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Indian Journal of Medical Sciences

(INCORPORATING THE MEDICAL BULLETIN)
DECEMBER 2006

VOLUME 60

NUMBER 12

ORIGINAL CONTRIBUTIONS

EVIDENCE FOR NON-HFE LINKED HEMOCHROMATOSIS IN ASIAN INDIANS

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ABSTRACT

BACKGROUND: Hereditary hemochromatosis is commonly due to two HFE1 (Histone Family E1) gene mutations – H63D and C282Y. Mutations in the Asian Indians are less well studied. AIMS: The aim of this preliminary study was to find out the prevalence of HFE gene mutations in nonviral liver cirrhosis patients. SETTINGS AND DESIGN: Unexplained liver cirrhosis cases with transferrin saturation >45%, attending the gastroenterology clinic in the years 2004 and 2005 were subjects of the prospective study. Asymptomatic individuals with negative family history of hemolytic anemia or liver disease served as controls. MATERIALS AND METHODS: The clinical presentation was recorded in the patients. Transferrin saturation was estimated by standard colorimetric technique. The two common mutations in HFE1 gene and Y250X mutation of TFR (transferrin receptor) gene were studied by polymerase chain reaction based methods. RESULTS: A majority of the cases were sporadic, but family history was positive in four patients. In one family with multiple affected members, there was clear evidence of autosomal dominant inheritance. Seven out of 31 (22.6%) of unexplained cirrhosis cases were positive for mutations. One was homozygous for H63D. In healthy controls, prevalence was 8.1% (6/74). None of the patients or controls was positive for C282Y mutation of HFE1 or Y250X of TFR gene. CONCLUSIONS: Thus, in a number of cases of hemochromatosis in Indians, a gene with dominant inheritance may be involved in causation of the phenotype. The prevalence of HFE mutations in Indians is comparable to that reported from neighboring countries. It is worth studying other mutations in HFE gene and other iron overload genes in cryptogenic cirrhosis cases.

Key words: Chronic hepatitis, cirrhosis, ferroportin, mutations, transferrin saturation

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Inusha Panigrahi, 46/ Y1, C-block, Dilshad Garden, Shahdara, Delhi - 110 095, India. E-mail: inupan@yahoo.com Histone Family E (HFE) gene mutations are important in causation of primary hemochromatosis in Caucasians^[1] (HFE, OMIM 235200). Mutation H63D (C \rightarrow G transversion) increases relative risk of developing hemochromatosis in individuals heterozygous for C282Y mutation. Mutations in the HFE protein alter affinity of the transferrin receptor (TFR) for its ligand transferrin and thus attenuate uptake of transferrin-bound iron from plasma. There are limited studies^[2-4] on the role of HFE gene in iron overload in Asian Indians and they have identified mainly the H63D mutation. The aim of this study was to determine the role of two common HFE gene mutations and transferrin receptor mutation Y250X in cryptogenic cirrhosis cases.

MATERIALS AND METHODS

The cryptogenic cirrhosis cases were consecutive nonviral hepatitis cases attending the gastroenterology clinic in the years 2004 and 2005 at a tertiary care center. In the prospective study, the cases included were those in which MRI was suggestive of pigment deposition, transferrin saturation was >45% and tests for autoimmune markers were negative and serum ceruloplasmin and α 1 anti-trypsin were normal. The diagnosis of cirrhosis was based on clinical assessment. ultrasonographic and MRI findings. Patients with history of chronic alcoholism and those positive for the viral hepatitis markers-HbSAg, anti-HCV and autoimmune markersanticardiolipin antibody and lupus anticoagulant were excluded from the study. Transferrin saturation was quantitated by standard colorimetric technique. The

aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured using commercially available kits. Controls were healthy staff of the hospital and included the permanent staff, residents and Ph.D. students with negative family history of liver disease and hemolytic anemia. Consent was taken while filling the pro forma. Ethical clearance was obtained from the Review Board.

The DNA (deoxyribonucleic acid) extraction was done from citrated blood by standard phenol chloroform method. The molecular analyses for HFE mutations H63D and C282Y were done by polymerase chain reaction (PCR) using allele specific primers described by Mullighan et al.[5] The positive cases were confirmed by haplotype analysis and PCR-RFLP method,^[6] with enzymes Mbol and Rsal respectively. The mutation 63D abolishes the *Mbol* site and the mutation 282Y creates an additional Rsal site in the PCR product. Electrophoresis was done on 2% agarose gel for allele-specific PCR products, following Mbol digestion. Following Rsal digestion, the product was run on 7% polyacrylamide gel electrophoresis (PAGE). The Y250X mutation of the TFR gene was studied by PCR-RFLP technique,^[7] using restriction enzyme Mael.

RESULTS

The age of the liver cirrhosis patients in the present study ranged from 28 to 59 years (median 38 years). There were 19 males and 12 females. There were 74 normal controls. Most patients were symptomatic from the second decade of life, with an average age

of 26.5 years at onset. The transferrin saturation (TS) was greater than 45% in all cases (range 47-73%). The TS between 20 and 45% was taken as normal.

All the patients had hyperpigmentation of skin and liver dysfunction with increased transaminase levels. AST and ALT levels were 102.3 and 114.1 units respectively. In four families, there was a strong family history of liver disease; and in one, with pedigree with multiple generations affected, suggestive of autosomal dominant inheritance. Seven of 31 cases (22.5%) were positive for H63D mutation. One was homozygous for H63D. In contrast, in controls, 6 of 74 (8.1%) were heterozygous for H63D mutation. None of the patients or controls showed the C282Y mutation of HFE gene or Y250X mutation of TFR2 gene. Figure 1 shows the size of HFE haplotype product to be around 2 kb. The sizes of products obtained after digestion with Rsal for

1 2 3 4 5 2600 bp 2000 bp 1500 bp 1200 bp 1000 bp 500 bp

Figure 1: Lanes 1 and 2: HFE normal haplotype products; lane 3: DNA molecular marker XIV (Roche Diagnostics, GmBH, Germany); lanes 4 and 5: 2 kb and 1.2 kb amplification products from factor VIII gene. Sequence comparison from NCBI Blast site revealed HFE haplotype product size to be 1,997 bp C282Y mutation are shown in Figure 2. There was no difference in mean transferrin saturation in the patients with and without the H63D mutation (46.3 *vs.* 45.6%).

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DISCUSSION

The HFE1 gene at 6p21.3 is commonly implicated in hereditary hemochromatosis, with two mutations H63D and C282Y present in majority of cases in the Western population. In the present study, most patients were heterozygous for H63D mutation. Comparable frequency has been reported from Italy, Finland, Africa, Australian population^[1,8] and Turkish population.^[9] A previous study in liver disease from India also did not find C282Y mutation.^[3]

The H63D allele frequency was 4.1% (6/148 chromosomes) in control population in the present study. This is lower than that reported in North Europeans^[6] but comparable to that



Figure 2: Lane 1: pBR Msp I digest (Bangalore Genei); lanes 2-4: digested products 185 and 122 bp. None of the patients in the gel showed the 156 bp product, suggestive of presence of mutant allele

of Thailand.^[10]

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In a previous report by Rees *et al*,^[11] 1 of 47 thalassemia intermedia cases from this region, a resident of Punjab, was positive for C282Y mutation; however, more recent studies have found absence of this mutation,^[3,4] which is comparable to the present study.

In genetic hemochromatosis patients from Western Australia, the C282Y mutation was homozygous in 64 of 72 cases.^[12] In an Italian study on patients with increased transferrin saturation (>50% in males and >45% in females), overall C282Y allele frequency was 9% and that of H63D and S65C was 22.2 and 1.4% respectively.^[13] In noncirrhotic HBV patients, HFE mutations were not found to have significant role in the causation of iron overload,^[14] though 27% patients had elevated transferrin saturation. In another study,^[15] patients who were heterozygous for C282Y and H63D mutations exhibited higher iron serum parameters than subjects without these mutations. H63D mutation in heterozygote state has also been reported in NASH patients in an Indian study.[16]

In haplotype analysis in the present study, the product size was more than that mentioned in reference.^[5] The exact size was found to be 1,997 bp by comparison with sequence obtained from the NCBI blast site (www.ncbi.nlm.nih.gov/BLAST). Since the sizes of products obtained after digestion with *Rsal* were not found from any available reference, the sequence analysis of the gene from above NCBI site revealed PCR product size to be 307 bp and sizes after enzymatic digestion to be 185 and 122 bp for the normal allele and 156, 122 and 29 bp for the mutant allele.

Transferrin receptor mutations (OMIM, 604250) are also associated with hemochromatosis. None of the patients in the present study showed Y250X mutation. Camaschella *et al* first reported this mutation from Sicily.^[17] TFR2 mutations have also been reported in Sardinians and Italians.^[7] The mutations are likely to be different in different populations.

The other genes causing severe iron-loading disorder are hepcidin (HAMP) on 9q13 and hemojuvelin (HJV) on 1q21.^[18] Many cirrhotic patients in the present study had severe presentation and earlier onset of disease manifestations. Mutations in ferroportin gene [HFE4 (OMIM 606069)] have been associated with autosomal dominant hemochromatosis. This gene needs to be further studied on a larger patient population. There is also a possibility of mutations in the other genes or digenic inheritance of mutations.

ACKNOWLEDGMENTS

We thank all the patients for participating in the study and the residents in gastroenterology for providing the samples.

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Source of Support: Nil, Conflict of Interest: None declared.