range between (1.7 - 2.0) nm which It was detected by Nanodrop device and the results of electrophoresis on agarose gel (2%) showed the success of the DNA amplification process for the regions under study. Using primers.

Primer selection and design

Primers were chosen based on several studies and other research conducted in Iraq, as well as neighboring and non-neighboring countries (Chan *et al*., 2010; Al-Allawi *et al*., 2015; Jarjour *et al*., 2014; Fettah *et al*., 2013). The primers were designed using the NCBI/Primer-BLAST program and were then compared to previous research using the same primers to determine their sequence. It had forward and reverse primers for detecting mutations.

Table - 1: The primer is used to detect the Thalassemia mutations

Primers	Primer sequence and orientation 5′- 3′
P1 (forward)	CTTAGAGGTTCATTGAATCACGGCTGTCATCACTTAGAC
P2 (reverse)	TATGACATATTTCGGATCGCCTCCCCTTCCTATGACATGA
P3 (forward)	CAATGTATCATGCCTCTTTGCACC
P4 (reverse)	GAGTCAAGGCTGAGAGATGCAGGA

Conventional- PCR Programmes

The final PCR reaction volume was 20 µl, which included 5 µl of master mix (Bioneer, Korea), 5µl of template DNA, and 1µl of all designed primers. The thermal cycle is composed of 35 cycles: denaturation at 95°C for 30 seconds, primer annealing at 58°C for 45 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 7 minutes.

Statistical Analysis

The study results and data (represented as mean ± SD) were analyzed using the Statistical Package for the Social Sciences (SPSSversion 22). An independent ANOVA test was used to assess statistically significant differences between male healthy and sick groups at significance levels of 5% (p = 0.05) and 1% (p= 0.01) to clarify significant differences between variables. In addition, the completely random design method (F-test) was used and the averages were compared using the LSD model (0.05) to analyze the results of the studied traits. As well as using the Bioedit V.7.2.6 Multiple Alignment programs to study the genetic forms and their effect (Hall, 1999).

3. Results

The results in the tables below show the effect of genetic diversity on the chemical parameters represented by the functional enzymes of the liver and kidneys and some chemical variables of calcium. and phosphorous and ferritin as follows:

DNA Isolation Results

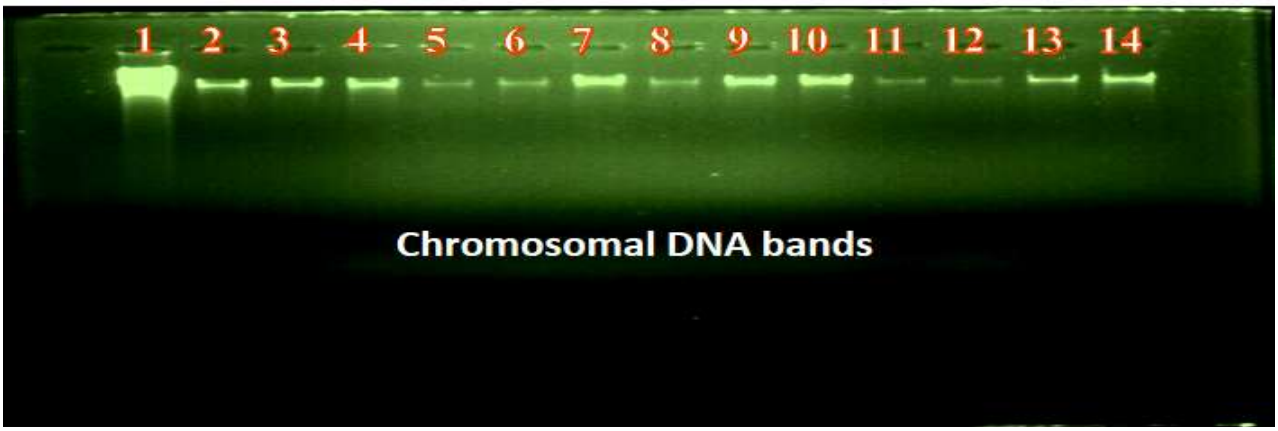

Figure - 1: Chromosomal DNA bands on 0.8 % agarose gel at 70 voltages for one hour

Functional liver enzymes according to genetic polymorphisms

Table (2): shows the effect of thalassemia and the effect of the resulting genotypes due to mutations 205.C>T, 294.T>C, 389.G>A, 49.T>C and 624.C>A on the concentration of functional enzymes. Liver in the blood.

Mutations		Total Mean± SD (Concentration of LFT)			P- value		
		(Patient 1) n=20	(Control) n=10	Polymorphisms	Group (A)	Polymorphisms (B)	A*B
205.C>T	ALT	40.06 ± 15.77	14.81 ± 4.30	31.64 ± 17.75	0.006**	0.365	0.285
	ALP	179.62 ± 84.63	85.31 ± 12.98	148.18 ± 82.40	0.055*	0.540	0.965
	AST	53.78 ± 34.51	18.43 ± 4.85	42.81± 33.04	0.006 **	0.345	NS
294.T>C	ALT	40.06 ± 15.77	14.81 ± 4.30	31.64 ± 17.75	0.000**	0.09	NS
	ALP	179.62 ± 84.63	85.31 ± 12.98	148.18 ± 82.40	0.003**	0.123	NS
	AST	53.78 ± 34.51	18.43 ± 4.85	42.81± 33.04	0.004 **	0.011 *	NS
389.G>A	ALT	40.06 ± 15.77	14.81 ± 4.30	31.64 ± 17.756	0.000**	0.013*	NS
	ALP	179.6 ±84.63	85.3 ±12.98	148.18 ±82.40	0.005**	0.907	NS
	AST	53.78 ± 34.51	18.43 ± 4.85	42.81 ± 33.04	0.019 *	0.033 *	NS
49.T>C	ALT	40.06 ± 15.77	14.81 ± 4.30	31.64 ± 17.75	0.002**	0.262	0.242
	ALP	179.62 ± 84.6	85.3 ±12.98	148.18 ± 82.40	0.038 *	0.890	0.886
	AST	53.78 ± 34.51	18.43 ± 4.85	42.81± 33.04	0.003 **	0.114	NS
624.C>A	ALT	40.06 ± 15.77	14.81 ± 4.30	31.64 ± 17.75	0.000**	0.052	0.164
	ALP	179.62 84.63	85.31 ±12.98	148.18 ± 82.40	0.004**	0.557	0.838
	AST	53.78 ± 34.51	18.43 ±4.85	42.81 ± 33.04	0.046 *	0.557	NS

N.S Not Significant, * Significant (P≤0.05), ** High Significant (P≤0.001), LFT: Liver function test

Biochemical parameters according to genetic polymorphisms

Table - 3: Effect of genetic polymorphisms caused by mutations 205.C>T, 294.T>C, 389.G>A, 49T>C and 624.C>A on the concentration of biochemical

Mutations		Total Mean± SD Concentration of biochemical			P- value		
		Patients n=20	Control n=10	Polymorphisms	Group (A)	Polymorphisms (B)	A*B
205.C>T	Urea	21.86 ± 6.48	26.131±7.74	23.28 ± 6.48	0.446	0.201	0.019*
	Creatinine	3.05±11.75	0.85±0.22	2.32 ± 9.57	0.778	0.883	0.762
	Ca	9.24 ± 1.02	9.71 ± 1.19	9.40 ± 1.08	0.957	0.731	0.370
	P	5.56 ± 1.54	4.01 ± 0.26	5.04 ± 1.46	0.04 *	0.814	0.746
	Ferritin	4747.41±3030.98	16.49±3.30	3170.43±3341.28	0.011*	0.698	0.406
294.T>C	Urea	21.86 ± 6.48	26.13±7.74	23.28±7.09	0.012*	0.006*	NS
	Creatinine	3.05± 11.75	0.85±0.22	2.32± 9.57	0.485	0.846	NS
	Ca	9.24 ± 1.19	9.71 ± 1.19	9.40 ± 1.08	0.203	0.462	NS
	P	5.56 ± 1.54	4.01 ± 0.26	5.04 ± 1.46	0.013 *	0.031 *	NS
	Ferritin	4747.41±3030.9	16.49± 3.30	3170.43±3341.28	0.000**	0.085	NS
389.G>A	Urea	21.86 ± 6.48	26.13±6.74	23.28 ±7.09	0.426	0.244	NS
	Creatinine	3.05± 11.75	0.85±0.22	2.32 ±9.57	0.357	0.626	NS
	Ca	9.24 ± 1.02	9.71 ± 1.19	9.40 ± 1.08	0.757	0.231	NS
	P	5.56 ± 1.54	4.012 ± 0.26	5.04 ± 1.46	0.033	0.345	NS
	Ferritin	4747.41±3030 .9	16.49±3.30	3170.43±3341.28	0.001**	0.178	NS
49.T>C	Urea	21.86± 6.48	26.13 ± 7.74	23.28 ± 7.09	0.991	0.613	0.107
	Creatinine	3.05±11.75	0.85± 0.22	2.32±9.57	0.690	0.813	0.677
	Ca	9.24 ± 1.02	9.71 ± 1.19	9.40 ± 1.08	0.951	0.547	0.496
	P	5.56 ± 1.54	4.01 ± 0.26	5.04 ± 1.46	0.103	0.555	0.664
	Ferritin	4747.41±3030 .9	16.49±3.30	3170.43±3341.28	0.001**	0.125	0.765
624.C>A	Urea	21.86±4.48	26.13±7.74	23.28± 7.09	0.475	0.385	0.472
	Creatinine	3.05± 11.75	0.85±0.22	2.32 ±9.57	0.929	0.124	0.981
	Ca	9.24 ± 1.02	9.71 ± 1.19	9.40 ± 1.08	0.885	0.326	0.228
	P	5.56 ± 1.54	4.012 ± 0.26	5.04 ± 1.46	0.022*	0.966	0.799
	Ferritin	4747.41 ±3030.98	16.49±3.30	3170.43±3341.28	0.001**	0.537	0.660

Table - 4: Detected mutations, specific genotypes and polymorphisms in the NCBI Gene Bank and the DDBJ Gene Bank

Mutations (Polymorphism)		Gen Bank	
		Accession Number	Size (bp)
205. C>T	CC	LC727508	657 bp
	CT	LC727509	657 bp
	TT	LC727510	657 bp
294.T>C	TT	LC727511	657 bp
	TC	LC727512	657 bp
	CC	LC727513	657 bp
389.G>A	GG	LC727514	657 bp
	GA	LC727515	657 bp
	AA	LC727516	657 bp
49.T>C	TT	LC727517	777 bp
	TC	LC727518	777 bp
	CC	LC727519	777 bp
624. C>A	CC	LC727520	777 bp
	CA	LC727521	777 bp
	AA	LC727522	777 bp

Genetic forms of mutations and their percentages of patients with beta thalassemia

Table - 3.21: Explains the effect of mutations on the genotypes of thalassemia patients

β-thalassemia mutation	*Homozygous*		*Heterozygous*	
	N	*%*	*N*	*%*
HBB: 205. C >T	*16*	*16*	*4*	*4*
294.T>CHBB:	*17*	*17*	*3*	*3*
HBB: 289.G>A	*17*	*17*	*3*	*3*
HBB: 49 T > C	*13*	*13*	*7*	*7*
624.C>AHBB:	*15*	*15*	*5*	*5*
Total	*78*	*78*	*22*	*22*

The results of the statistical analysis indicated that the group of beta-thalassemia patients was superior in the concentration of ALT enzyme in the blood compared to the healthy group (the control group). Where the P value was recorded (0.006, 0.001, 0.001, 0.002, 0.001). for mutants 205.C>T and 294.T>C, 389.G>A, 49T>C and 624.C>A respectively which are highly significant statistical differences. The plasma ALT concentrations were 40.06 U/L and 14.81 U/L between the two groups of patients and the control group, respectively. Regarding the effect of the genetic polymorphism caused by 205. C>T mutation. The results did not indicate any significant effect of the CC, CT and TT genetic polymorphisms on the concentration of ALT enzyme in the blood plasma of the two groups of patients and the control group, where the P value was (0.365) and also did not. The results of the statistical analysis indicate that there is a significant effect of the genetic variants of the 294.T>C mutation for the group of patients compared to the control group. The homozygous TT genotype was the only one in the control group, with a concentration of 14.81 U/L. As for the mutation 389.G > A, the results of the study indicated a significant effect of the genetic polymorphisms, where the p-value was (0.013). The study indicated that there is a clear superiority of the

genotype (AA) 50.80 units/liter for the mutation 389.G >A compared to the genotypes (GG, AG) with values of (39.73, 23.5) units/liter, respectively. It was also noted that the homozygous GG genotype was the only one in the control group with a value of (14.81 units/liter) for the 389. G>A mutation. The study did not indicate any significant effect between the genetic forms of the 49. T>C mutation on the concentration of ALT enzyme in the blood plasma, where the P-value was (0.262). Also, the results of the statistical analysis did not indicate any significant effect of the genetic forms resulting from the 624.C>. A mutation in the two study groups (the patient group and the control group), where the P value was (0.052). Also, the results of the study did not indicate a significant effect of the interaction between the effect of the group (patients and the control group) with the effect of any of the genetic polymorphisms 205.C>T, 294.T>C, 389.G>A, 49.T>C and 624. C > A. The concentration of ALT in the blood plasma).

The results of the statistical analysis of our study indicated that the group of patients (thalassemia patients) was superior to the healthy group (the control group) in ALP enzyme concentrations in the blood, as the P value (0.055, 0.003, 0.005, 0.038, 0.004) was recorded for the mutations 205.C>T and 294.T>C, 389.G>A, 49T>C, and 624.C>A, respectively, as these were highly significant differences for the two mutations (294.T>C, 389.G>A and 624.C>A) Significantly for the two mutations (205.C>T and 49T>C), the concentration of ALP in the blood plasma was 179.62 units/l and 85.31 units/l between patients and the control group, respectively. The study indicated the effect of a genetic polymorphism caused by a mutation of 205 C>T. The results of the study showed that there was no significant effect between the genetic forms of all the mutations under study on the concentration of ALP enzyme in the blood plasma of the two groups of patients and the control group, where the P-value was (0.540, 0.123, 0.907, 0.890, 0557) respectively, And the TT genotype (TT) of mutation 294.T>C was recorded as a single phenotype for the control group. As for the 389.G>A mutation, the homozygous GG genotype was recorded as a single phenotype in the control group. The results did not indicate any significant effect of the interaction between the effect of the group (patients and the control group) with the effect of any of the genotypes of the 205.C>T, 294.T>C, 389.G>A, 49.T>C and 624.C >A mutation. The concentration of ALP enzyme in the blood plasma.

The results of the statistical analysis of our study showed the superiority of the patient's group (thalassemia patients) compared to the healthy group (the control group) in the concentration of AST in the blood, as it recorded highly significant P values (0.006, 0.00 4, 0.003) for mutations (205.C >T, 294.T>C, 49.T >C) respectively and with significant differences for the two mutations (389.G > A and 624.C > A). The plasma AST concentrations were 53.78 units/l and 18.43 units/l between the two groups of patients and the control group, respectively. The study indicated that there was no significant relationship between the effect of the genetic forms CC, CT, and TT of the C > T.205 mutation on the concentration of AST enzyme in the blood plasma of both (patients group and control group) where the P-value was (0.345). Regarding the effect of the genotypes caused by the 294.C>T mutation, the results of the statistical analysis indicated a significant effect of the P-value (0.011) between the TT, TC, and CC genotypes of the patient group. As for the 389.G>A mutation, the resulting genotypes GG, GA and AA recorded a significant effect as well, as the P-value was (0.033). One homozygous genotype (GG) was recorded in the control group. The study did not indicate a significant effect between the genotypes TT, TC, and CC resulting from the 49T>C mutation on the concentration of AST enzyme in the blood plasma, where the P-value was (0.114). Also, the results of the statistical analysis did not indicate any significant effect between the CC, CA, and AA genetic variants caused by the 624.C>A mutation in both groups of patients and controls, where the P-value was (0.557). The results did not indicate any significant effect of the interaction between the effect of the group (patients and the control group) with the effect

of any of the mutations 205.C>T, 294.T>C, 389.G>A, 49.T>C and 624.C>A The concentration of AST in the blood plasma (Abdulwahid and Hassan, 2013).

The results of the statistical analysis of our study showed a significant superiority of the patient's group (thalassemia patients) compared to the healthy group (the control group) in serum urea concentration (Urea) for only one mutation (294T>C), which recorded a P-value (0.012). The mean urea concentration was (21.86 g/dL, and 26.13 g/dL) respectively for the patients and the control group, and the study did not indicate significant differences for the rest of the other mutants (205.C>T, 389.G.>A, 49T>C and 624.C>A) for the study group (patients and control group). As for the effect of genetic variations resulting from genetic mutations, the study showed that there is one significant relationship to the effect of genetic forms of the study group (patients and healthy people), for the mutation (294. T > C) where the P-value (0.019) was recorded, while there was no Any significant relationship for the rest of the other genetic forms of mutations on the study group (patients and healthy control people) for the remaining mutations, and the study also showed a significant relationship for the interaction between the effect of the group (patients and healthy people) and the mutation (205.C >T) and the results did not indicate any significant effect of the interaction Between the effect of the group (patients and control group) by the effect of any of the remaining mutations and 294.C>C, 389.G>A, 49T>C and 624.C>A in urea concentration.

The results of the statistical analysis of our study did not indicate a significant difference between the group of patients (thalassemia patients) compared to the healthy group (the control group) in the concentration of (creatinine), as the (P-value) (0.778, 0.485, 0.357, 0.690, 0.929) respectively for mutations 205.C>T, 294T>C, 389.G>A, 49T>C and 624.C>A were the mean total creatinine concentrations (3.05, 0.85), respectively (for patients and control). The results did not indicate any effect of the genetic polymorphisms dependent on the mutations (205.C>T, 294T>C, 389.G>A, 49.T>C and 624.C>A) on the creatinine concentration of the group of patients and healthy controls. The results also did not indicate any significant effect of the interaction between the effect of the group (patients and control) and the genetic forms of the mutations (205.C>T, 294.T>C, 389.G>A, 49T>C and 624.C>A) on the concentrations of creatinine; Any of the spikes in creatinine concentration (Shaalan *et al*., 2022).

The results of the statistical analysis of our study did not indicate a significant difference for the thalassemia patients group) compared to the healthy group (the control group) in the calcium concentration as the (P-value) was (0.957, 0.203, 0.757, 0.951, 0.885, respectively for the mutations 205.C>T, 294T>C, 389.G>A, 49T>C and 624.C>A respectively and the mean total calcium concentration were (9.24, 9.71), respectively for patients and control. The results did not indicate any significant effect of the genetic polymorphisms of the mutations (205. C>T, 294T>C, 389.G>A, 49.T>C and 624.C>A (patients and controls group) on calcium concentration. The results also did not indicate any significant effect of the interaction between the group (patients and control) and the genetic morphology of the mutations (205.C>T, 294.T>C, 389.G>A, 49T>C and 624.C>A) for calcium concentration.

The results of the statistical analysis of our study showed a significant superiority of the patient group (thalassemia patients) compared to the healthy group (the control group) in the concentration of inorganic phosphorus in the blood for four of the detected mutations: (205.C>T, 294.T>C, 389.G>A and 624.C >A) P-value (0.04, 0.013, 0.033, 0.022) respectively. While the study did not indicate a statistically significant relationship for the study group (patients and control group) with regard to the mutation (49.T >C), where the P value (0.103) was recorded, and the average phosphorous concentration (5.56 g/dL, 4.01 g/dL) was recorded. respectively for patients and the control group. As for the effect of genetic forms resulting from genetic mutations, the study

showed that there is one significant relationship between the genetic forms of the study group (patients and healthy individuals) and these genetic forms of the mutation (294.T > C), while there is no significant relationship for the rest of the genetic forms in the study group. (Patients and healthy controls (controls) for the remaining mutations and the results did not indicate any significant effect of the interaction between the effect of the group (patients and control group). With the effect of any of the mutations 205.C>T and 294T<C, 389.G>A, 49T>C. and 624.C>A in inorganic phosphorous concentration

The results of the statistical analysis of ferritin for our study showed highly significant superiority of the study group (thalassemia patients and the control group) in the concentration of ferritin in the blood, where the P-value was recorded (0.011 0.000, 0.001, 0.001, 0.001) respectively for the mutations. 205.C>T, 294.T>C, 389.G>A, 49T>C and 624.C>A, respectively as the ferritin concentration was (4747.41 ng/dL, 16.49 ng/dL) respectively for patients and the control group. The study also showed that there was no significant relationship for genotypes due to four mutations (205.C>T, 389.G>A, 49T>C, and 624.C>A) where the P-value was (0.01, 0.178, 0.125. 0.537), respectively. While there was a significant relationship for the genetic polymorphisms due to the 294 T>C mutation, where the P value was (0.085) and the results did not indicate any significant effect of the interaction between the effect of the group (patients and the control group) with the effect of any of the remaining mutations.

4. Conclusions

The present study found a disturbance, significant changes and an increase in the activities of ALT, ALP, AST, ferritin, inorganic phosphorous and calcium, which could be caused by the breakdown of red blood cells, excessive blood transfusion, and the resulting increase in iron concentration. As a result, iron is deposited in the liver and kidneys. And the rest of the body, we conclude that these enzymes can be used as one of the diagnostic indicators for thalassemia major, as well as predicting the extent of damage caused by this disease. We also detected five distinct mutations in the -globin gene. This study shows the need to intensify efforts to follow up, investigate and reduce the causes of the disease.

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