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World J Gastroenterol 2007 June 7; 13(21): 2956-2959
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Frequency of primary iron overload and HFE gene mutations (C282Y, H63D and S65C) in chronic liver disease patients in north India

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Received: 2007-01-28 Accepted: 2007-02-14

Abstract

AIM: To identify the frequency of iron overload and study the three mutations in the HFE gene (C282Y, H63D, and S65C) in patients with chronic liver disorders (CLD) and controls.

METHODS: To identify patients with iron overload (transferrin saturation > 45% in females and > 50% in males and serum ferritin > 1000 ng/mL) we evaluated 236 patients with CLD, including 59 with non-alcoholic steatohepatitis (NASH), 22 with alcoholic liver disease (ALD), 19 of cirrhosis due to viruses (HBV, HCV), and 136 with cryptogenic cirrhosis. Mutations of the HFE gene were analyzed by PCR-RE. hundred controls were screened for iron status and the mutations.

RESULTS: Seventeen patients with CLD showed evidence of iron overload. Fifteen cases of iron overload had cryptogenic cirrhosis and two had ALD. None of the controls showed iron overload. We did not find any individual with 282Y or 65C either in the cases or in the controls. The prevalence of H63D heterozygosity was 12% in normal individuals, 14.8% in 236 patients (16.9% in NASH, 13.6% in ALD, 26.3% in viral and 12.5% in cryptogenic cirrhosis) and the overall prevalence was 13.98%. Only two of the 17 patients with primary iron overload were heterozygous for H63D. One patient with NASH and one normal individual who were homozygous for H63D showed no iron overload.

CONCLUSION: Primary iron overload in Indians is non-HFE type, which is different from that in Europeans and further molecular studies are required to determine the defect in various iron regulatory genes.

Key words: HFE gene mutations; C282Y; H63D; S65C; Population genetics

Dhillon BK, Das R, Garewal G, Chawla Y, Dhiman RK, Das A, Duseja A, Chandak GR. Frequency of primary iron overload and HFE gene mutations (C282Y, H63D and S65C) in chronic liver disease patients in north India. *World J Gastroenterol* 2007; 13(21): 2956-2959

http://www.wjgnet.com/1007-9327/13/2956.asp

INTRODUCTION

Hereditary hemochromatosis (HH) is one of the most common genetic disorders encountered in the Northern European population. Its inheritance is autosomal recessive and results in disordered iron metabolism leading to enhanced iron absorption and progressive iron deposition in parenchymal organs, most notably in liver[1]. Excess iron may cause damage to parenchymal organs, with an increased risk of developing diabetes mellitus, arthropathy, liver cirrhosis and ultimately hepatocellular carcinoma[2,3]. The HFE gene encodes a protein, which is highly similar to HLA class 1 molecules. Two missense mutations (C282Y, H63D) have been described on the HFE gene in patients suffering from HH on the basis of phenotypic data. The predominant mutation in the Caucasians, C282Y, is a G-A transition at nucleotide 845 of the open reading frame that changes the amino acid cysteine to tyrosine[4].

H63D is a C-G transition at nucleotide 187 of the HFE gene which results in a histidine to aspartic acid substitution. It has been found to be present with a frequency of 3.3%-15.2% in the general population across the world[5-7]. A third mutation in the HFE gene, S65C, has been found in eight French HH patients[7]. However, its clinical importance remains controversial as the S65C variant is associated with increased percent transferrin saturation in healthy Canadian blood donors.

The allelic frequency of S65C is 0.6%-1.95% in Caucasian population[8].

There are only a few studies from India on the frequency of the known HFE gene mutations[9-12]. We attempted to study the prevalence of these mutations in patients with various liver disorders and healthy controls. We also determined the frequency of primary iron over load in patients with various liver disorders.

MATERIALS AND METHODS

A prospective study was undertaken in the Department of Haematology of the Postgraduate Institute Medical Education & Research, Chandigarh, a referral hospital in North India by studying cases of chronic liver disease (CLD) from the Hepatology Department, for identifying subjects with primary iron overload based on iron studies and liver biopsy wherever possible.

hundred controls were screened for iron status and the three known HFE gene mutations, namely C282Y, H63D and S65C. The controls were unrelated individuals from the indigenous population of north India without any disease or biochemical abnormality and willing to enter the study. Two hundred and thirty-six patients with various types of liver disorders were screened for iron status, including 59 cases of non-alcoholic steatoheptitis (NASH), 22 cases of alcoholic liver disease (ALD), 19 cases of cirrhosis due to viral etiology (HBV, HCV), and 136 cases of cryptogenic cirrhosis.

Collection of samples

A complete history was taken with special emphasis on the duration of illness, disease activity, and complications such as loss of blood. The patients were excluded if they had a history of multiple blood transfusions and hemoglobinopathy or if they were on iron supplements. Overnight fasting blood samples were collected for iron study, 6-8 mL blood sample was taken in iron free tube and 5 mL was taken in liquid EDTA for DNA extraction. Institutional ethical clearance and informed consent from all the patients and controls were obtained for the study.

Iron studies

Serum iron and total iron binding capacity (TIBC) were measured by the colorimetric method with ferrozine chromogen as described by Dacie *et al*[13] and percentage transferrin saturation was calculated. Serum ferritin was measured using an enzyme immunoassay kit (Orgentec diagnotika GmbH, Germany). Percentage transferrin saturation (%TS) of > 55% in males and postmenopausal females and > 45% in premenopausal females, serum ferritin > 1000 μg/mL were taken as biochemical criteria for the diagnosis of primary iron overload. Liver biopsy was performed for confirmation of parenchymal iron overload by haematoxylin and eosin stain and Perls Prussian blue stain.

DNA analysis

Genomic DNA was extracted from the peripheral blood leucocytes by standard phenol chloroform method. HFE gene mutations (C282Y, H63D and S65C) were determined by specific polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described previously[4,7]. The PCR products were digested with restriction enzymes *Rsa*- I , *Bcl*- I and *Hinf*- I to identify the C282Y, H63D and S65C variants respectively[4,7].

Table 1 Prevalence and allelic frequencies of the H63D mutation in controls and chronic liver disorder patients

Group	Prevalence				Allele frequency
	-/-	-/+	+/+	%	
Controls (*n* = 100)	88	11	1	12	6.5
Cryptogenic (*n* = 136)	119	17	0	12.5	6.2
NASH (n =59)	49	9	1	16.9	9.3
Viral (*n* = 19)	14	5	0	26.3	13.1
ALD (*n* = 22)	19	3	0	13.6	6.8
Total (*n* = 336)	289	45	2	13.98	7.29

RESULTS

No case of iron overload was encountered in the 100 controls (58 males and 42 females). Iron deficiency was found in 26 individuals, of them six were males (10.3%) and 20 (47.6%) were females. Two hundred and thirty-six patients with liver disorder were analyzed for iron parameters, namely percentage transferrin saturation (%TS) and serum ferritin as well as the HFE genotypes, namely, C282Y, H63D and S65C. Out of the 236 chronic liver disease patients, 17 showed biochemical iron overload. The clinical features of the 17 cases were consistent with a diagnosis of primary iron overload. Fifteen out of the seventeen patients had cryptogenic cirrhosis and two had alcoholic cirrhosis. The overall percentage of iron overload in cryptogenic cirrhosis was 11% (15/136). Four patients presented with associated diabetes mellitus. A positive family history with more than one family member affected was found in only one patient. Liver biopsy could be performed in 12 cases and could not be carried out in 5 cases because of their low platelet counts. All the biopsies showed 3+ to 4+ parenchymal deposition of iron on Perls' staining. There were 14 males and 3 females (M:F = 4.6:1) and the mean age of the males was 48 years and that of the 3 females was 68, 54 and 49 years, respectively.

All the controls and patient group were detected to have the wild type of C282Y and S65C. Hundred normal controls showed the prevalence of H63D (11 individuals were heterozygous and one individual was homozygous). Out of the 236 patients with liver disorders, 34 were heterozygous and one was homozygous for the H63D mutation. The prevalence of this mutation in the patients with liver disorders was 14.8% (normals = 12%, NASH = 16.9% viral = 26.3%, ALD = 13.6%, cryptogenic = 12.5%) and the overall frequency was 13.98%. The Odd' s ratio was found to be 1.27 (95% confidence interval was 0.63-2.57). The prevalence and allelic frequencies of the H63D in the normals and chronic liver disorder patients are summarized in Table 1. Only two individuals, one in the control and one with NASH were found to be homozygous for H63D. However, these two individuals

did not show iron overload. Overall, in the 17 patients with primary iron overload only two showed heterozygosity for H63D.

DISCUSSION

Primary iron overload is uncommonly encountered in Indians and happens to be common in the Caucasians of North Europe. In the west, the C282Y mutation of the HFE gene is associated with HH in majority of cases. Variations in prevalence of the HFE gene mutations (C282Y and H63D) have been established in many European populations and descent (United States, Canada, Australia, South Africa). Few studies are available from India on the prevalence of these mutations in the general population[9-12].

We attempted to study the prevalence of iron overload in different types of liver disorders and the frequency of the three known point mutations of the HFE gene. Our study shows that of all the groups of chronic liver disorder, cryptogenic cirrhosis showed the highest frequency (15/136, 11%) of iron overload. Two patients with alcoholic cirrhosis showed iron overload. The other groups of NASH and viral cirrhosis did not reveal iron overload. We have shown that iron does not have any etiological role in the development of NASH[14]. The lower frequency of primary iron overload in our population may be due to a high frequency of iron deficiency anemia encountered in Indians.

On screening for the HFE gene mutations (C282Y, H63D and S65C) in 672 alleles of our population, no case of C282Y and S65C was identified. No C282Y mutation has been identified in Asian people including Hong Kong Chinese, Taiwanese aboriginals and Indonesians[15]. In addition, a study involving 252 Japanese subjects confirmed complete absence of the C282Y[16]. The prevalence of HH seems to be low in people of Asian origin. Few HH patients of Japanese origin, a small series of Chinese patients[17] and single Chinese women with marked iron overload were negative for the HFE C282Y mutation[18]. Therefore, absence of the Hfe C282Y mutation supports the hypothetical existence of non-Caucasian haemochromatosis, which seems to be non-HLA linked. However, it remains to be defined at the genetic level.

In the present study, the overall prevalence of H63D was 13.98% (12% in normal and 14.8% in patients with chronic liver disease). Thirty-four of the 236 patients with liver disease and 11 normal subjects were H63D heterozygous, and one in each group was homozygous. Neither the patients with NASH nor the normal individuals who were homozygous for H63D showed iron overload. A pilot study was previously conducted by us in 58 normal subjects, 154 subjects with beta thalassemia trait (BTT) and 9 with HH for the HFE mutations (C282Y and H63D). No individual was found to be positive for the C282Y and the prevalence of H63D mutation in the 212 subjects (normal and BTT) was 16.5%[9]. Since primary iron overload exists in our population and there is paucity of molecular information in these patients, more studies to delineate this defect are warranted in our population. These frequencies are similar to those found for this genotype in liver disorder subjects and controls[19].

In conclusion, primary iron overload is uncommonly encountered in our population. Of the known HFE gene polymorphisms, both C282Y and S65C are absent in our population. H63D is present in a frequency of 13.9% in our population but is not associated with iron overload even in the homozygous state. Our study reiterates the fact that primary iron overload in Indians is the non-HFE type and further molecular studies are required to determine the exact defect in various iron regulatory genes, like the transferrin receptor 2, hepcidin, ferroportin, ferritin and hemojuvelin.

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S- Editor Liu Y **L- Editor** Wang XL **E- Editor** Liu Y