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Original article

A Study of Hepcidin Gene Polymorphism in Beta Thalassemia Major Egyptian Patients Refractory to Iron Chelation

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ABSTRACT

Background: Beta-thalassemia major (TDT) is a hereditary blood disease marked by a severe case of anaemia and the requirement for regular red blood cell transfusions. Iron overload resulting from transfusions and inefficient iron chelation therapy poses a significant challenge in managing these patients. Hepcidin polymorphisms may contribute to variations in iron homeostasis and susceptibility to iron overload. **Objective:** To examine the correlation between Hepcidin gene polymorphism (C.-582A>G) and Iron overload in patients with TDT who are unresponsive to iron chelating treatment. **Methods:** This case-control study included 50 patients with TDT receiving regular iron chelation therapy and 50 healthy subjects matched for age and socioeconomic status as the control group had been genotyped for the Hepcidin gene variants (C.-582A>G) and were analyzed using the RFLP-PCR technique. **Results:** All results statistically analyzed and tabulated in the study. **Conclusions:** Hepcidin gene polymorphism (C.-582A>G) is correlated with iron overload in refractory TDT patients. The GG genotype shows an elevation in serum iron and ferritin levels in contrast to the AA/AG genotypes, along with a significant reduction in hemoglobin and mean corpuscular volume (MCV) levels. Despite chelation treatment, Iron overload is still considered as a major complication of thalassemia.

Keywords: Hepcidin, Gene Polymorphism, Beta Thalassemia Major, Refractory, Iron Chelation.

Introduction

Beta-thalassemias constitute a collection of hereditary conditions that affect the production of hemoglobin, leading to persistent anemia. The extent of the anemic condition was classified as transfusion-dependent β-thalassemia (TDT) or non-transfusion-dependent β-thalassemia (NTDT) [1]. In terms of geographical distribution, beta-thalassemia exhibits higher occurrence rates in nations situated around the Mediterranean, Southeast Asia, and Eastern Europe. Notably, in Egypt, it poses a substantial healthcare challenge, with an estimated annual occurrence of around 1000 cases for every 1.5 million live births [2]. Individuals with transfusion-dependent thalassemia (TDT), encompassing individuals with beta-

thalassemia major and the severe manifestations of HbE/beta-thalassemia, necessitate continuous transfusion treatment throughout their lives to ensure their survival. Although these transfusions have enhanced longevity, they also bring about an excessive accumulation of iron in the body, leading to harm to vital organs (heart and liver) and elevated risk of mortality [3].

Ensuring equilibrium of iron levels within the body occurs by three processes, absorption, movement, and reutilization of iron. A key modulator in this iron equilibrium is hepcidin, an antimicrobial peptide gene, whose predominant expression is found in the liver [4]. Originating from the HAMP (Hepcidin Anti-Microbial Peptide) gene, hepcidin performs its action by binding to ferroprotein forming a complex and then preventing the duodenum from absorbing iron, decreasing the liberation of iron from macrophage and, that leading to accumulation of iron within the cell. In thalassemia patients, the hepcidin level was decreased as its production was diminished in the liver by various factors such as hematopoiesis, anemia, and excessive iron overload [5].

The HAMP gene is found on chromosome 19's long arm (19q13), prior research indicated that the polymorphism (C.-582A>G) in the HAMP promotor may be involved in iron metabolism since it is situated in the E-box, a location that is positive for transcription factors. This could result in a decrease in the synthesis of the protein so (C.-582A>G) gene polymorphism reduces expression of HAMP gene, leading to iron overload [6].

The aim of our study was to investigate the association between Hepcidin gene polymorphism (C.-582A>G) and iron overload in beta-thalassemia major patients resistant to treatment with iron chelation.

Declaration of the World Medical Association (ethical approval code no :Ms 13-6-2022)

Patients and methods

Study Design: This case-control research comprised 50 patients diagnosed with TDT, who were chosen through the Internal Medicine Department and outpatient clinic at Benha University Hospitals during the period from May 2022 to August 2023. Additionally, 50 apparently healthy subjects were chosen as a control group, matched with the patient group in terms of age and socio-economic level.

Ethical consideration: The Benha University Faculty of Medicine's Ethical Committee gave its approval for this study. All subjects provided signed informed consent, and the study adhered to the ethical guidelines outlined in the Helsinki

The inclusion criteria for the study were as follows: both sexes, age above 14 years old, patients with transfusion-dependent β-thalassemia major who required regular red blood cell transfusions to survive, and patients receiving regular iron chelating therapy such as deferoxamine (Desferal®) or deferasirox (Exjade®)**.** Patients with other types of anemia and uncooperative patients were excluded from the study.

Participants and Groups:

The participants were split into two equal groups: Group A comprised of 50 patients (27 female and 23 male) ,their age range between (19-40)with TDT receiving iron chelating therapy, and Group B included

50 apparently healthy subjects (26 female and 24 male),their age range between(20-40) matched in terms of age and sex.

Every patient was subjected to the following:

1-History-taking including personal history (residence, social level, age, and gender) and a medical history of the current condition, with particular focus on jaundice, fatigue, clinical presentation, duration of symptoms, blood transfusion length, and iron chelating therapy used, and its duration, Family history also taken.

2-Clinical Examination: Thorough clinical examinations were conducted, including general examination (awareness level, pallor, jaundice, and cyanosis), vital signs, and anthropometric measurements.

3-Laboratory Investigations:

Five ml of venous whole blood was drawn in an entirely aseptic environment from each participant and distributed as follows:

3 ml were evacuated into a serum separator tube and were left for 30 min till clotting then centrifuged at 1500×g for 10 min. The separated serum was employed for the estimation of kidney function (Urea - Creatinine), liver function (ALT- AST- total billirubin and direct billirubin), and iron status (serum iron levels, serum ferritin, and TIBC) using a chemical auto analyzer (Abbott, Architect c 4000 Serial No.02p24-01, Japan.).

2ml were collected into EDTA blood tube and divided into 2 tubes.

- 1 ml was evacuated into an EDTA-blood tube and was used for CBC using a blood counter (Sysmex Xs800, Japan, Serial No. 63387), hemoglobin electrophoresis using (SEBIA minicap Serial No. 2447, Evry _ France) and reticulocyte stained with brilliant cresyl blue, and counted manually, the rest of blood was stored at -80°c. till used for subsequent evaluation of the HAMP gene variant(C.-582A>G) using RFLP-PCR.

Gene study:

The assessment of Hepcidin Antimicrobial Peptide (HAMP) gene variant (C.-582A>G) (rs10421768) was performed using the RFLP-PCR technique.

1. DNA Extraction:

Blood samples were subjected to DNA extraction in accordance with the manufacturer's procedure utilizing the Gene JET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Germany), catalogue numbers (#k0781, #k0782).

2. Amplification of purified DNA PCR technique and restriction digestion of resulting PCR amplicon:

Amplification was performed using Cosmo PCR RED M.mix (Willofort, Uk, catalog No,Wf 10203001), by adding DNA polymerase**,** dNTPs, and optimized buffer, the Amplification mixture comprised of Cosmo Master mix a total volume of **25µl** : (Cosmo PCR m. Mix 12.5µl, Forward Primer PF1 **1.5µl**, Reverse Primer PR1 **1.5µl**, H2o **6.5µl**, Sample **3µl**).

Forward Primer:

´5GTGCTGGGCCATATTACTGCT´3

Reverse Primer:

´5CACGTGCATAGGTTCTGGCA´3

The following conditions were used for the PCR: 5 minutes at 94 °C, 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and a final extension at 72 °C for a total of 10 minutes (Veriti 96 Well Thermal Cycler Applied Biosystem, Singapore).

Following PCR amplification, the output PCR amplicon was digested using Bstul restriction enzyme. The reaction mixture was prepared by adding the following

ingredients: 5 µL of the PCR reaction mixture, 16.5 µl nuclease-free water, 2.5 µl 10x buffer, and 1 µl Bstul restriction enzyme, Following gentle mixing and brief spinning, the PCR amplicon was incubated at 60 °C for three hours before being denatured at 4°C by the restriction enzyme at the appropriate restriction sites on the DNA into fragments of the necessary size.

3. Separation of DNA fragments by gel electrophoresis technique:

Ethidium bromide-stained 2% agarose gel was used to separate the digested products. then the power supply was turned on for the gel run, which used an ultraviolet light transilluminator to view the separated digested DNA fragments into distinct bands. The 3 expected genotypes appeared as follows: one band at (179Bp) represents the AA genotype, two bands at (102Bp) and (77Bp) represent the GG genotype, while three bands at (179BP), (102Bp), and (77Bp) represent the AG genotype.

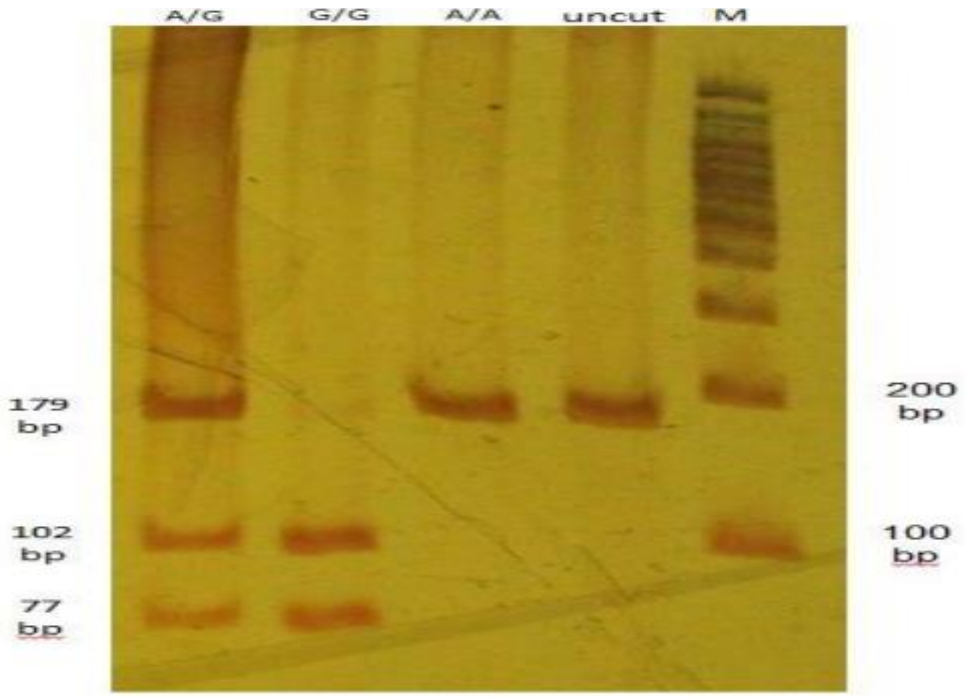

Figure (1): The Hepcidin c.-582 A>G polymorphism was identified via RFLP-PCR utilising promoter primers specific to Hepcidin and digestion with restriction enzyme Bstu1. Lane M: DNA ladder (100bp). Lane uncut: PCR product without digestion. Lane A/A: homozygote for wild-type allele that does not be digested (179 bp). Lane G/G: homozygote for c.-582A>G variant exhibiting digested bands (102bp and 77bp). Lane A/G: heterozygotes for c.-582A>G exhibiting normal undigested band (179bp) and the digested c.-582A>G variant (102 bp and 77bp)

Statistical Analysis:

Version 18 of the SPSS program (SPSS Inc., PASW statistics for Windows version 18) was utilized to analyze the data (Chicago Illinois, USA). An appropriate analysis was carried out based on the kind of data that was obtained for each parameter. Number and percent values were utilized to determine the qualitative data. After determining the normalcy of the quantitative data utilizing Kolmogorov-Smirnov test, the data were reported

utilizing the median (minimum and maximum) for non-normally distributed data and the mean and standard deviation for normally distributed data. Using the results, the P value was determined; a value of less than 0.05 was considered statistically significant. When appropriate, Chi-Square and Monte Carlo tests were performed to contrast qualitative data between groups. For normally distributed data, student t-test was utilized to contrast two independent groups. When comparing the outcomes of more than two independent groups, the One Way ANOVA test was employed.

Quanto Calculator software was utilized to determine the sample size (Version 1.2.4), For Sample Size or Power For Association Studies *in 2006*. [7].

The sample size calculations was conducted under minor allele frequency of healthy subjects as 0.026 as reported in Asian race *(NCBI, 2022),* AA= 55, AG=41, GG=6, as reported in *2020* [8], calculated minor allele frequency in thalassemic cases=26%, calculated odds ratio for allelic model 4.185, prevalence of beta thalassemia major of 4% as reported in *2000* [9], 80 % power and a 0.05 level of significance were utilized in the case-control study. For each group, a minimum sample size of 49 is needed.

RESULTS

Regarding clinicodemographic data, there was no significant difference between both groups concerning age and sex (p-value >0.05). In the patients group, anemia was present in 100%, Jaundice in 30%, hepatosplenomegaly in 84%, bone abnormalities in 86%, skin bronzing in 24%, and endocrinal disturbance in 14%, as shown in table (1).

As described in Table (2), there was a significant difference between both groups concerning reticulocyte count, LDH, AST, total and indirect bilirubin p-value <0.001), also there was a significant variation between both groups concerning urea and creatinine (p-value <0.002,0.295 respectively), while, there was no significant variatin between both groups concerning ALT and direct bilirubin (p-value > 0.05).Regarding hb electrophoresis,there was significant difference between both groups as A band was significant decrease in case group compared to control group, while A2,F bands were significant increase in case group compared to control group. The iron profile showed a significantly higher serum iron and ferritin level in the thalassemia group than the control group (p-value <0.001), while TIBC was significantly decreased in thalassemia group than the control group (p-value <0.001)

In table (3) on assessment of the Hardy-Weinberg equilibrium for (C.-582A>G) polymorphism.

The P-value for the cases group is 0.768, as well as for the control group is 0.335, indicating

that the observed genotype frequencies in the cases as well as the control group are not significantly different from the expected frequencies under HWE.

Regarding the hepcidin gene variant (C.-582A>G)), the GG and AG genotypes showed

significantly elevated frequency in thalassemia patients in contrast to the control group. While the AA genotype showed significantly reduced frequency in thalassemia patients contrasted with the control group (p-value <.001), as described in table(3)

In table (4) Concerning refraction to iron chelation therapy, the AG and GG genotypes of the hepcidin variant (C.-582A>G) were significantly higher in

patients not responding to iron chelation compared to patients who responded to iron chelation therapy. as shown in table (4).

Table (1): The demographic and clinical data of the studied groups.

	Cases group (n = 50)	Control group (n = 50)	Test of significance	P value
Age			**t**	**0.699**
Mean ± SD	31.4 ± 5.05	31.8 ± 4.74	**0.388**	
Min. – Max.	19.0 – 39.0	19.0 – 39.0		
Sex			χ^2	**0.841**
Male	23	24	**0.040**	
	46.0%	48.0%		
Female	27	26		
	54.0%	52.0%		
Anemia				
No	0	50		
	0.0%	100.0%		
Yes	50	0		
	100.0%	0.0%		
Jaundice				
No	35	50		
	70.0%	100.0%		
Yes	15	0		
	30.0%	0.0%		
Hepatosplenomegaly				
No	٨	50		
	١٦.0%	100.0%		
Yes	٤٢	0		
	٨٤.0%	0.0%		
Bone abnormalities				
No	٧	50		
	١٤.0%	100.0%		
Yes	٤٣	0		
	٨٦.0%	0.0%		
Skin bronzing				
No	38	50		
	76.0%	100.0%		
Yes	12	0		
	24.0%	0.0%		
Endocrinal disturbance				
No	43	50		
	86.0%	100.0%		
Yes	7	0		
	14.0%	0.0%		

T:Independent t test **Chi square test X**:

Table (2): Comparison between the studied groups regarding laboratory data

	Case group(n=50)	control group(n=50)	test of significance	p value
Hb gm/dl				
Mean ± SD	7.8 ± 0.39	11.2 ± 0.54	**t**	**<0.001***
Min. – Max.	7.1 – 8.5	10.2 – 12.0	**34.934**	
RDW%			**t**	**<0.001***
Mean ± SD	19.5 ± 1.64	13.4 ± 1.25	**20.843**	
Min. – Max.	16.0 – 22.0	12.0 – 15.0		
Reticulocyte after correction %			**t**	**<0.001***
Mean ± SD	3.6 ± 0.38	1.0 ± 0.38	**33.889**	
Min. – Max.	2.8 – 4.1	0.6 – 2.0		
LDH IU/L			**U**	**<0.001***
Median (IQR)	405.0 (380.0 – 425.75)	128.0 (121.25 – 211.25)	**0.0**	
AST (U/L)			**U**	**<0.001***
Median (IQR)	46.0 (42.0 – 50.0)	27.0 (22.0 – 29.0)	**0.0**	
ALT (U/L)			**t**	**0.701**
Mean ± SD	25.7 ± 6.44	25.3 ± 3.51	**0.386**	
Min. – Max.	18.0 – 45.0	18.0 – 32.0		
Direct bilirubin (mg/dl)			**t**	**0.821**
Mean ± SD	0.26 ± 0.03	0.26 ± 0.03	**0.227**	
Min. – Max.	0.21 – 0.29	0.2 – 0.3		
Indirect bilirubin (mg/dl)			**t**	**<0.001***
Mean ± SD	1.2 ± 0.16	0.4 ± 0.29	**19.808**	
Min. – Max.	0.85 – 1.5	0.2 – 0.9		
Total bilirubin (mg/dl)			**t**	**<0.001***
Mean ± SD	1.4 ± 0.16	0.7 ± 0.29	**16.404**	
Min. – Max.	1.08 – 1.78	0.44 – 1.7		
Urea (mg/dl)			**t**	**<0.002***
Mean ± SD	21.9 ± 3.10	19.5 ± 1.76	**3.238**	
Min. – Max.	17.0 – 29.0	17.0 – 22.0		
Creatinine (mg/dl)			**U**	**0.295**
Median (IQR)	0.9 (0.8 – 1.05)	0.8 (0.675 – 0.9)	**1100.0**	

Continue of table (2):

	Case group(n=50)	control group(n=50)	test of significance	p value
Hb electrophrosis				
A			**U**	**<0.001***
Median (IQR)	2.0 (0.0 – 11.25)	96.0 (96.0 –96.8)	**36.0**	
A2			**t**	**<0.001***
Mean ± SD	5.5 ± 0.74	2.5 ± 0.33	**26.071**	
Min. – Max.	4.5 – 7.0	2.0 – 3.0		
F			**U**	**<0.001***
Median (IQR)	91.5 (83.5 – 94.5)	1.25 (1.0 – 1.625)	**0.0**	
S. Iron (μg/dl)			**U**	**<0.001***
Median (IQR)	190.0 (180.0 – 210.0)	115.0(104.75-156.0)	**146.0**	
Ferritin (ng/ml)			**U**	**<0.001***
Median (IQR)	438.0 (406.0 – 520.0)	110.0(93.75-206.25)	**94.0**	
TIBC (μg/dl)			**U**	**<0.001***
Median (IQR)	210.0 (206.75 – 214.25)	281.0(250.0-350.0)	**0.000**	

IQR: Interquartile range t: Independent t test U: Mann Whitney U test

***p≤ 0.05 (Statistically significant)**

Table (3): comparison between the frequency of the (C.-582A > G) hepcidin gene variants in studied groups

	Cases group (n = 50)	Control group (n = 50)	Total (n = 100)	Test of significance	P value
C-582A > G				**MC**	**<0.001***
AA	20	38	58		
	40.0%	76.0%	58.0%		
AG	24	12	36		
	48.0%	24.0%	36.0%		
GG	6	0	6		
	12.0%	0.0%	6.0%		
HW p	X2=0.087	X2=0.930			
	P=0.768	0.335			

MC :Monte Carlo Exact test ***p≤ 0.05 (Statistically significant).** **HW p:Hardy-Weinberg equilibrium**

Table (4) : Refraction to iron chelation based on the genotype of c.-582A > G variant

	AA (n = 20)	AG (n=24)	GG (n = 6)	Test of significance	P value
Response to iron Chelation thearpy				**MC**	**<0.001***
Not Respond	5	24	6		
	25.0%	100.0%	100.0%		
Respond	15	0	0		
	75.0%	0.0%	0.0%		

MC: Monte Carlo Exact test ***p ≤ 0.05 (Statistically significant)**

Table (5): The demographic and clinical data of the participants based on genotype of (c.-582A > G) variant

	AA (n = 58)	AG (n=36)	GG (n = 6)	Test of significance	P value
Age				**Kruskal Wallis**	**0.989**
Median (IQR)	32.0 (28.0 – 36.0)	31.5 (28.25– 36.5)	33.0 (27.5 – 36.25)	**0.023**	
Sex				**MC**	**0.895**
Male	26	18	3		
	44.8%	50.0%	50.0%		
Female	32	18	3		
	55.2%	50.0%	50.0%		
Anemia					
Yes	20	24	6		
	100.0%	100.0%	100.0%		
Jaundice				**MC**	**0.912**
No	15	16	4		
	75.0%	66.7%	66.7%		
Yes	5	8	2		
	25.0%	33.3%	33.3%		
Hepatosplenomegaly				**MC**	**0.256**
No	4	2	2		
	20.0%	8.3%	33.3%		
Yes	16	22	4		
	80.0%	91.7%	66.7%		
Bone abnormalities				**MC**	**0.738**
No	3	4	0		
	15.0%	16.7%	0.0%		
Yes	17	20	6		
	85.0%	83.3%	100.0%		
Skin bronzing				**MC**	**0.246**
No	17	18	3		
	85.0%	75.0%	50.0%		
Yes	3	6	3		
	15.0%	25.0%	50.0%		
Endocrinal disturbance				**MC**	**0.860**
No	18	20	5		
	90.0%	83.3%	83.3%		
Yes	2	4	1		
	10.0%	16.7%	16.7%		

Table (6): Laboratory data of the participants based on genotype of (c.-582A > G) variant

	AA (n=58)	AG (n=36)	GG (n=6)	Test of significance	P value
Hb(g|dl)				**Kruskal Wallis 12.835**	**0.002***
Median (IQR)	10.75 (8.0 – 11.0)	8.3 (7.625 – 10.7)	7.9 (7.325 – 8.275)		
	p1 = 0.004* p2 = 0.007* p3 = 0.219				
MCV(Fl)				**Kruskal Wallis 16.351**	**<0.001***
Median (IQR)	84.0 (72.0 – 90.0)	73.5 (70.0 – 83.0)	70.0 (67.75 – 70.0)		
	p1 = 0.010* p2 = <0.001* p3 = 0.027*				
MCH (pg.)				**Kruskal Wallis 9.393**	**0.009***
Median (IQR)	27.5 (23.0 – 29.0)	24.0 (21.0 – 27.75)	23.5 (21.75 – 24.25)		
	p1 = 0.009* p2 = 0.039* p3 = 0.457				
MCHC (%)				**Kruskal Wallis 14.030**	**0.001***
Median (IQR)	33.0 (31.0 – 34.0)	31.0 (29.0 – 32.75)	30.0 (29.75 – 32.0)		
	p1 = 0.001* p2 = 0.013* p3 = 0.391				
RDW%				**Kruskal Wallis 10.578**	**0.005***
Median (IQR)	14.5 (12.75 – 18.25)	18.5 (15.0 – 20.0)	19.5 (17.75 – 20.0)		
	p1 = 0.007* p2 = 0.022* p3 = 0.353				
S. Iron (µg/dl)				**Kruskal Wallis 7.893**	**0.019***
Median (IQR)	152.5(113.0– 191.25)	180.0(150.0 – 195.0)	190.0(186.25–206.25)		
	p1 = 0.087 p2 = 0.001* p3 = 0.016*				
Ferritin (ng/ml)				**Kruskal Wallis 19.926**	**<0.001***
Median (IQR)	207.5(105.0– 406.0)	416.0(218.75–520.0)	463.0(360.0–1652.5)		
	p1 = <0.001* p2 = 0.005* p3 = 0.407				
TIBC (µg/dl)				**Kruskal Wallis 10.026**	**0.007***
Median (IQR)	250.0 (214.25 – 310.0)	211.0 (207.25 – 258.25)	216.5 (212.25 – 218.5)		

IQR: Interquartile range ***p ≤ 0.05 (Statistically significant)**

(p1: between AA & AG, p2: between AA & GG, p3: between AG & GG)

MC: Monte Carlo Exact test

Table (5) shows the association between the hepcidin gene variant (C.-582A>G) and the clinicodemographic data, there was no significant difference between the hepcidin gene variant (C.-582A>G) with age, sex, and clinical data (p-value> 0.05). A significant correlation was observed between the hepcidin gene variant (C.-582A>G) and laboratory data, GG genotype showed a significant decrease with Hb, MCV, MCH, MCHC, TIBC, and a significant rise with RDW, serum iron, and ferritin than AG, AA genotypes (p-value <.001) as described in Table (6).

Discussion

β-thalassemia is one of the most common types of thalassemia, characterized by a quantitative impairment in haemoglobin β chain formation. β-thalassemia is distinguished by the presence of anaemia, the severity of which is contingent upon the location of the gene encoding the β chain, which is situated on chromosome $_{11}$p15.4 and comprises two introns and three exons[8].

β-thalassemia major patients treated by iron chelation therapy ,but it's not effective in some patients due to considering factors like treatment adherence and individual variability in response to treatment ,and this lead to iron overload[10]

The long term outcomes of iron overload are cardiomyopathy which is the most common cause of death ,diabetes ,skeletal deformities ,splenomegaly ,pituitary failure ,hypothyroidism and liver failure .[11]

Hepcidin is a significant regulator of iron hemostasis, particularly in thalessemia, and is implicated in a number of iron metabolic pathways [12].

The impact of single nucleotide polymorphisms situated in the promoter region of HAMP on hepcidin expression has been observed in multiple investigations [13].The study was done in 2010 [6] found that the(C.-582A>G) variant reduces the expression of HAMP, and this lead to iron overload causing multiple organ damage especially in liver and heart like cirrhosis , diabetes and heart failure , also causing endocrinal disturbances , bone and skin abnormalities.

The objective of this research was to examine the correlation between iron overload and the Hepcidin gene polymorphism (C.-582A>G) in patients with significant β-thalassemia who were resistant to iron chelating therapy.

Our findings indicated that the frequency of hepcidin gene variant (C.-582A>G), GG and AG genotypes were significantly increased in thalassemia patients who regularly blood transfused and on iron chelation therapy in contrast to control group, while AA genotype showed significantly reduced frequency in thalassemia patients compared to control group (P value <0.001), our results were compatible with a study's findings that was done in 2020 [8]. who discovered that the frequency of (C.-582A>G) GG,AG genotypes were higher frequency in thalessemia patient compared to control group, while AA genotype showed a significant lower frequency in thalessemia patient compared to control group.

In our study, the (C.-582A>G) GG genotype exhibited higher mean serum iron, ferritin, and lower TIBC than AA & AG genotypes, this is consistent with a study in 2020[8] who showed that the C.-582A>G variant's GG genotype is linked to a high level of heart iron overload. Additionally, the serum ferritin level was assessed, and ferritin levels > 1000 ng/ml were found in all patients with the GG genotype. The findings of their investigation support the notion that iron accumulation in the liver and heart can occur in certain people even in the presence of large doses of chelating medication, even when used regularly. This can be linked, at least partially, to the patients' genetic heritage. For these patients, mini-hepcidin may be used as an alternative to a large dose of chelating treatment. Furthermore, they reported that iron overload in β-thalassemia major patients was associated with three SNPs in the promoter of HAMP, c.-582A > G, c.-443C > T, and c.-153C > T. They also found that homozygous patients for the G allele had significantly more iron deposition in cardiac tissue (p = 0.02). [8]

In contrast to our study, a study was done in 2009 [14] showed no significant variations were noted in the ferritin, iron, and transferrin levels between the AA&AG in c.-582A > G GG genotypes. Nonetheless, the reason for this disparity may be traced back to the role that polymorphism plays in determining the degree of gene expression, resulting in varying levels. Moreover a study was done in 2010 [15], claimed that, in comparison to the presence of "A" at the same position, the less prevalent "G" at -582 was linked to greater liver iron concentrations (LIC), as measured by liver biopsy, and higher serum ferritin in patients. This distinction was found exclusively among patients (52 males and 45 females) who received iron chelator treatment sporadically; regular iron chelation treatment is likely capable of superseding any potential variations. The clinical manifestation of iron overload in

patients with beta-thalassemia can be influenced by various factors, such as chelation regimens, blood transfusions, and iron absorption resulting from erythropoietic expansion. Nevertheless, upon careful examination of many factors, the authors postulate that in theory, the hepcidin levels of these patients could be lower than those of the similar patients lacking the polymorphic alteration.

On the contrary, the Galician study in 2010 [6] reported that There was no statistically significant correlation observed between the c.-582A > G genotype and levels of blood iron, transferrin, transferrin saturation, or ferritin. This finding may indicate that there are no variations in the concentration of iron in the liver; this contrariety may be due to difference in study design, population characteristics, methodology and genetic interactions with other factors.

Furthermore, a study done in 2019 [16] found that there was a difference in blood ferritin levels between the two genotypes, but no significant correlation was found between the blood ferritin level and the (C.-582A>G) polymorphism in the patients who normally ingested chelator. This suggests that the polymorphism may have an impact on the HAMP promoter's function, which can be overpowered by chelator ingestion on a frequent basis (environmental effect on the gene). Nevertheless, the function of the hepcidin protein may vary, which was not examined in their study, and other factors may also have an impact on the rise in iron overload, contributing to the variation in ferritin levels.

In our study, serum ferritin exhibited significant rise in B thalassemia major patients in contrast to control groups (P-value <0.001).Supporting our results, studies were done in 2014, 2017 and 2021 [17-19], reported that, concerning pre-transfusion iron overload parameters, they declared that serum ferritin levels were raised in β- thalassemia major patients and thalassemia intermediate patients respectively in contrast to normal controls.

Concerning refraction to iron chelation therapy, the AG & GG genotypes of hepcidin variant (C.-582A>G) were significantly higher in patients not responding to iron chelation in comparison to patients responding to iron chelation therapy.

This study is useful as clinical management strategy by using Hepcidine gene polymorphism (C.-582A>G) as a marker for iron overload in other populations or to explore its potential use in other conditions associated with iron metabolism disorders[20]

Conclusion

The present research demonstrates a possible association between the Hepcidin gene polymorphism (C.-582A>G) and excessive iron accumulation in β-Thalassemia major patients who are unresponsive to iron chelation therapy. Particularly, the GG genotype of this genetic variation is correlated with elevated serum iron and ferritin levels in contrast to the AA/AG genotypes, Therefore, the Hepcidin gene polymorphism (C.-582A>G) could serve as a valuable marker for identifying β-Thalassemia major patients at risk of iron overload, potentially benefiting from more aggressive iron chelation therapy.

Recommendation

To conclude the true association, a wide scale study will be conducted with a large cohort of Cases and controls.

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