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The Relevance of rs34598529 SNP of HBB Gene among β-thalassemic Patients Dependent on Blood Transfusions in Thi-Qar Governate

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Abstract: Thalassemia is a recessive hereditary disease which is a massive serious threat on general health besides the financial costs to settle down their patients. Thalassemia is causing anemia with many complications, pluse the effects of blood transfusion on health. Hemoglobins morphology and functionally are controled by one of others which is human beta globin (HBB) gene located on chromosome 11. By polymorphisms or single nucleotide polymorphism (SNP), alteration such as insertion, deletion, or rearrangement of a nucleotide which impact the gene expression. The researchers regard (SNP) as a genetic marker for many diseases. rs34598529 identified among some patients whom suffered of beta (+) thalassemia, also known as codon 29 (A>G), which has been seen more frequently in patients with clinical diagnosis than in populations of healthy people. Therefore, this study conducted by collecting 80 blood samples of β-thalassemic patients whom treated by The Thalassemia & Hereditary Blood Diseases Centre in Thi-Qar/Iraq, plus 50 blood samples of healthy individual. The biological molecules analysis of this study indicate the SNP (rs34598529) genotypes AA/AG significantly affected among beta thalassemic major only, also the alleles A/G significantly rose for beta thalassemia major. Regarding the gender finding there is no significant among β-thalassemia which is not a sex-linked disease and affects both sexes equally. On the other hand, the age can indicate significant affects by beta thalassemia among patients by grown old. Finally, β-thalassemia major and intermedia not associated by variant rs34598529 regarding Hemoglobin concentration therefore, no correlation to the blood transfusion among β-thalassemic patients major nor intermedia.

Keywords: Antimicrobial peptide, Burns, checkerboard, LL-37, *Pseudomonas aeruginosa*, synergism.

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Introduction

Thalassemia is refered to blood disorders that are characterized by low levels or the absence of typical globin chains in the normal red blood cell protein hemoglobin. There are four kinds of globin chains: alpha (α) beta (β) gamma (γ) and delta (δ). The thalassaemias are classified as α-, β-, γ-, δ -, δβ-, or εγδβ-thalassaemias depending on which chain of production is disrupted. The most prevalent forms are α- and β-thalassaemias, which occur from a lack of α- or β-globin proteins, which are required for the formation of a normal hemoglobin molecule (HbA, α2β2) in an adult person (1). Hemoglobin is a metalloprotein (Hb or Hgb) present inside the red blood cells (RBCs) of almost of vertebrates and some invertebrates that serves as an oxygen transporter (2). Basically, thalassemia causes severe anemia and a lifetime dependency on blood transfusions due to a lack of hemoglobin. Chelation therapy is

usually required for people with thalassemia to remove excess iron from their bodies on a regular basis. There are several forms of thalassemia, based on the defective hemoglobin gene (alpha/beta) and severity, with beta-thalassemia major being the most prevalent but also one of the most severe. Thalassemia is always fatal if not treated, however patients who have regular access to therapy are now living into their 50s, 60s, and even 70s, implying that thalassemia is no longer the life-threatening disease it once (3). The reasons of beta thalassemia caused by point mutations, or rarely by deletions in the HBB gene in the region of chromosome 11p15.5 effecting the missing (beta 0) synthesis of the beta-chains of hemoglobin (Hb) mutations causing BT major which are homozygous (4). Globin gene is made up of a string of nucleotide bases that is separated into three coding sequences called exons and two noncoding portions called introns or intervening sequences (IVS). The HBB gene is regulated by a 5' promoter region that contains the classical TATA, CAAT and duplicated CACCC boxes. Upstream of the beta-globin cluster is another regulatory element for HBB, namely the locus control region (LCR)(5). The β-thalassemia molecular pattern revealed a wide range of mutations affecting the HBB, and new mutations are being uncovered as diagnostic technologies improve (6). Single nucleotide polymorphisms (SNPs) are genetic modifiers (7). Understanding the functions of SNPs can greatly help to understand the genetics of the human phenotypic variation and especially the genetic basis of human complex diseases. Understanding the functions of SNPs can greatly help to understand the genetics of the human phenotypic variation and especially the genetic basis of human complex diseases(8). SNP distribution has been a key biomarker in a variety of applications, including population genetics, pedigree analysis, and the identification of quantitative trait loci (9). Therefore, this study was important to address part of this SNP (rs34598529) to cover some gaps about the thalassemia disease related to blood transfusion dependency, plus the old studies uncover this field completely. The aims of the study o examine the association of the genotypes and alleles polymorphisms of the HBB Gene (rs34598529) of thalassemic patients and control groups by calculation the odds ratio for each genotype. It correlates the relationship between gene polymorphism of the SNP (rs34598529) among blood transfusion dependency by hemoglobin concentration and ferritin level of thalassemia patients and healthy controls. It finds the relevant between the gene polymorphism of the SNP (rs34598529) among the thalassemia patients and healthy controls by the gender and age.

Material and methods

This study has two patients' groups including 80 patients of beta thalassemic patients, in addition to the control group comprises of 50 apparently healthy subjects randomly. Patients' groups were selected from The Thalassemia Clinic Center (Hereditary Blood Diseases center in Thi-Qar) Inclusion Criteria of beta thalassemia were:

- The diagnosed patients by specialist divided equally for two groups which were β-thalassemia major and intermedia, excluding β-thalassemia minor for result's simplicity.

- The control group comprises of 50 healthy subjects randomly.
- Samples blood collected in EDTA tube after hemoglobin and ferritin laboratory analysis kept freezing at (-20) Degrees Celsius for little while.
- Analyzed the Hemoglobin concentration by SYSmex which is Hematology Automated Analyzer using multi-dimensional scattergrams and side fluorescent light into electrical impulses to analyze and calculate measurements.
- Determination of Serum Ferritin levels by miniVIDAS Biomerieux Automated using enzyme linked fluorescent assay (ELFA) technique.
- Then used for DNA extraction, primers design, as shown in Table (no.1). (Tetra-ARMS PCR), and agarose gel electrophoresis, eventually analysis the data.
- Finally getting the result statistically.

Table (1): Primers in the present study

Primer	Sequence
Forward inner	GAGGGCAGGAGCCAGGGCTGGGCCTA
Reverse inner	AGCAATAGATGGCTCTGCCCTGACTGTC
Forward outer	AGCCAGTGCCAGAAGAGCCAAGGACAGG
Reverse outer	CTTGATACCAACCTGCCCAGGGCCTCAC

Statistical analysis

Data were investigated with SPSS software, version 27. Categorical variables were displayed as frequencies and percentages, while continuous variables were reported as the mean ± standard deviation (SD). Normality was evaluated through the Shapiro–Wilk method for continuous variables.

Chi-square test was applied to assess genotype and allele frequencies between patients and controls. The genotype and allele distributions were determined in each group, and odds ratios (OR) with 95% confidence intervals (95% CI) were calculated. The genotype distributions of rs34598529 were tested for deviation from the Hardy-Weinberg equilibrium in controls (10). A p-value of <0.05 was considered statistically significant at a confidence interval (CI) of 95% (11).

Result and discussion

Genotype and allele frequency for rs34598529

The genotyping AA results showed that the protective factor is high among the control 52%, while the thalassemia major is lowest 17.5% and the medium is the β-thalassemic intermediate 27.5%. On the other hand, the risk factor which shows the highest 77.5% among β-thalassemic major similarly intermediate 72.5% instead the lowest is among control samples 44%, otherwise the GG control show 4% beside thalassemia major 5% and intermediate are 0% respectively. Giving that the allele A among control 74%, then thalassemia major 56.25%, while intermediate 63.75%. Additionally, the allele G shows control 26%, thalassemia major 43.75% and intermediate 36.25%. So, compares between genotypes of control and thalassemia major shows there are significant differences at both homozygous AA and heterozygous AG same as both alleles A and Gas significant, opposite that genotype homozygous GG which shows non-significant at all. On the whole comparison between control and the thalassemia intermediate the results show no significant at all the

homozygouses AA, GG and heterozygous AG as well as both alleles A and G.

rs34598529 (codon–29A-G) resulted in a 25% affected the normal globin production in the erythroid cells (12). When compared to healthy individuals, the rate of association of complexes containing TATA binding protein/TATA boxes (TBP/TATA boxes) with this SNP is reduced, and value, which characterizes TBP/TATA box affinity is decreased. Although that affected the β-thalassemia intermedia homozygotes for this allele, and do not depend on blood transfusions (12). Human HBB genes contain the largest number of SNP markers including rs34598529 of resistance to malaria and thalassemia caused by under expression of the genes (13) as shown in Table (2).

Table (2): Genotype and Allele Frequency of for β-thalassemia Major, intermediate and apparently controls for HBB gene (rs34598529)

Genotypes and allele	Groups			G2 vs G1		G3 vs G1	
	G1 Control N=50(%)	G2 β-TM N=40(%)	G3 β-TI N=40(%)	P value	OR(CI)	P value	OR(CI)
Genotypes							
AA Wild	26(52)	7(17.5)	11(27.5)	0.001*	0.1958(0.0730 to 0.5251)	0.02	0.3501(0.1440 to 0.8514)
AG Hetero	22(44)	31(77.5)	29(72.5)	0.001*	4.3838(1.7318 to 11.0971)	0.16	1.75(0.7961 to 3.8803)
GG Mutant	2(4)	2(5)	0(0)	0.81	1.2632(1.700 to 9.3867)	0.36	0.2395(0.0112 to 5.1334)
Alleles							
A	74(74)	45(56.25)	51(63.75)	0.01*	0.4517(0.2410 to 0.8468)	0.13	0.6179(0.3264 to 1.1699)
G	26(26)	35(43.75)	29(36.25)	0.01*	2.2137(1.1810 to 4.1495)	0.13	1.6184(0.8548 to 3.0641)

Significant difference at P<0.0.
β-TM: β-thalassemia major.
β-TI: β-thalassemia intermediate.

Association of patients and control according to gender

Regarding the gender of (β-TM) beta-thalassemia major males' number is 26 (65%), besides the female's number is 14 (35%). Furthermore, the gender of (β-TI) beta thalassemia intermiate males' number is 72.5 (29%), while the number of the females is 11 (27.5%). Moreover, the controls males 64 (32%), on the other hands females' number of 18 (36%). Overall, the table (3) shows no significant of gender at this study, agreeable with the study of Alali and Faraj (14) who found that there was highly significant difference between gender groups when they investigated the prevalence of of β-thalassemia patients in Missan/Iraq the results in their study demonstrated that males were more affected than females. Nevertheless, the present result was confirmed by observation of Lucarelli *et al.* (15) and Raftopoulos *et al.,* (16) who announced that thalassemia is an autosomal recessive disease caused by abnormalities in the β-globin gene located on chromosome 11, so β-thalassemia is not a sex-linked disease and affects both sexes equally.

Table (3): Distribution of gender of β-thalassemia patients and apparently healthy control

Groups	Total No.	Gender (No. and %)	
		Male	Female
Beta thal. Major	40	26(65)	14(35)
Intermediate	40	29(72.5)	11(27.5)
Control	50	32(64)	18(36)
Calculated P value	*0.663		

*** No Significant difference at P<0.05.**

Association about age

This study shows there is a significant about age of thalassemia major frequency 5% of age 2-11, 32.5% for 12-21 age, 40% for 22-31 of age, while 7.5% for 32-41 age and 15% about 42-52 age. Thalassemia intermediate distributes 30% for 2-11 age, 35% for 12-21 age, 22.5% about 22-31 of age, 7.5% of 32-41 age and 5% for 42-52 age. Finally, the control shows 0% for 2-11 age, 8% of 12-21 age, 44% of 22-31 age, 30% for 32-41 age, finally 18% of 42-52 age. Eventually there is significant distribution for the patients age and control as table (3). This finding suggested that regular blood transfusions could help people live longer lives. However, because there was a severity range, some patients may only be mildly affected. In untreated β-thalassemic patients, however, hemolytic and profound anemia cause marrow hypertrophy and hyperplasia. Anemia can be so severe that death occurs within the first five years. Long-term erythrocyte breakdown causes chronic bilirubin overproduction, which predisposes the patient to pigmentary gallstone formation and hemosiderosis caused by excess iron deposition in the reticuloendothelial system, particularly the myocardium, liver, pancreas and hemosiderosis that causes death which can occur before the 25 years of age (17,18,19). Numerous research agreed with this conclusion (20,21.22) who made sure that the main factor in β-thalassemia was a hereditary illness that often did not show until individuals were older than 6 months. As additional research was conducted in other locations throughout the globe.

Table (4): Distribution of age of β-thalassemia patients and apparently healthy control

Groups	Total No.	age (No. and %)				
		2-11	12-21	22-31	32-41	42-52
Beta thal. Major	40	2(5)	13(32.5)	16(40)	3(7.5)	6(15)
Intermediate	40	12(30)	14(35)	9(22.5)	3(7.5)	2(5)
Control	50	0(0)	4(8)	22(44)	15(30)	9(18)
Calculated P value		<0.001*				

*** Significant difference at P<0.05.**

Distribution of HB level and ferritin concentration

The frequency is significant of the association about the Hemoglobin level and Ferritin concentration among patients and control.

The Hb level at control is higher average 14.4 gm%, thalassemia major 8.6, then thalassemia intermediate at 8.4 gm%. About the Ferritin concentration the highest is thalassemia major 1877 ng/mL, intermediate 1627 ng/mL then the lowest is control 75.7 ng/mL.

Compatable with (23) who suggest that genetic factors play a significant role in hemoglobin concentration variation, these preliminary findings from genome-wide association and linkage studies indicate that variation in hemoglobin concentration in the general population is influenced by gene variants that differ from the polymorphisms that cause the major hemoglobinopathies as shown in Table (5).

Sala, *et al.* (24) reported an intriguing finding that hemoglobin concentration varied geographically, in addition to providing heritability estimates for hemoglobin concentration.

Through some association studies, trying to figure out what genetic factor controls the level of Hb. These studies have discovered that genetic variation affects Hb concentration in healthy persons. The iron metabolism, globin gene regulation, and erythropoiesis are three processes that are all affected by the discovered SNPs. When it comes to Hb concentrations and other hematological parameters, the frequency of the discovered SNPs varies among populations and has a negligibly modest impact. Most research has been done in European populations (25,26,27).

Table (5): HB level and Ferritin concentration of β-thalassemia patients and apparently healthy control.

Groups	HB (gm%)	Ferritin (ng/mL)
β-thalass. Major	8.62±1.97a	1877.2±1760a
β-thalass.Intermediate	8.47±1.81a	1627.023±1336a
Control	14.46±1.09b	75.76±55.4b
Calculated P value	<0.001*	<0.001*

Different letters between any two means denote to the significant difference at p<0.05 (mean±SD).
Significant difference at P<0.05.

HB level and ferritin conc. distribution of thalassemia major and intermediate

Regarding the rs34598529 Hemoglobin concentration and ferritin level mean of heterozygous AG, homozygous GG and homozygous AA showed for both major and intermedia that were no significant.

It has been known for a long time that genetic factors affect Hb concentration and the risk of anemia. Similar to what has been seen in other phenotypes, some genetic variations have significant effects, frequently resulting in monogenic diseases, while other genetic variations have a minor impact on the variation in Hb concentration. The hemoglobin (Hgb) concentration is a result of the interaction of genetic variation and environmental elements, such as nutritional status, sex, age, pollution and altitude. The complex genetic diversity that affects this protein varies greatly between populations. Anemia is a risk factors include variations linked to abnormal Hb or altered erythrocyte properties (28). Also extended as genes involved in Hb synthesis, erythropoiesis, and iron metabolism frequently have polymorphisms that regulate the interindividual variation of Hb concentrations. These variations' effects on Hb concentrations have been relatively mild. Studies in other populations around the world are necessary because the majority of research on the underlying genetic

influences in Hb variation has been done in European populations.

A primary keyword search uncertain about hemoglobin deficiency as a biochemical marker for rs34598529 SNP (29,30) as the tables below.

Table (6): HB and ferritin frequency of β-thalassemia major (rs34598529)

Parameters	Genotype (mean±SD)			Calculated P value
	AA	AG	GG	
HB	7.46±2.52a	8.83±1.88a	8.75±1.06a	0.301*
Ferritin	1277.5±644a	1946.2±1867.4a	2572.5±2725.8a	0.601*

Similar letters between any two means denote to the no significant difference at P<0.05
*** No Significant difference at P<0.05.**

Table (7): HB and ferritin frequency of β-thalassemia Intermedia (rs34598529)

Parameters	Genotype (mean±SD)		Calculated P value
	AA	AG	
HB	7.82±1.38a	8.65±1.90a	0.229*
Ferritin	1747.2±810.2a	1592.1±1463.4a	0.764*

Similar letters between any two means denote to the no significant difference at P<0.05
*** No Significant difference at P<0.05**.

Conclusion

This study indicated that rs34598529 was pathological factor specially among beta thalassemia major, on the other hand there was no linkage regarding the blood hemoglobin concentration or the ferritin level. Besides the gender was no influence relation by SNP (rs34598529). Also, correlation of age the study found no association by both beta thalassemia mojor neither intermedium. Finally, this research indicated that no correlation of HBB gene mutation (rs34598529) SNP among β-thalassemic patients dependent on blood transfusions in Thi-Qar Province/Iraq.

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