

ASSOCIATION OF HFE GENE MUTATION IN THALASSEMIA MAJOR PATIENTS

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ABSTRACT

BACKGROUND

Thalassemia major patients are dependent on frequent blood transfusion and consequently develop iron overload. HFE gene mutations (C282Y, H63D and S65C) in hereditary haemochromatosis has been shown to be associated with iron overload. The study aims at finding the association of HFE gene mutations in β -thalassemia major patients.

MATERIALS AND METHODS

A descriptive observational pilot study was conducted including fifty diagnosed β -thalassemia major cases. DNA analysis by PCR-RFLP method for HFE gene mutations was performed.

RESULTS

Only H63D mutation (out of three HFE gene mutations) was detected in 8 out of 50 cases. Observed frequency of H63D mutation was 16%. While frequency of C282Y and S65C were 0% each.

CONCLUSION

The frequency of HFE mutation in β -thalassemia major is not very common.

KEYWORDS

Thalassemia Major, HFE Gene Mutation, PCR-RFLP Method, H63D, C282Y, S65C.

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BACKGROUND

HFE gene mutations have been frequently detected in Hereditary Haemochromatosis (HH). It is an autosomal recessive disorder characterised by increased intestinal absorption of iron and progressive iron overload.^{1,2} HFE gene associated mutations are- C282Y (cysteine to tyrosine substitution), H63D (histidine to aspartate substitution), S65C (serine to cysteine substitution).² This prevents 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 -thalassemia major patients is not well studied.^{4,5}

There is an occasional study on coexistence of HFE gene mutation in β -thalassemia syndrome. Most of the studies conducted earlier are related to β -thalassemia trait and intermedia.^{6,7,8} The interaction of HFE gene and β -globin gene mutations may have synergistic effect, increasing iron

absorption and storage in β -thalassemia major patients.⁹ The present study is designed to determine the association of HFE gene mutation in β -thalassemia major patients.

AIMS AND OBJECTIVES

To evaluate association of HFE gene mutation in thalassemia major patients.

MATERIALS AND METHODS

Inclusion Criteria

All diagnosed cases of β -Thalassemia major of either sex were included in study.

Exclusion Criteria

Non-thalassemic patients with iron overload, thalassemia minor and intermedia patients.

This study was conducted in the Departments of Pathology and Paediatrics (Thalassemia Day Care Centre), University College of Medical Sciences (UCMS) and Guru Teg Bahadur Hospital, Delhi, from October 2011 to March 2013. This is a descriptive observational pilot study. Venous blood samples were collected in EDTA vial (5 mL) for DNA extraction using standard phenol-chloroform extraction method.¹⁰ 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.¹¹

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RESULTS

Total number of β -thalassemia major cases were 50, in which 25 were male patients. The age of males ranged from 5 to 23 years with mean (\pm SD) age of 11.44 (\pm 4.7) years. Total number of female cases was also 25. Their age ranged from 5 years to 24 years with mean age of 12.56 (\pm 4.7) years. Most of the patients (28/50) were between age group of 6 years to 15 years.

H63D mutations was detected in 8 (5 males and 3 females) out of 50 cases (16%). C282Y and S65C were not detected in any patients. Cases without HFE gene mutations were 42 (20 males and 22 females) with age ranged from 5 to 23 yrs. Mean (\pm SD) age of patients were 11.7 (\pm 4.7) years (table 1, chart 1). Age of male patients with mutation ranged from 5 to 20 years with mean (\pm SD) age 12.6 (\pm 5.5) years, while age of females ranged from 12 to 16 years with mean (\pm SD) age 14.6 (\pm 2.3) years (table 2, chart 2).

DISCUSSION

Thalassemia 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 -thalassemia gene mutations.^{12,13} In Europe, they are particularly prevalent in inhabitants of Italy and Greece.

The normal HFE gene encodes for the production of normal HFE protein, which is expressed in crypt cells in the duodenum and in reticuloendothelial cells.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.

HFE gene mutations have been frequently detected in Hereditary Haemochromatosis (HH). Hereditary

haemochromatosis, an autosomal recessive disorder, is characterised by increased intestinal absorption of iron and progressive iron overload.^{1,2} HFE gene associated mutations are- C282Y, H63D, S65C.² This prevents 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

We have studied the association of HFE gene mutations (H63D, C282Y and S65C) in fifty thalassemia major cases. In our study, the H63D mutation was seen in 8 (16%) cases out of 50 thalassemia major cases. This is near to similar in observation of Agarwal et al⁷ (15.9% cases positive for H63D mutation out of 46 Indian cases of thalassemia syndrome), Kaur et al⁸ (12% cases positive for H63D mutation out of 75 Indian cases of thalassemia major) and Filomena et al¹⁵ (12.7% cases positive for H63D mutation out of 71 Italian cases of thalassemia major).

We did not see even a single case of C282Y or S65C mutation. This is similar to observation of Garewal et al⁶ (no cases positive for C282Y mutation out of 215 Indian cases of thalassemia trait) and Agarwal et al⁷ (no cases positive for C282Y mutation out of 46 Indian cases of thalassemia syndrome). However, Kaur et al⁸ reported 3 (4%) cases positive for C282Y mutation of total 75 Indian cases of thalassemia major. Hence, this probably indicates C282Y mutation is rare in India.

CONCLUSION

We conducted descriptive pilot study to determine the frequency of C282Y, H63D and S65C mutations in 50 patients of β -thalassemia major including equal number of males and females by PCR-RFLP using specific primers. Observed frequency of HFE mutation of C282Y, H63D and S65C were 0%, 16% and 0%, respectively. Concluding thus; frequency of HFE mutation in β -thalassemia major is not very common. Our observation of HFE gene mutation need to be replicated on larger patient population. This may play a contributory role to iron overload in these patients and predict prognosis.

| Age (Years) | Male (n=20) No (%) | Female (n=22) No (%) | Total (n=42) |
|---------------|--------------------|----------------------|----------------|
| 0-5 | - | 2 (4.8%) | 2 (4.8%) |
| 6-10 | 10 (23.8%) | 8 (19.1%) | 18 (42.8%) |
| 11-15 | 7 (16.7%) | 9 (21.4%) | 16 (38.1%) |
| 16-20 | 1 (2.4%) | 1 (2.4%) | 2 (4.8%) |
| 21-25 | 2 (4.8%) | 2 (4.8%) | 4 (9.5%) |
| Mean \pm SD | 11.2 \pm 4.5 | 12.3 \pm 4.9 | 11.7 \pm 4.7 |
| Range | 6-23 | 5-23 | 5-23 |

Table 1. Distribution of Cases of β -Thalassemia Major Without HFE Gene Mutation (n=42)

| Age (Years) | Male (n=5) No (%) | Female (n=3) No (%) | Total (n=8) | H63D | S65C | C282Y |
|---------------|-------------------|---------------------|----------------|------|------|-------|
| 0-5 | 1 (12.5%) | - | 1 (12.5%) | 1 | - | - |
| 6-10 | - | - | - | - | - | - |
| 11-15 | 3 (37.5%) | 1 (12.5%) | 4 (50%) | 4 | - | - |
| 16-20 | 1 (12.5%) | 2 (25%) | 3 (37.5%) | 3 | - | - |
| 21-25 | - | - | - | - | - | - |
| Mean \pm SD | 12.6 \pm 5.5 | 14.6 \pm 2.3 | 13.6 \pm 2.9 | | | |
| Range | 5-20 | 12-16 | 5-20 | | | |

Table 2. Distribution of Cases of β -Thalassemia Major Patients with HFE Gene Mutation (n=8)

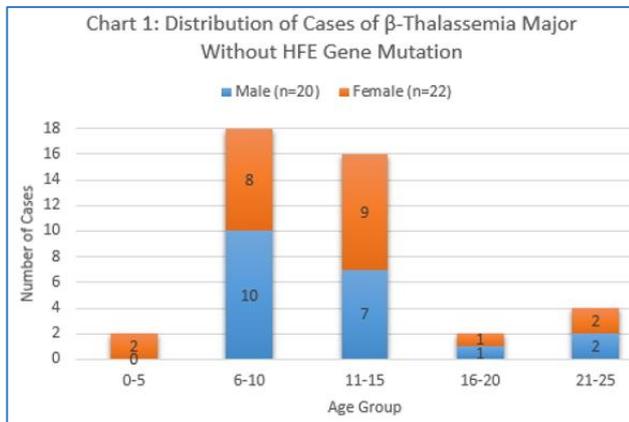


Chart 1. Distribution of Cases of β -Thalassemia Major Without HFE Gene Mutation

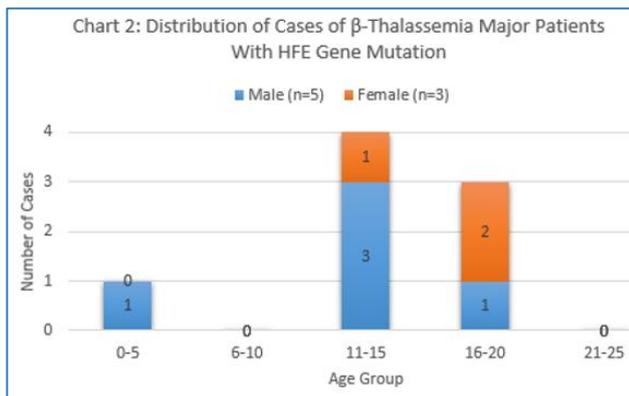


Chart 2. Distribution of Cases of β -Thalassemia Major Patients with HFE Gene Mutation

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