

THE EFFECT OF HAEMOCHROMATOSIS MUTATION ON IRON OVERLOAD IN THALASSAEMIA MAJOR PATIENTS

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ABSTRACT

BACKGROUND

Haemochromatosis is a genetic form of iron overload due to a defective HFE gene. Secondary iron overload is the main complication in transfusion-dependent thalassaemia major patients. This study aims at evaluating the degree of iron overload in β -thalassaemia major patients with and without HFE mutations (C282Y, H63D and S65C).

MATERIALS AND METHODS

A descriptive observational study was conducted including fifty diagnosed β -thalassaemia major cases. Detailed clinical history and iron profile was estimated. DNA analysis by PCR-RFLP method for HFE gene mutations was performed.

RESULTS

After DNA analysis of all the thalassaemia major cases, two groups were identified, one with HFE gene mutation and other without HFE gene mutation. Iron profile of both the groups (with and without HFE gene mutation) was estimated and compared. Only H63D mutation (out of three HFE gene mutations) was detected in 16% cases (8 out of 50 cases), which comprised the group with mutation. Comparison of iron parameters between two groups (with and without HFE gene mutation) showed significant difference in percent transferrin saturation ($p=0.02$), while other iron parameters (serum iron and serum ferritin) did not show significant difference.

CONCLUSION

No significant difference between serum ferritin values (a marker of iron overload) of groups with and without mutation (mean ferritin level 4641 ± 2166 ng/mL and 4170 ± 2461 ng/mL, respectively) was found ($p=0.61$), in a patient population in whom transfusion protocol and proper chelation regimen was followed.

KEYWORDS

Thalassaemia Major, Haemochromatosis, HFE Gene Mutation, PCR-RFLP Method, H63D, C282Y, S65C, Iron Overload.

HOW TO CITE THIS ARTICLE: Tapas Ranjan B, Amit Kumar T, Amar Bahadur S, et al. The effect of haemochromatosis mutation on iron overload in thalassaemia major patients. J. Evid. Based Med. Healthc. 2016; 3(90), 4895-4898. DOI: 10.18410/jebmh/2016/1032

BACKGROUND

Hereditary haemochromatosis is characterised by increased intestinal absorption of iron and progressive iron overload.^{1,2} HFE gene mutations are- C282Y (cysteine to tyrosine substitution), H63D (histidine to aspartate substitution), S65C (serine to cysteine substitution).² These mutations prevent the abnormal HFE protein from binding to beta 2-microglobulin on the cell surface. Excessive iron is then absorbed through the crypt cells and passed into

circulation.³ Coexistence of HFE gene and globin gene mutations and its effect on severity of iron overload in β -thalassaemia major patients is not well studied.^{4,5}

Most of the studies conducted before are not specifically related to β -thalassaemia major, but they are related to trait and intermedia cases.^{6,7,8} The interaction of HFE gene and β -globin gene mutations together may produce iron overload in β -thalassaemia major patients.⁹ The present study aims to determine the influence of haemochromatosis mutations on iron overload in thalassaemia major patients.

AIM AND OBJECTIVES

To evaluate the effect of HFE gene mutation on iron overload in thalassaemia major patients.

MATERIALS AND METHODS

Inclusion Criteria

Fifty diagnosed cases of β -Thalassaemia major of either sex were included in study.

Financial or Other, Competing Interest: None.

Submission 06-10-2016, Peer Review 12-10-2016,

Acceptance 24-10-2016, Published 08-11-2016.

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DOI: 10.18410/jebmh/2016/1032



Exclusion Criteria

Non-thalassaemic patients with iron overload, thalassaemia minor and intermedia patients, patients with acute infection.

This study was conducted in the Departments of Pathology and Paediatrics (Thalassaemia Daycare Centre), University College of Medical Sciences (UCMS) and Guru Teg Bahadur Hospital, Delhi, from October 2011 to March 2013. This is a descriptive observational study. Venous blood samples were collected in: 1. iron free tube (5 mL) for: serum iron level (ICSH 1978),¹⁰ UIBC (Ressler et al 1958)¹¹ and serum ferritin level (ELISA); 2. EDTA vial (5 mL) for DNA extraction using standard phenol-chloroform extraction method.¹² The mutation analysis was carried out by PCR using specific primers for HFE gene mutations. The amplified product was digested with specific restriction enzyme to find out HFE gene polymorphism.¹³

RESULTS

In our study, number of β -thalassaemia major cases were 50. The age ranged from 5 to 24 years. H63D mutations were detected in 8 (5 males and 3 females) out of 50 cases (16%) only. C282Y and S65C were not detected in any patients. Age at first transfusion in cases with haemochromatosis mutation vary from 0.5 years to 2.5 years while it was 0.5 to 6 years in patients without mutation. Total number of transfusions received in cases with haemochromatosis mutation varied from 40 to 233 with an average of 153 units, while the range was from 53 to 280 with an average of 145 in patients without mutations. The cases with haemochromatosis mutation showed mean (\pm SD) serum iron of 219.9 (\pm 34.4) μ g/dL with a range of 173 μ g/dL to 280 μ g/dL, while cases without haemochromatosis mutation showed mean (\pm SD) serum iron of 224.9 (\pm 35.9) μ g/dL with a range of 140 μ g/dL to 304 μ g/dL. The difference in which was not significant ($p=0.71$) (Table 1).

The cases with haemochromatosis mutation showed mean (\pm SD) percent transferrin saturation value of 69.6 (\pm 15.6)% with a range of 46.8% to 90.5%, while cases without HFE gene mutation showed mean (\pm SD) percent transferrin saturation value of 59.4 (\pm 10.5)% with a range of 49.2% to 90.9%. The difference was found to be significant ($p=0.02$). The cases with haemochromatosis mutation showed mean (\pm SD) serum ferritin value of 4641 (\pm 2166) ng/mL with a range of 1711 ng/mL to 8304 ng/mL, while cases without haemochromatosis mutation showed mean (\pm SD) serum ferritin value of 4170 (\pm 2461) ng/mL with a range of 1800 ng/mL to 14786 ng/mL. The difference in which was not found to be significant ($p=0.61$).

DISCUSSION

Our study shows effect of haemochromatosis mutation to be noncontributory towards iron overload in thalassaemia major patients in whom transfusion protocol and proper chelation regimen was followed. The normal HFE gene encodes for the production of normal HFE protein, which is expressed in crypt cells in the duodenum.¹⁴ In a healthy individual with adequate body iron stores, the HFE protein binds to beta 2-microglobulin, which decreases the affinity

of cell membrane transferrin receptor for transferrin, its ligand. This results in decreased iron absorption. In an iron-deficient individual, synthesis of HFE protein is decreased. There is less binding of HFE protein to beta 2-microglobulin. Hence, increased affinity of transferrin receptor for transferrin and duodenal crypt cells absorb more iron.

Thalassaemia is considered the most common single genetic disorder worldwide. It occurs at a particularly high frequency in a broad belt extending from the Mediterranean basin through the Middle East, Indian subcontinent, Burma, Southeast Asia, Malaysia and the Islands of the Pacific. About 3% of the world's population (150 million people) carries β -thalassaemia gene mutations.^{15,16} In Europe, they are particularly prevalent in inhabitants of Italy and Greece.

In our study, the H63D mutation was seen in 8 cases (16%). This is approximately similar in observation of Agarwal et al⁶ (15.9% cases positive for H63D mutation out of 46 Indian cases of thalassaemia syndrome), Kaur et al⁸ (12% cases positive for H63D mutation out of 75 Indian cases of thalassaemia major) and Filomena et al¹⁷ (12.7% cases positive for H63D mutation out of 71 Italian cases of thalassaemia major).

C282Y or S65C mutations were not seen in our study. This finding is similar to observation of Garewal et al⁶ (no cases positive for C282Y mutation out of 215 Indian cases of thalassaemia trait) and Agarwal et al⁷ (no cases positive for C282Y mutation out of 46 Indian cases of thalassaemia syndrome). On contrary, Kaur et al⁸ reported 3 (4%) cases positive for C282Y mutation. Probably, C282Y mutation is rare in India. The presence of haemochromatosis mutations has been shown to adversely affect the course of disorders like porphyria cutanea tarda,¹⁸ sideroblastic anaemia¹⁹ and beta-thalassaemia intermedia.^{20,21}

Severe iron overload was reported in a 61-year-old Indian patient with thalassaemia intermedia heterozygous for C282Y.²⁰ These outcomes suggest speculation that haemochromatosis mutation may modify gene of thalassaemia major.

Borgna-Pignatti et al has recently been shown that haemochromatosis mutations do not modify the clinical picture of regularly transfused and chelated thalassaemia major patients suggesting that optimal medical treatment is able to overcome the potential effect on iron absorption caused by the defective HFE gene.²² This finding is similar in observation to our study. There are a few limitations of this study. The smaller sample size and possible selection bias could have been eliminated by including larger number of cases under the study. Nevertheless, this is among the few studies on haemochromatosis mutation in thalassaemia major.

CONCLUSION

We conducted descriptive study to determine the influence of HFE gene mutations on iron overload in 50 patients of β -thalassaemia major by PCR-RFLP using specific primers and iron profile. Observed frequency of HFE mutation of C282Y, H63D and S65C were 0%, 16% and 0%, respectively. Comparison of iron parameters between two groups (with

and without HFE gene mutation) showed significant difference in percent transferrin saturation ($p=0.02$), while other iron parameters (serum iron and serum ferritin) did not show significant difference.

This study is the first report of significant difference in percent transferrin saturation between patients with and without haemochromatosis gene mutation. Our observation of influence of HFE gene mutation on iron overload in thalassaemia major patients, however, needs to be replicated on larger patient population.

Iron Studies

Parameters	Cases with Mutation (n=8)	Cases without Mutation (n=42)	P value
	Mean±SD (Range)	Mean±SD (Range)	
S. iron (µg/dL)	219.9±34.4 (173-280)	224.9±35.9 (140-304)	0.71
Percent transferrin saturation (%)	69.6±15.6 (46.8-90.5)	59.4±10.5 (49.2-99.9)	0.02
S. ferritin (ng/mL)	4641±2166 (1711-8304)	4170±2461 (1800-14786)	0.61

Table 1. Iron Parameters in Cases of β-Thalassaemia Major With and Without Haemochromatosis Mutation

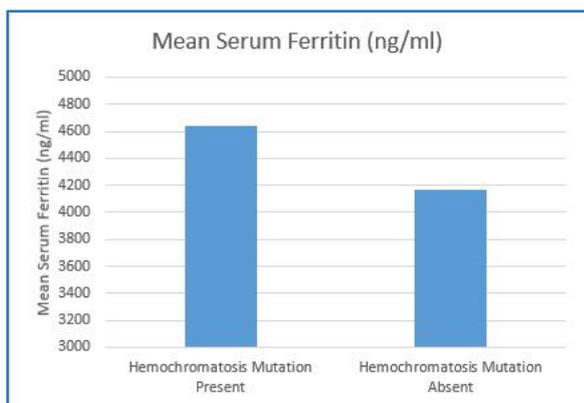


Chart 1. Mean Serum Ferritin Level of Patients With and Without Haemochromatosis Mutation

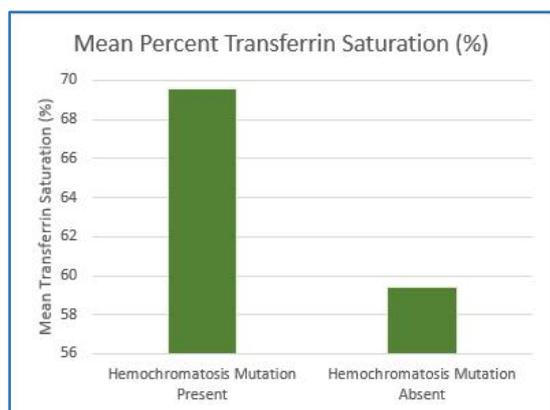


Chart 2. Mean Transferrin Saturation of Patients With and Without Haemochromatosis Mutation

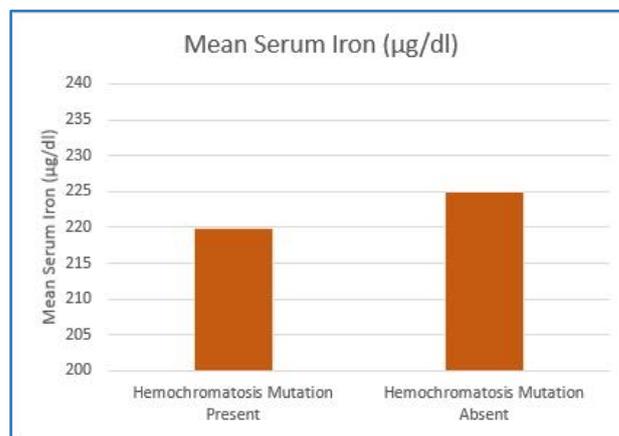


Chart 3. Mean Serum Iron of Patients With and Without Haemochromatosis Mutation

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