Prevalence of the HFE Gene Mutation in the Liver Transplanted and Primary Hemochromatosis Patients in the Southern Iran

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Abstract

Background: Primary hemochromatosis is an inherited disorder. Mutation in this gene is accompanied with iron overload in the body leading to organ failure that primarily affects liver. Individuals with homozygote HFE gene mutation are prone to developing the end stage liver disease. Concomitance heterozygote HFE mutation with the other hepatic risk factors may accelerate hepatic damage, leading to cirrhosis. The aim of this study was to find out the spectrum and frequency of the HFE gene mutations in the liver transplantation (end-stage liver disease [ESLD]) candidate groups.

Methods: Totally, 170 individuals were studied for HFE gene mutations including 87 ESLD patients with various etiologies from Division of Liver Transplant in Nemazee Hospital affiliated to Shiraz University of Medical Sciences. Seventy four randomly selected healthy blood donors were evaluated as the control group, and 9 hemochromatosis patients who referred to our lab for genetic analysis due to their high serum ferritin levels and clinical diagnosis were surveyed in a period of one year.

Results: HFE gene mutation was found in 57 (~33%), 14 (~9%), and 15 (~83%) chromatids of the ESLD group, control sample, and hemochromatosis patients, respectively. The allele frequency of H63D is about 0.085 among these people. The HFE mutation H63D in the ESLD is significantly higher than that of the control group (W/H63D: odds ratio 5.70, 95% CI= 2.6 – 12.55; H63D/H63D: odds ratio 6.39, 95% CI= 0.77-53.1).

Conclusion: In compliance with our previous report, the C282Y mutation is very uncommon in the southern population. This prevalence could be due to a significant aggravating effect of H63D for liver disease in these patients and may contribute to the poor liver transplantation outcome.

Keywords: HFE gene; Hemochromatosis; Liver transplant

Introduction

Primary hemochromatosis is an inherited disorder introduced by von Recklinghausen in 1889. The disturbed iron metabolism and its accumulation in the organs lead to iron overload. The excessive iron overload can lead to organ failure that primarily affects the liver. Several known gene defects and some loci are responsible for primary iron overload. All of these gene products interact cooperatively in iron regulation.

Since 1996, discovery of the HFE gene, at least four additional genes considered as the causative genes in the diverse type of hemochromatosis have been described. These HFE genes (6p21) include hemojuvelin (1q21), Hepcidin (19q13), Transferrin Receptor 2 (7q22), and Ferroprtin (2q32) which may cause iron overload with distinct clinical entities. All these protein-encoding genes are inherited as autosomal recessive except Ferroprtin (FPN1/SLC40A1) that has autosomal dominant pattern.

The prevalent clinical presentation of iron overload in the adults is primary hemochromatosis (type

I), which is presented by a slow accumulation of iron in the tissues. Hemochromatosis type I is caused by a malfunctioning HFE gene. The gene product is a transmembrane protein similar to HLA class I. HFE protein has a high affinity to transferrin receptor in the duodenal crypt cellsi.[1] The 5-10 fold affinity reduction in transferrin receptor affinity to its ligand,[2] diminishes the uptake of transferrin bound iron. This iron deficiency state will increase the expression of divalent metal transporter which raises iron absorption from the duodenum.[3] Abnormality in the HFE protein can disturb iron regulation and the phenotype of hemochromatosis will appear in around the fifth decade of life.[4]

The predominant HFE gene mutation is C282Y followed by H63D and S65D mutations in western patients. Since the liver is the major site of iron deposition, HFE associated hemochromatosis and potential oxidative iron damage hepatic cells; thus, homozygote HFE mutations are prone to developing the end stage liver disease.

In comparison to the normal HFE gene patients, coexistence of HFE gene mutation and viral hepatitis not only increases the development and progression of chronic hepatitis,[5,6] but also advances cirrhosis at younger ages.[5] In addition to the co-mortality effect of iron overload and other causes on the developing advanced hepatic failure, the liver transplantation outcome is affected by the presence of HFE gene mutation, either homozygote or compound heterozygote, and decreases 5 year survival about 20%.[7]

We hypothesized that concomitant presence of HFE mutation with the other hepatic risk factors might accelerate hepatic damage and lead to cirrhosis in early stages or adverse effects on the liver transplantation outcome.

The aim of this study was to assess the coexisting of the HFE gene mutations in the end stage liver disease (ESLD) as the aggravating factor and transplantation outcome in the affected patients.

Material and Methods

A group of ESLD patients (n=87; age=18-59 years) with various etiologies who had enrolled for liver transplantation were selected from the Division of Liver Transplant in Nemazee Hospital affiliated to Shiraz University of Medical Sciences in Shiraz, southern Iran for evaluation of the impact of HFE gene mutation on the development of liver disease. A group of normal population among the healthy blood donors (n=74) and a group of patients (n=9; age=34-51 years) who referred to our lab for genetic analysis due to their high serum ferritin levels and clinical diagnosis of primary hemochromatosis were evaluated during one year.

For 87 ESLD subjects, genomic DNA from the stored frozen blood samples was extracted with the use of QIAamp DNA Blood Kit (Qiagen Inc.) and all DNAs of the control samples and referred patients were extracted from fresh peripheral blood samples by salting out method.[8]

All the samples were genotyped for nucleotide changes of C282Y, H63D, and S65D in the HFE gene by PCR methods, using of ARMS,[9] and RFLP techniques.[10] PCR based amplified fragments of exon 2 and 4 from HFE gene were analyzed for subjected mutations by ARMS and RFLP methods simultaneously.

To determine the haplotype linked to the H63D mutation, 5 di-allelic polymorphisms in the HFE gene (Figure 1) were studied as described previously.[11,12] All the polymorphic sites were determined by RFLP method, using the following enzymes: Bby (for –984); RsaI (IVS2); Sau 96I (for IVS4); Ban I (for IVS5) and Rsa I (for poly A +5).

Results

Analysis of 340 chromatids from the mentioned groups for HFE gene mutations reveals mutation in
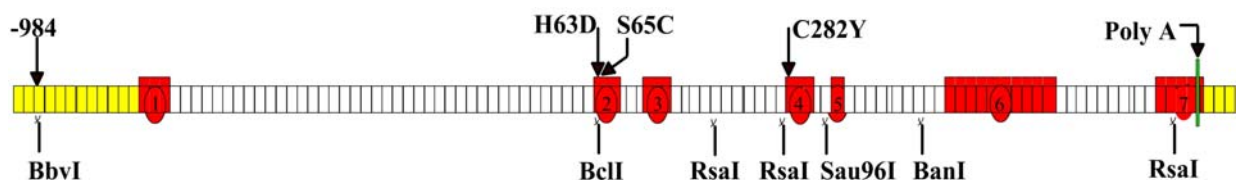

Fig. 1: HFE gene structure is showing the location of the polymorphic sites used in the haplotype analysis. Exons are shown as red boxes. The H63D mutation results in exon 2 and C282Y in exon 4. The BbvI, RsaI, Sau96I, and BanI restriction sites detect non-coding, or silent, substitution mutations.

the 89 chromatids. While 9.5% of the chromatids in the control samples showed HFE gene variants, 83% and 33% of the chromatids from hemochromatosis patients and ESLD group had affected HFE gene correspondingly. Totally, hemochromatosis patients represented with one C282Y, four H63D homozygotes, and four compound heterozygotes (one C282Y/H63D and 2 H63D/S65D). The ESLD group revealed 41 H63D heterozygotes, 7 H63D homozygotes and 1 compound heterozygote C282Y/H63D (Table 1).

In this study, we could not characterize any mutation in three chromatids from a 56 year-old female and a 16 year-old male. She was from Marvdasht (Shiraz) and presented heterozygote H63D mutation that does not explain her clinical presentation and phenotype (Her serum ferritin was 458 µg/mL). A large deletion or other candidate gene mutation in this case is under study. Haplotype analysis on 11 homozygote H63D individuals reveals that this mutation is mainly linked to the haplotype VI (54.5%). The rest are linked to the haplotypes VII (27.3%), II (9%) and I (9%).

Discussion

The effective mechanisms for excreting iron are negligible in human beings and the most important maintaining iron balance is regulated by intestinal iron absorption to contest body iron requirements.

A non-heme iron is absorbed after its reduction to the ferrous form and transported across the brush border of enterocyte by DMT1. Then, ferroportin 1 (FP1) in conjunction with hephaestin, a ferroxidase, starts its entry from enterocyte into the circulation. The crypt cell has been proposed to be an iron-sensing cell,

body iron storage. Hepcidin is a circulatory peptide secreted by the liver and has an inhibitory effect on iron absorption. The cross-communication between hepatocytes and reticuloendotheilal macrophages regulates hepcidin levels. The expressed HFE protein on the hepatocytes and macrophages surface modulates this communication.[13,14]

The most important mutations in the HFE gene that are associated with significant clinical hemochromatosis are C282Y and H63D but a mild form of clinical presentation is seen with the allele S65C. Poor cell surface expression of HFE protein in the homozygous C282Y patients, due to intracellular degradation, and expression of distorted HFE protein in cases of H63D and S65C, disturbs the regulation of hepcidin, leading to iron accumulation.

Limited studies on the frequency of HFE alleles from various parts of Iran with a complex variety of ethnic groups are available. The present study did not reveal any C282Y allele in the control group but both the patient and ESLD groups had C282Y allele which indicates a low frequency of this allele in the southern population. In spite of low frequencies of C282Y and S65C, the frequency of H63D in agreement with another study,[12] is high (allele frequency= 0.088).

In comparison to the control group, both patients and ESLD groups presented with a high frequency of homozygote and heterozygote H63D (odd ratio xx and xx, respectively).

It seems that in the absence of hereditary hemochromatosis (HH), the end-stage cirrhosis is associated with moderate to marked hepatic iron overload, especially in the liver disease because of alcohol and/or hepatitis C. Co-existence of HFE gene mutation acts as an aggravating factor and accelerates

Table 1: Prevalence of HFE Genotypes According to the studied Group

Group	No. of Participants	C282Y/C282Y		C282Y/H63D		H63D/H63D		H63D/S65D		H63D/W	
		No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %
Hemochromatosis Patients	9	1	0.10-0.12	1	0.10-0.12	4	0.28-0.61	2	0.08-0.46	1	0.10-0.12
End-Stage liver Disease	87	0	-	1	0.001-0.024	7	0.05-0.11	0	-	41	0.42-0.53
Control individuals	74	0	-	0	-	1	0.0004-0.0278	1	0.0004-0.0278	10	0.10-0.17

which regulates iron transporters in response to the

the severity of liver disease, particularly fibrosis, sig-

nificantly in those individuals.[15] The clinical severity and age onset clearly depends on several parameters including sex, nature of gene mutation and its penetrance, and other genetic factors such as TfR2 and ferroportin 1 gene as well as non-genetics factors.

There are several studies that report an unfavorable outcome after liver transplantation in patients with end-stage liver disease and increased hepatic iron.[16,17] The survival rate after liver transplantation of these patients in comparison to recipients for other conditions appears to be decreased, based on reports of several centers.[18-23] A decrease of about 25% in the 1-year and 5-year survival rates of liver transplanted patients with concomitant hemochromatosis has been reported from 37 transplant centers.[19]

Acknowledgment

Conflict of interest: None declared.

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