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# Study of HFE Gene Mutation at C282Y and H63D Locus with Special Reference to Thalassemia Patients

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**Abstract**

Hemochromatosis disorder of iron metabolism leads to excess iron levels in body which is extremely toxic to all cells of the body and can cause serious and irreversible damages. Clinical complications of hemochromatosis include cirrhosis of the liver, congestive cardiac failure and cardiac arrhythmias, endocrine pancreatic disease. Hemochromatosis is classified as Primary and secondary hemochromatosis. One of the type of primary hemochromatosis is type I also refereed as hereditary hemochromatosis (HH) and is a autosomal recessive disorder of iron metabolism. Three recurrent mutations in hemochromatosis gene (HFE) : C282Y, H63D and S65C are known for hereditary hemochromatosis. C282Y is considered the most relevant mutation responsible for hemochromatosis. In secondary or acquired hemochromatosis, reasons for excess iron accumulation are repeated blood transfusions or enhanced iron absorption produced by thalassemia or both, if thalassemic patients are having mutations in HFE gene repeated blood transfusions may aggravate the condition hence, it is important to screen the thalassemic patients for HFE gene mutations.

The present study was carried out to study mutation in HFE gene at C282Y and H63D locus in thalassemic patients. The detection technique includes isolation of DNA from peripheral blood of the mentally retarded patients of Surat and Anand regions of Gujarat state. PCR-RFLP was used for detection of mutation. For C282Y locus genotypes observed in thalassemic patients were AA , AB and in normal individuals AA, BB and BC. For H63D genotypes observed in thalassemic patients and normal individuals are BC and CC. Wild type pattern observed for C282Yand H63D shows absence of mutation at C282Yand H63D locus of HFE gene in thalassemic patients and normal individuals.

**Highlights:** Thalassemia patients are at high risk of secondary hemochromatosis as blood transfusions is the most practiced treatment hence, screening for HFE gene mutation is important in them.

**Keywords:** HFE gene, hemochromatosis, H63D mutation, C282Y mutation.

Hemochromatosis is disorder of iron metabolism leading to iron overload, Clinical consequences of iron overload include cirrhosis of the liver, congestive cardiac failure and cardiac arrhythmias, and pancreatic disease (Melchiori *et. al.,* 2010).

Hemochromatosis is classified as primary hemochromatosis and secondary hemochromatosis. The primary hemochromatosis which is an inherited to next generation and secondary hemochromatosis, which is caused by some other disease or underlying condition. Secondary hemochromatosis is frequently observed in beta-thalassemia major due to an increased rate of iron absorption by gastrointestinal tract and frequent blood transfusions. In beta-thalassemia trait, there is some degree of ineffective erythropoiesis, which leads to heightened erythropoietic activity and increased iron absorption (Demir *et.al.,* 2004).

Three recurrent disease associated mutations in hemochromatosis gene (HFE) are: C282Y, H63D and S65C. C282Y is considered the most relevant mutation responsible for hemochromatosis a cysteine to-tyrosine amino acid substitution caused by an G>A transition at codon 282 within exon 4 (Cys282Tyr, C282Y). In H63D C to G transversion at nucleotide 187, which changes

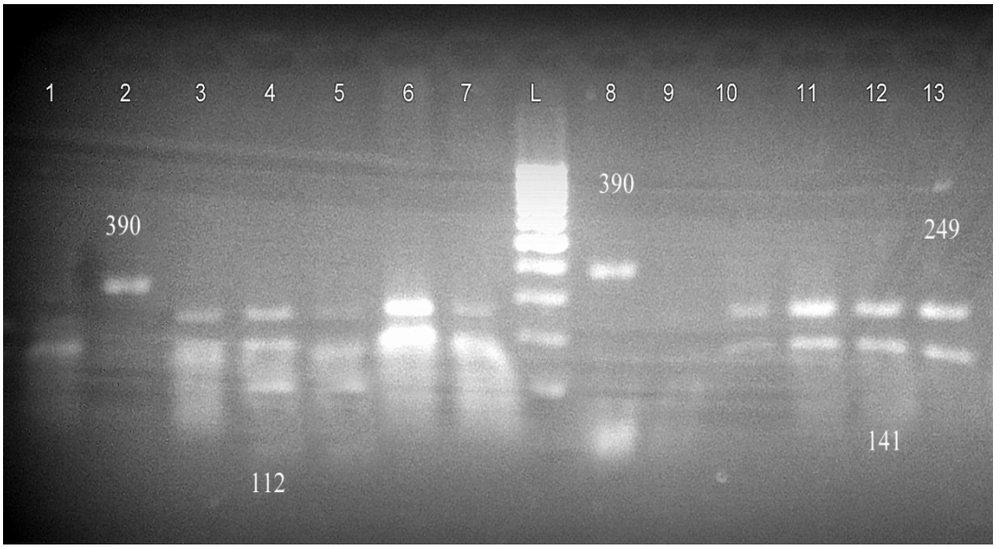


**Plate 1: Restriction digestion of C282Y of HFE gene (exon 4) locus fragment of 390bp electophoresed on 3% agarose in 0.5 X TBE at 100V Lane 1,2,3,4,5,6,7,10,11,12,13- Digested Product, Lane 8 - PCR product, Lane L- 100bp DNA Ladder . Lane 2-AA- 390bp , 4-BC 249bp, 141,112,29 . 12,13-BB -249,141bp**

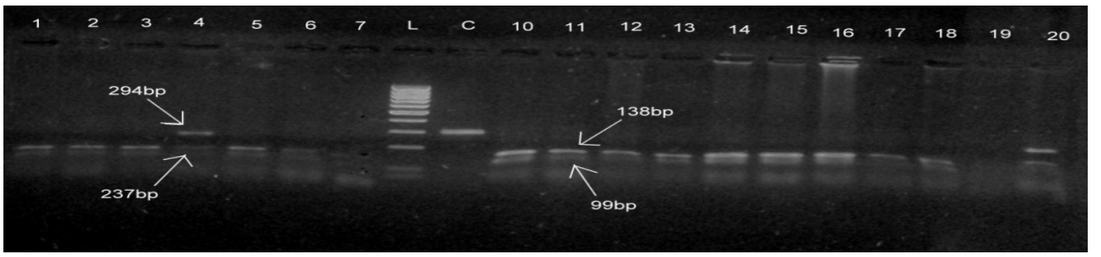


**Plate 2: Restriction digestion of H63D exon 2 locus fragment electophoresed on 3% agarose in 0.5 X TBE at 100 V Lane: 1,2,3, 4,5,6,7,10, 11,12,13, 14, 15,16, 17, 18,19,20- digested Products. Lane-4-BC -294bp,237bp,57bp and Lane 11-AA -138bp,99bp,57bp Lane 9- PCR product (control) Lane L- 100bp DNA Ladder**

amino acid 63 from histidine to aspartic acid, in S65C serineto-cysteine substitution generated by a 193 A>T transversion in exon 2 (Bomford, 2002). The HFE gene is located on the short (p) arm of Chromosome 6 (Powell *et.al*., 2000). The gene encodes a protein that is found on the surface of epithelial cells and some immune cells. The HFE protein is involved in regulating the absorption of iron by the intestinal cells; it also influence the expression of a second iron-regulatory protein, hepicidin (Townsend and Drakesmith, 2002).

If thalassemic patients are having mutations in hemochromatosis gene repeated blood transfusions may aggravate the condition hence, it is important to screen the thalassemic patients for HFE gene mutations.

As the published information on HFE gene mutations which causes hemochromatosis is very scanty for thalassemia patients in Indian population. The present study has been undertaken with objectives to screen the thalassemia patients for C282Y and H63D mutations in HFE gene by PCR_RFLP method, as thalassemia patients are at high risk of secondary hemochromatosis.

## Materials and Methods

The present study was carried out to detect mutation at C282Y and H63D locus of HFE gene by PCR-RFLP technique in Thalassemia patients. The blood samples for the study were obtained from the 30 unrelated thalassemia patients from Vadodara, Surat districts of Gujarat and blood samples of 30 unrelated normal individuals were obtained from ARIBAS College from volunteer students, New V.V.Nagar with informed consent. Methods of collection and use of human samples were approved by the institutional ethics committee. The blood samples obtained were brought to college laboratory on ice for further use.

Genomic DNA was extracted from peripheral blood leukocytes by standard phenol/chloroform method (Sambrook, and Russell, 2001).

The PCR reaction was carried out to amplify C282Y of HFE gene (exon 4) by using primers reported by Baiget *et. al.,* (1998) and to amplify selected regions of H63D genes by using primers reported by Arsov *et. al.,* (1998).

PCR amplification of DNA was amplified using 10pmol/µl of each primer, 2X PCR master mix, DNAse free water 7.5 µl and DNA template 30ng/µl. This sample mix was subjected to thermocycler consisting of denaturation at 94°C for 5 min, annealing at 60°C for 45sec for C282Y and annealing at 59°C for 45sec for H63D followed by extension at 72°C for 45sec and finally to 35 PCR cycles. PCR products were subsequently digested with the restriction enzyme RsaI and MboI.

## Results and Discussion

PCR product of C282Y (exon4) of 390bp and H63D (exon 2) of 294 bp was amplified successfully. C282Y (exon4) of 390bp fragment was screened for *RsaI* RFLP and H63D (exon 2) of 294 bp fragment was screened for *MboI* RFLP.

C282Y (exon4) of 390bp fragment was screened for *RsaI* RFLP and genotyping was done as per Baiget *et. al.,* (1998). All the samples of thalassemic patients as well as normal individuals were screened for *RsaI* mutation at C282Y exon4 were polymorphic containing A, B and C allele at C282Y exon4 locus with AA, AB, BB and BC genotypes (Plate 1) (AA, BB wild type genotypes). In the present study genotypes observed at C282Y in thalassemic patients are AA (25%), AB (10%), BB (65%) and in normal individuals AA (25%), BB (65%), and BC (10%). Here the most observed genotype in normal as well as in thalassemic patients were wild type AA and BB.

H63D exon 2 digested with *MboI* genotyping was done as reported by Arsov *et.al*. (1998) generates five band patterns, yields two fragments of 237 bp and 57 bp in wild type and mutated allele 237 bp is further digested in to 138bp and 99bp. In the present study out of the five restriction patterns two were observed in Thalassemic patients and normal individuals i.e. genotypes BC and CC (Plate 2). In thalassemic patients genotypes observed were 10% BC and 81.1% CC and in normal patients 9.1% BC and 90.9% CC. Here the most observed genotype in normal as well as in thalassemic patients is CC (CC wild type genotype).

More wild type pattern observed shows absence of mutation at C282Y (exon4) and H63D locus of HFE gene in thalassemic patients and normal individuals.

The results of the present study for H63D mutation are in accordance with Arsov *et.al,*(1998 ) that H63D mutation is very rare in the populations outside Europe and America.

The results of the present study are in accordance for C282Y mutation and in contrast for H63D with Kaur *et al.* 2003; Thakur *et al.* 2004; Garewal *et al.* 2005; Dhillon *et al.* 2007 that absence of C282Y mutation in native Indians and H63D to be the major mutation present ranging from 9.1% to 13.9%.

Possible explanations for the divergent findings include small sample sizes and differences across ethnic groups.

## Conclusion

More wild type pattern observed for C282Yand H63D shows absence of mutation at C282Yand H63D locus of HFE gene in thalassemic patients and normal individuals

As the study concluded on small sample size and hence, for mutation detection in C282Y and H63D association studies with thalassemia, study can be verified by taking large sample number of normal as well as thalassemia

patients.

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