ORIGINAL ARTICLE

Frequency of H63D Mutations in Different Patients with Increased Bone Marrow Iron

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ABSTRACT

Aim: To study the frequency of H63D mutations in different patients with increased bone marrow iron.
Study design: Descriptive cross sectional.
Setting: The study was carried out on patients with increased bone marrow iron at Hematology Department, Armed Forces Institute of Pathology Rawalpindi (AFIP, Rawalpindi).
Methods: A total of 65 patients with increased bone marrow iron were studied. Genomic DNA was extracted from whole blood by using DNA extraction kit. All samples were genotyped for H63D mutations by PCR-RFLP methods. Polymerase chain reaction was done and amplification products were digested with the restriction enzymes (Mbo 1 for H63D mutation). Digestion products were resolved on polyacrylamide gel and stained with silver nitrate.
Results: H63D mutation was detected in 10/65 (15.4%) patients. 8/65 (12.30%) were found to be heterozygous and 2/65 (3.07%) were homozygous for H63D mutation.
Conclusion: A significant number of patients (15.4%) with increased bone marrow iron have hereditary hemochromatosis.
Keywords: H63D, bone marrow iron, PCR

INTRODUCTION

Type 1 or classical hemochromatosis is an autosomal recessive disorder[1]. It is due to increased absorption of iron in intestinal mucosa causing iron over load[2]. Increased iron is deposited in organs like liver and heart etc[3]. Deposited iron is toxic and causing abnormal effects and failure of different organs[4,5]. A gene called HFE, is located on the short arm of chromosome 6[7,8]. There are two mutations in the HFE gene called C282Y and H63D. Cysteine to tyrosine substitution at amino acid 282 results in C282Y mutation in exon 4[8]. Histidine to aspartic acid substitution at 63 results in H63D mutation[6]. 60 – 70 years usually is age of onset[9]. Due to menstruation and pregnancy, females are less frequently involved[10].

METHODOLOGY

Bone marrow iron stores of 3+ to 6+ patients were included. The study patients were those who presented to AFIP for bone marrow aspiration and were found to have increased bone marrow iron (3+ to 6+). Patients with known cause of iron overload like blood transfusion, thalassaemia major, myelodysplastic syndrome, hemolytic anemia, megaloblastic anemia etc. were excluded. For extraction of DNA and CBC, 3ml blood was taken in EDTA vials. By PCR, genotyping was done for H63D mutation.

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RESULTS

Detail of results were given in tables 1,2,3.

Table 1: Clinical data

Clinical data	No.
Pyrexia	20
Hepatomegaly	06
Splenomegaly	04
Lymphadenopathy	35
Total	65

Table 2: Bone marrow iron and mutations

B.M. iron	H63D (homo)	H63D (hetero)	Normal
+++	No	No	10
++++	No	No	20
+++++	No	07	25
++++++	02	01	No

Table 3: H63D mutation positive cases

Cases	n =	%age
H63D (homozygous)	02	03 %
H63D (heterozygous)	08	12.4%
Negative cases	55	84.6%
Total cases	65	100%

DISCUSSION

The most reliable method of detection of stored iron in the body is perl's stain. Bone marrow iron stores were assessed according to grading system. Patients with 3+ (numerous small particles in reticulum) to 6+ grade (very large clumps and cellular iron) were considered as having iron overload. In this study, males are more as compared to females. The reason may be the early checkup by males. In this study, H63D mutation is present in 15.4 % patients. In one study, H63D mutation is less severe as compared to C282Y mutation[13]. In another study, the frequency of H63D mutation is 14.8%[11.] A study by Tsui et al[12,] 9.8 % H63D mutation was observed.

CONCLUSION

H63D mutation was present in a significant proportion of patients with iron overload (15.4 %). The most probable cause for iron overload in these patients appears to be hereditary hemochromatosis due to HFE gene mutation.

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