Original Article

Association of HFE Gene Polymorphism and Ferritin with the Iron Overload in β-Thalassemia Major Patients.

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Abstract

Background: The human hemochromatosis protein HFE is encoded by the HFE gene and participates in iron regulation. **Objectives:** to detect the most frequent HFE gene mutations in a control population and genotype thalassemia major. **Material and Methods:** A total of 100 individuals (50 normal and 50 patients) were examined. Genetic diagnosis of HFE gene polymorphisms was performed by Conventional polymerase chain reaction (PCR) analysis. **Results:** The heterozygous rs1799945 (CG) genotype was significantly among β-TM patients than in controls (p = 0.041) and the homozygous (GG) genotype was significantly higher among β-TM patients than in controls (p = 0.008). Odds ratio [OR](CG, GG) = 0.28 and 0.28, 95% confidence interval [CI] = [0.08-0.95], [0.11-0.72], respectively). The results show that there is a highly significant allelic frequency difference between case and control groups (P= 0.0003). The heterozygous rs1800562 (GA) genotype was significantly higher among β-TM patients than in controls (p = 0.028) and the homozygous (AA) genotype was significantly among β-TM patients than in controls (p = 0.052). Odds ratio [OR] (GA, AA) = 0.27 and 0.35, 95% confidence interval [CI] = [0.08-0.87], [0.12-1.01] , respectively). The results show that there is highly-significant allelic frequency difference between case and control groups (P= 0.003). Serum levels of ferritin in patients with β-thalassemia major patients were higher than control the mean ± standard deviation patients groups (2008.44 ± 690.39) and (84.59 ± 21.27) in control group, the difference was highly significant (P< 0.001). **Conclusion:** This study shows that as there is a correlation between HFE mutation and ferritin levels the presence of HFE mutation may be a predictor of susceptibility to iron overload due to high level of ferritin in Beta thalassemia patient.

Keyword: HFE; beta-thalassemia; hereditary hemochromatosis; iron metabolism

Introduction

β-thalassemia is defined as a relative excess of α chains with a decreased or nonexistent β-globin chain synthesis. As these chains build up and precipitate in the erythroid precursors, they form inclusion bodies that, when attached to the membrane skeleton, cause the red blood cell precursors in the bone marrow to undergo extensive premature destruction through apoptosis, leading to oxidative membrane damage and ineffective erythropoiesis [1]. Iron overload, hyperbilirubinemia, and osteoporosis are a few of these known side effects [2–4]. The most prevalent genetic iron overload condition in Caucasians is called hereditary hemochromatosis (HH). The HFE gene has the most frequent mutations, while other variants may also cause the clinical condition [5]. HFE, which is connected to the major histocompatibility complex (MHC) on chromosome 6p, was

discovered in 1996 by Feder and associates via positional cloning [6]. Hepcidin is the primary regulator of iron metabolism, and its correct regulation in the liver depends on the HFE protein [1]. In white people descended from Western Europe, about 90% of hemochromatosis phenotypes are caused by common HFE mutations [6]. The 845G polymorphism, which results in an amino acid change (C282Y) in the p.Cys282Tyr region of the HFE protein, is the most common disease-causing mutation in the general population. In European populations, homozygosity for this single missense alteration (p.Cys282Tyr) accounts for 60–100% of instances of HH [5]. Using HFE gene sequencing techniques, further HFE mutations with various pathogenic implications have been found. Among them are the changes to the amino acids H63D and S65C [7]. In patients with b-thalassemia, the coexistence of HFE gene-associated hereditary hemochromatosis and b-thalassemia can worsen iron overload and iron-related problems. It has been noted that b-thalassemia and hemochromatosis are commonly linked. Numerous investigations have demonstrated that the combination of b-thalassemia and hereditary hemochromatosis can cause these patients to have an augmented response to iron absorption and storage [8]. Two frequent missense mutations, c.845G>A (p.C282Y; rs1800562) and c.187C>G (p.H63D; rs1799945), have been identified in patients with HFE-associated hereditary hemochromatosis. The distribution of these variants exhibits notable ethnic diversity, with the p.C282Y mutation primarily occurring in North Europe. This variation is thought to be extremely rare in populations of Asian, African, and Australian descent. It is demonstrated that the p.H63D mutation has a frequency of 3.3%−15.2% and a cosmopolitan distribution worldwide [9,10]. Iron overload and related diseases can result from inefficient erythropoiesis and excessive iron absorption in beta-thalassemia major caused by repeated blood transfusions. A common observation in beta-thalassemia major is hemochromatosis [11]. The erythroid regulator, which adjusts intestinal iron absorption in response to erythron demands, and the storage regulator, which manages iron buildup, are the two separate routes that have been hypothesized to control iron metabolism [12,13]. Erythroid regulator in these patients boosts iron absorption due to inefficient erythropoiesis; nonetheless, there is debate regarding the impact of these mutations on iron burden in b-thalassemia major patients [14].

In order to look at how these mutations affect serum ferritin levels, we determined the prevalence of HFE gene mutations among patients with b-thalassemia major in this study.

Materials and Methods

Study group

A total of 100 individuals were reported for major β-thalassemia in the Maternity and Children's Hospital in Hilla city, Babylon province. from January 2023 to April 2023. Experimental work was carried out at private laboratories in Babylon , Iraq. The volunteers in the current study were divided based on simple randomization into two groups: healthy volunteers as the control group (n=50) and β-thalassemia patients as the case group (n=50). Groups were matched based on gender and age. The inclusion criteria were suffering from major β-thalassemia, whereas the exclusion criterion was any other type of anemia or any other related diseases.

Genomic DNA Analysis (Genotypic Analysis)

Detection of HFE (rs1799945) and (rs1800562) gene polymorphisms was done by Conventional

polymerase chain reaction (PCR) analysis. Two milliliters of venous blood were collected on EDTA vacutainer tubes. Samples were stored at -20° C till DNA extraction which was performed using the Blood Genomic DNA Purification Kit (favorgen).

This was followed by amplification of the extracted DNA for detection of different polymorphisms using Conventional polymerase chain reaction (PCR) was used to amplify the target DNA using a specific primer for both (HFE (rs1799945) and (rs1800562)). It includes three consecutive steps that are repeated for a specific number of cycles to get PCR product (amplicon), which can finally be visualized after agarose gel electrophoresis. The thermal cycling conditions are mentioned in Table 1. Sequencing of polymerase chain reaction products Forty microliters of PCR product were sent to Macrogene/ Korea for Sanger sequencing. After trimming each sequence, the results of the trimmed sequence were blasted in NCBI to check the similarities and differences with the database.

Table 1: Primers sequences of rs1800562 and rs 1799945 SNP for HFE gene.

Primer name	Primer sequence 5′ to 3′	Annealing Temp.	Product size	Reference
rs1799945	F-5-GGACTGCAACTC ACCCTTCA-3 R-5-CTCTTCCCTGCTC CCACAAG-3	59.5	506 bp	Designed by this study
rs1800562	F-5-TCCCCTCTCCTCA TCCTTCC-3 R-5TCCAATGACTAGG GTGCCAG-3	58.7	500 bp	Designed by this study

Statistical analysis

Statistical package for the Social Sciences (SPSS) version 25 (IBM Corp., Armonk, NY, USA) was used for Coding, entering data and statistical analysis. Data were summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Genotype and allele frequencies were compared between the disease and the control groups. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. P-values less than 0.05 were considered as statistically significant. Multi-variate linear regression analysis was done to examine possible confounders in significant relations 9 e.g., patient age, age of onset of transfusion, transfusion duration, chelation therapy duration, chelation therapy compliance and serum ferritin level.

Ethical Approval

Ethical Approval in this study was carried out through it, Ethical committee in the College of Medicine, Al-Qadisiyah University, and Verbal of agreement consent from each patient as well as control. Subject information, and consent form were reviewed and approved by a local ethics committee according to document number 30/1237, dated January 10, 2023, to get this approval.

Results

Serum ferritin level in patients and control groups.

The comparison of serum ferritin level between β-thalassemia patients and healthy controls groups has been carried out and the results were demonstrated in table (2) serum levels of ferritin in patients with β-thalassemia major patients were higher than control the mean ± standard deviation patients groups (2008.44 ± 690.39) and (84.59 ± 21.27) in control group, the difference was highly significant (P< 0.001).

Table 2: Distribution of ferritin among study population; β-thalassemia major patients and healthy control.

Parameter	Study groups	No.	Mean±SD	T test	P Value
Ferritin	β-thalassemia major patients	50	2008.44 ± 690.39	24.11	< 0.001
	Healthy control	50	84.59 ± 21.27		
*(P<0.01), SD: standard deviation.					

Statistical Analysis Association of HFE Gene polymorphism with β-thalassemia major

The prevalence of the HFE (rs1799945) and (rs1800562) gene polymorphisms among the cases and control subjects are listed in Table 2. The heterozygous rs1799945 (CG) genotype was significantly among β-TM patients than in controls (p = 0.041) and the homozygous (GG) genotype was significantly higher among β-TM patients than in controls (p = 0.008). Odds ratio [OR](CG,GG) = 0.28 and 0.28, 95% confidence interval [CI]=[0.08-0.95], [0.11-0.72], respect-ively. The results show that there is highly-significant allelic frequency difference between case and control groups (P= 0.0003).

The heterozygous rs1800562 (GA) genotype was significantly higher among β-TM patients than in controls (p = 0.028) and the homozygous (AA) genotype was significantly among β-TM patients than in controls (P=0.052). Odds ratio [OR] (GA,AA) = 0.27 and 0.35, 95% confidence interval [CI] = [0.08-0.87], [0.12-1.01] .

The results show that there is highly-significant allelic frequency difference between case and control groups (P= 0.003).

Table 3: Genotype and allele frequency of *HFE* Gene polymorphism SNP (rs1799945) and (rs1800562).

SNP	Patients (n=50)	Controls (n=50)	Chi-square	P=value	Odd (95%CI)
rs1799945					
CC	20(40%)	35(70%)	Reference		
CG	10(20%)	5(10%)	2.03	0.041*	0.28(0.08-0.95)
GG	20(40%)	10(20%)	2.62	0.008*	0.28(0.11-0.72)
Allele					
C	50 (50%)	75(75%)	3.59	0.0003*	0.33(0.18-0.60)
G	50(50%)	25(25%)			
rs1800562					
GG	25(50%)	38(76%)	Reference		
GA	12(24%)	5(10%)	2.18	0.028*	0.27(0.08-0.87)
AA	13(26%)	7(14%)	1.94	0.052	0.35(0.12-1.01)
Allele					
G	62(62%)	81(81%)	2.93	0.003*	0.38(0.20-0.72)
A	38(38%)	19(19%)			

Figure 1: Electrophoresis pattern of Allele Specific PCR of HFE Gene SNP (rs 1799945) genetic polymorphism showed: Lane M: DNA ladder 100 - 1500 bp, Lane 1 to 5.

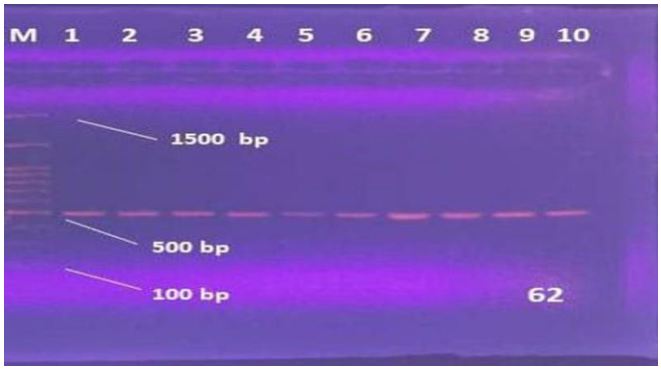

Figure 2: Electrophoresis pattern of Allele Specific PCR of HFE Gene SNP (rs rs1800562) genetic polymorphism showed: Lane M: DNA ladder 100 - 1500 bp, Lane 1 to 5.

Association of Novel SNP of HFE gene polymorphism with β-thalassemia major.

Novel SNP of HFE (rs1799945) (C\T87, T\C134 and A\T376) and (rs1800562) (T\C 86, C\T237

and T\A410) gene polymorphisms among the cases and control subjects are listed in Table 4, 5. The heterozygous and homozygous of rs1799945 (C\T87) (CT, TT) genotype was significantly higher among β-TM patients than in controls (p = 0.005, 0.029). Odds ratio [OR] (CT,TT) = 0.16 and 0.33 , 95% confidence interval [CI] = [0.04-0.56], [0.12-0.91], respectively). Allele frequency difference between case and control groups showed that there is highly-significant between case and control groups (P= 0.001). Odds ratio [OR](C, T) = 0.34 , 95% confidence interval [CI] = [0.18-0.64] , respectively).

T\C134 and A\T376 heterozygous and homozygous T\C134 (TC, CC) and A\T376 (AT, TT) genotype was non-significant among β-TM patients than in controls (p = 0.14, 0.59) and (0.08, 0.37). Odds ratio [OR] = (0.42, 0.70) (0.38, 0.62). 95% confidence interval [CI] = [0.13-1.36], [0.19-2.52] [0.13-1.16], [0.21-1.78], respectively. Showed that there is no-significant between case and control groups in Allele frequency (P= 0.20) (0.11). Odds ratio [OR] (T,C)(A,T) = (0.62, 0.11). 95% confidence interval [CI] = [0.30-1.29], [0.31-1.12], respectively).

Table 4: Genotype and allele frequency of *HFE* Gene polymorphism of novel SNP (rs1799945) (C\T87, T\C134 and A\T376).

SNP 1799945	Patients (n=50)	Controls (n=50)	Chi-square	P=value	Odd (95%CI)
C\T87					
CC	22(44%)	38(76%)	Reference		
CT	14(28%)	4(6%)	9.41	0.005*	0.16(0.04-0.56)
TT	14(28%)	8(16%)	4.75	0.029*	0.33(0.12-0.91)
Allele					
C	58 (58%)	80(80%)	11.31	0.001*	0.34(0.18-0.64)
T	42(42%)	20(20%)			
T\C134					
TT	34(68%)	40(80%)	Reference		
TC	10(20%)	5(10%)	2.14	0.14	0.42(0.13-1.36)
CC	6(12%)	5(10%)	0.28	0.59	0.70(0.19-2.52)
Allele					
T	78 (78%)	85(85%)	1.62	0.20	0.62(0.30-1.29)
C	22(22%)	15(15%)			
A\T376					
AA	28(56%)	36(72%)	Reference		
AT	12(24%)	6(12%)	2.95	0.08	0.38(0.13-1.16)
TT	10(20%)	8(16%)	0.78	0.37	0.62(0.21-1.78)
Allele					
A	68 (68%)	78(78%)	2.53	0.11	0.59(0.31-1.12)
T	32(32%)	22(22%)			

The heterozygous of rs1800562 T\C 86 (TC) genotype was highly significant among β-TM patients than in controls (p = 0.002). Odds ratio [OR](TC) = 0.14 . 95% confidence interval [CI] = [0.03-0.54]. Homozygous T\C 86 (CC) genotype was non-significant among β-TM patients than in controls (p = 0.75). Odds ratio [OR] = (0.85). 95% confidence interval [CI] = [0.32-2.29], respectively. Allele frequency difference between case and control groups showed that there is significant between case and control groups (P= 0.034). Odds ratio [OR] = 0.53. 95% confidence interval [CI] = [0.29-0.95], respectively).

Heterozygous and homozygous C\T237genotype was non-significant among β-TM patients than in controls (p = 0.11, 0.77). Odds ratio [OR] = (0.45, 1.20). 95% confidence interval [CI] = [0.17-1.22] [0.34-4.17], respectively. Allele frequency difference between case and control groups showed that there is no significant between case and control groups (P= 0.61). Odds ratio [OR] = 0.84. 95% confidence interval [CI] = [0.44-1.62] respectively.

The heterozygous T\A410 (TA) genotype was non-significant among β-TM patients than in controls (p = 0.15) odds ratio [OR] = 0.45. 95% confidence interval [CI] = [0.14-1.37] respectively. The homozygous (AA) genotype was significant among β-TM patients than in controls (p = 0.05). Odds ratio [OR] = 0.30. 95%

confidence interval [CI] = [0.08-1.05], respectively. The results show that there is highly-significant allelic frequency difference between case and control groups (P= 0.006). Odds ratio [OR] = 0.38. 95% confidence interval [CI] = [0.18-0.77], respectively.

Table 5: Genotype and allele frequency of *HFE* Gene polymorphism of novel SNP (rs1800562) (T\C 86, C\T237 and T\A410)

SNP rs1800562	Patients (n=50)	Controls (n=50)	Chi-square	P=value	Odd (95%CI)
T\C 86					
TT	25(50%)	35(70%)	Reference		
TC	15(30%)	3(6%)	9.62	0.002*	0.14(0.03-0.54)
CC	10(20%)	12(24%)	0.09	0.75	0.85(0.32-2.29)
Allele					
T	65 (65%)	73(73%)	4.49	0.034*	0.53(0.29-0.95)
C	45(45%)	27(27%)			
C\T237					
CC	30(60%)	35(70%)	Reference		
CT	15(30%)	8(16%)	2.47	0.11	0.45(0.17-1.22)
TT	5(10%)	7(14%)	0.08	0.77	1.20(0.34-4.17)
Allele					
C	75 (75%)	78(78%)	0.25	0.61	0.84(0.44-1.62)
T	25(25%)	22(22%)			
T\A410					
TT	30(60%)	40(80%)	Reference		
TA	10(20%)	6(12%)	2.02	0.15	0.45(0.14-1.37)
AA	10(20%)	4(8%)	3.81	0.05*	0.30(0.08-1.05)
Allele					
T	70 (70%)	86(86%)	7.45	0.006*	0.38(0.18-0.77)
A	30(30%)	14(14%)			

Discussion

The etiology of iron overload in BTM patients may be significantly influenced by mutations in the HFE gene. The purpose of this study was to identify the genotype and allele frequency of the common variations in the HFE gene, rs1799945 and rs1800562, in individuals with β-thalassemia. The frequency of HFE H63D and C282Y mutations in a cohort of 65 Iranian BTM patients and 200 healthy controls is presented here. H63D and C282Y mutation carrier frequencies were discovered to be 20% and 0% among BTM patients and 21% and 1.5% among control people, respectively. Therefore, there were no discernible variations between BTM patients and controls in the carrier frequencies of HFE H63D and C282Y mutations.. Other studies have shown that HFE gene mutations are common among β-thalassemia carriers compared with normal controls [15,16].

In a study of 33 Turkish patients with beta thalassemia major who reported a carrier frequency of 18.18% and 0% for H63D and C282Y mutations, respectively. [17] Also, the allelic frequencies found for H63D (12.31%) and C282Y (0%) mutations in present study were similar to the values obtained by Mokhtar et al, who reported an allelic frequency of 12.5% for HFE H63D mutation among Egyptian beta thalassemia patients. [18] Also, the prevalence of heterozygote and homozygote genotypes of HFE H63D (15.38%, 4.62%) and C282Y (0%, 0%) mutations were similar with the reported frequency from Turkish population [17] .The results of present study are in agreement with the some previously published studies that found no significant differences in genotypic and allelic distribution of HFE H63D and C282Y mutations between BTM patients and controls [18,19].

L´opez-Escribano *et al.*, and Mellouli *et al.* [20,21] A study to investigate the effects of HFE genotypes on iron markers in β-thalassemia carriers indicated that β-thalassemia was generally characterized by potential development of iron overload and tended to exacerbate with coinheritance of rs 1799945 polymorphism even in the heterozygous cases. [22] The βTM group showed a high frequency of the risk allele G. This result was in line with studies carried out in Egypt with 93 heterozygotes (21.1%) and Iraq with 14 heterozygotes (35%) in patient. [23]

According to Enein *et al.* and Zekavat et al. [19, 24] patients with TDT had a 39.5% frequency of H63D mutation. The hemochromatosis was often observed in β-thalassemia major. This will stop the aberrant HFE protein from attaching itself to the β2-microglobulin on the cell surface, which would allow the excess iron to be absorbed through the cell's crypt and into the bloodstream. Research on the function of H63D mutant polymorphism in β-thalassemia has been conducted in regions like southern Europe and Asia where the incidence of H63D is high. [25,26]

Conclusion

This study shows that as there is a correlation between HFE mutation and ferritin levels the presence of HFE mutation may be a predictor of susceptibility to iron overload due to high level of ferritin in Beta thalassemia patient.

References

[1] Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet. 2018; 391(10116): 155-167.

[2] Mettananda S, Higgs DR. Molecular basis and genetic modifiers of thalassemia. Hematol Oncol Clin North Am. 2018; 32: 177-91.

[3] Thein SL. Genetic modifiers of beta-thalassemia. Haematologica. 2005; 90: 649-60.

[4] Akkaif MA, Bitar AN, Al-Kaif LAIK, Daud NAA, Sha'aban A, Noor DAM, et al. The management of myocardial injury related to SARS-CoV-2 pneumonia. J Cardiovasc Dev Dis 2022;9:307

[5] Powell, L.W.; Seckington, R.C.; Deugnier, Y. Haemochromatosis. Lancet. 2016; 388: 706-716.

[6] Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996;13(4):399-408.

[7] Hollerer I, Bachmann A, Muckenthaler MU. Pathophysiological consequences and benefits of *HFE* mutations: 20 years of research. Haematologica. 2017; 102(5): 809-817.

[8] AlFadhli S, Salem M, Shome DK, Mahdi N, Nizam R. The Effects of *HFE* Polymorphisms on Biochemical Parameters of Iron Status in Arab Beta-Thalassemia Patients. Indian J Hematol Blood Transfus. 2017 Dec;33(4):545-551.

[9] Elmrghni S, Dixon RA, Williams DR. Frequencies of HFE gene mutations associated with haemochromatosis in the population of Libya living in Benghazi. Int J Clin Exp Med. 2011; 4: 200-4.

[10] Allen A, Premawardhena A, Allen S, Rodrigo R, Manamperi A, Perera L, et al. The p.H63D allele of the HFE gene protects against low iron stores in Sri Lanka. Blood Cells Mol Dis. 2019;76:72-7.

[11] Kosaryan MMR, Aliasgharian A, Mousavi M, Roshan P. Diagnostic value of fructosamine and glycosylated hemoglobin in estimating blood glucose level in diabetic patients with thalassemia major. mljgoums 2013;7:16–23.

[12] Steinberg MH, Forget BG, Higgs DR, Weatherall DJ. Disorders of hemoglobin: genetics, pathophysiology and clinical management. 2nd ed. Cambridge, UK: Cambridge University Press; 2009.

[13] Politou M, Kalotychou V, Pissia M, Rombos Y, Sakellaropoulos N, Papanikolaou G. The impact of the mutations of the HFE gene and of the

SLC11A3 gene on iron overload in Greek thalassemia intermedia and beta(s)/beta (thal) anemia patients. Haematologica 2004;89:490–2.

[14] Elmrghni S, Dixon RA, Williams DR. Frequencies of HFE gene mutations associated with haemochromatosis in the population of Libya living in Benghazi. Int J Clin Exp Med 2011;4:200–4.

[15] Madani HA, Afify RA, Abd El-Aal AA, Salama N, Ramy N. Role of HFE gene mutations on developing iron overload in beta-thalassaemia carriers in Egypt. East Mediterr Health J. 2011;17(6):546-51.

[16] Oliveira TM, Souza FP, Jardim AC, Cordeiro JA, Pinho JR, Sitnik R, et al. HFE gene mutations in Brazilian thalassemic patients. Braz J Med Biol Res. 2006; 39(12): 1575-80.

[17] Turedi A, Oymak Y, Meşe T, Yaman Y, Bayraktaroglu S, Alpman A, et al. The effect of HFE polymorphisms on cardiac iron overload in patients with betathalassemia major. Pediatr Hematol Oncol 2013; 30(8):755-760.

[18] Mokhtar DA, Hamdy MM, Badr AM. Frequency of human hemochromatosis (HFE) gene mutations in Egyptians with β-thalassemia. Egypt J Haematol 2013; 38: 36–40.

[19] Enein AA, El Dessouky NA, Mohamed KS, Botros SK, Abd El Gawad MF, Hamdy M, et al. Frequency of Hereditary Hemochromatosis (HFE) Gene Mutations in Egyptian Beta Thalassemia Patients and its Relation to Iron Overload. Open Access Maced J Med Sci 2016 15; 4(2):226-31.

[20] López-Escribano H, Ferragut JF, Parera MM, et al. Effect of co-inheritance of β-thalassemia and hemochromatosis mutations on iron overload. Hemoglobin. 2012; 36(1):85-92.

[21] Mellouli F, El Borgi W, Kaabi H, et al. HFE gene mutations in Tunisian major beta-thalassemia and iron overload. Transfus Clin Biol. 2006; 13(6):353-7.

[22] Hanson E, Imperatore G, Burke W. HFE gene and hereditary hemochromatosis HuGE review. Am J Epidemiol. 2001; 154(3):193-206.

[23] Al-Abedy NM, Salman ED, Faraj SA. Frequency of human hemochromatosis HFE gene mutations and serum hepcidin level in iron overload ?-thalassaemia Iraqi patients. Public Health 2019; 22:S275

[24] Zekavat OR, Jahromi MZ, Haghpanah S, Jahromi ZK, Cohan N. Association of HFE gene mutations with serum ferritin level and heart and liver iron overload in patients with transfusion-dependent beta-thalassemia. J Pediatr Hematol Oncol 2021; 43:e26–8

[25] Rahmani R, Naseri P, Safaroghli-Azar A, Tarighi S, Hosseini T, Hojjati MT. Investigation of correlation between H63D and C282Y mutations in HFE gene and serum ferritin level in beta-thalassemia major patients. Transfus Clin Biol 2019; 26:249–52

[26] Yang J, Lun Y, Shuai X, Liu T, Wu Y. Late-onset hemochromatosis: Co-inheritance of ?-thalassemia and hereditary hemochromatosis in a Chinese family: A case report and epidemiological analysis of diverse populations. Intern Med 2018; 57:3433–8.