Nonalcoholic steatohepatitis in Asian Indians is neither associated with iron overload nor with HFE gene mutations

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INTRODUCTION
Non-alcoholic steatohepatitis (NASH) is a clinicopathological entity that occurs in non-alcoholics but histologically simulates alcoholic hepatitis[1]. Mechanisms leading to inflammation, fibrosis, and cirrhosis from a relatively benign stage of only steatosis are poorly understood[2]. For this purpose, NASH is considered a two-hit process[3]. Initial hit leads to excessive fat accumulation in the liver parenchyma and the second hit initiated by an oxidative stress may lead to lipid peroxidation, thus damaging the cells and producing a variety of proinflammatory and fibrogenic byproducts[2,3]. This second hit may be initiated by various factors including insulin resistance and hepatic iron with or without HFE gene mutation of hemochromatosis[2,4].

The prevalence of HFE gene mutations and iron abnormalities in NASH patients have been linked to the ethnicity[5]. There is no study from India[6,7] on the role of iron abnormalities and HFE gene mutations in patients with NASH. We retrospectively studied 31 patients of NASH and analyzed the serum and liver iron abnormalities in them and also determined the mutations in the HFE gene.

MATERIALS AND METHODS
Thirty-one patients with NASH attending liver clinic of our Institute over a period of two years (April 2001- March 2003) were diagnosed on the basis of clinical examination biochemistry, ultrasonography and liver biopsy (n = 14) were included in the study. Serum iron parameters (n = 23) (iron, ferritin, total iron-binding capacity and transferrin saturation), Perls’ iron staining on liver biopsies (n = 14) and HFE gene mutations (C282Y and H63D) (n = 16) were studied in these patients. The association between iron staining, necroinflammatory activity and fibrosis stage on liver biopsies was also determined.

RESULTS: Elevated serum iron, ferritin and transferrin saturation above 55% were observed in 4.3% of patients. On histology, 71% of the patients had negative iron staining, 21.4% had 1+ staining, 7.2% had 2+ staining and none had 3+ or 4+ staining. There was no association between the degree of iron staining and necroinflammatory activity (P = 0.55) and fibrosis stage (P = 0.09) on histology. None of the patients had C282Y HFE gene mutation and four patients (25%) were found to be heterozygotes for H63D gene mutation.

CONCLUSION: Our study does not favor iron overload and HFE gene mutations as major factors in the pathogenesis of NASH in Asian Indians.

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Key words: Nonalcoholic steatohepatitis; Iron overload; HFE gene; Mutation

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in meter²) while waist hip ratio (W/H) for the central obesity was determined in 13 patients. Overweight, obesity, and abnormal waist hip ratio were defined as per the international criteria. A fasting plasma glucose ≥ 126 mg/dL or on more than one occasion or a random plasma glucose of more than 200 mg/dL in a symptomatic patient or a two-hour plasma glucose more than 200 mg/dL on glucose tolerance test (GTT) was defined as diabetes. Impaired glucose tolerance (2 h plasma glucose after 75 g of oral glucose between 140-200 mg/dL) and impaired fasting glucose (fasting plasma glucose between 110-126 mg/dL) were also defined. Dose and duration of drugs were recorded in patients who were known diabetics for a long duration. Lipid profiles including serum cholesterol, high-density lipoprotein (HDL), low-density-lipoprotein (LDL) and serum triglycerides were done in all patients (Table 1).

Table 1 Anthropometric, biochemical, serum iron parameters, liver iron staining and HFE gene mutations in 31 patients with NASH

| Parameter | Reference value | Abnormal-n (%) |
|-----------|-----------------|----------------|
| 1 BMI     | <25 kg/m²       | 23 (74.2)      |
| 1 Overweight | 25-29          | 20 (64.5)      |
| 2 Obesity | ≥30             | 3 (9.7)        |
| 2 W-H ratio (n = 13) | 12 (92)        |
| I. Males (n = 10) | <0.9           | 10 (100)       |
| II. Females (n = 3) | <0.85          | 2 (67)         |
| 3 Serum iron | 65-170 mg/dL   | 1 (4.3)        |
| 4 HDL    | 130-200 mg/dL   | 13 (41.9)      |
| 5 LDL    | 35-60 mg/dL     | 8 (25.8)       |
| 6 Triglycerides | 30-200 mg/dL | 10 (32.3)      |
| 7 Diabetes |                      | 3 (9.7)        |
| 8 Impaired glucose tolerance | 7 (22.6) |
| Serum iron parameters (n = 23) |                  |
| 9 Serum iron | 65-170 mg/dL   | 1 (4.3)        |
| 10 Transferrin saturation | <55%           | 1 (4.3)        |
| 11 Serum ferritin | 20-250 mg/dL  | 1 (4.3)        |
| Perls Prussian blue staining on liver biopsy (n = 14) |                  |
| 12 Iron staining positivity | 10 (71.4)     |
| 1+       | 3 (21.4)        |
| 2+       | 1 (7.2)         |
| 3+       | 0               |
| 4+       | 0               |
| 13 HFE gene mutations (n = 16) |                  |
| I. Normal/normal | 12 (75)        |
| II. C282Y/C282Y |                      | 0              |
| III. C282Y/H63D |                      | 0              |
| IV. H63D/H63D |                      | 0              |
| V. H63D/Normal |                      | 4 (25)         |

Statistical analysis

Chi square test was used to determine the association between the grade of iron staining and necroinflammatory grade and the stage of fibrosis on liver biopsies. P<0.05 was considered statistically significant.

RESULTS

There were 31 patients (M-25, F-6) with a mean age of 38.4 years (Range 19-70 years). Their clinical presentations included asymptomatic transaminases detected incidentally either during work up for other non-hepatic diseases (17 patients), generalized weakness and body aches (8 patients), and non-specific upper abdominal discomfort (6 patients). The rest of liver function tests were normal and upper GI endoscopy did not show varices in any patient. Results of the lipid profile, presence of diabetes and impaired glucose tolerance (IGT) are shown in Table 1. Abnormalities found in serum iron studies and on Perls Prussian blue staining of liver biopsies are also shown in Table 1. None of the 16 patients studied for HFE gene mutations revealed C282Y mutations, while four (25%) patients were found to be heterozygotes for H63D mutations. The relation between the grading, staging, and iron positivity on Perls staining of liver biopsies is shown in Table 2. There was no association between the iron staining and degree of inflammation (P = 0.55) and fibrosis stage (P = 0.09).

Table 2 Grade of iron staining, necroinflammatory grade and stage of fibrosis in liver biopsies of 14 patients with NASH

| Iron grade | Necroinflammatory grade | Fibrosis stage |
|------------|-------------------------|----------------|
| 0          | Mild                    | 2              |
| 1          | Mild                    | 3              |
| 0          | Mild                    | 2              |
| 0          | Moderate                | 2              |
| 0          | Mild                    | 2              |
| 0          | Mild                    | 1              |
| 0          | Mild                    | 0              |
| 2          | Mild                    | 1              |
| 0          | Mild                    | 1              |
| 0          | Mild                    | 2              |
| 1          | Mild                    | 2              |

No association between iron grade and necroinflammatory grade (P = 0.55) and stage of fibrosis (P = 0.09).

DISCUSSION

We did not find significant abnormalities of serum and liver iron in our patients with NASH. Only one patient had a high serum iron level, one having a high ferritin level and one having a high TS level above 55%, but the values were not high enough to either suggest hereditary hemochromatosis (HH) or an iron overload state. Though we did not measure the hepatic iron concentration (HIC) and did not calculate the hepatic iron index (HII = HIC/age), no significant positivity for Perls stain was found on liver histology (Table 1). Moreover, Perls staining on liver biopsies had no association with the degree of necroinflammatory activity (grade) and fibrosis (stage) (Table 2).

The two-hit hypothesis in the pathogenesis of NASH includes deposition of excessive fat in hepatocytes (first hit) and development of oxidative stress and lipid peroxidation (second hit). In addition to other factors like insulin resistance, oxidative stress may be initiated by excessive hepatic iron. With the saturation of mitochondrial β-oxidation by excess free fatty acids in the liver, peroxisomal β-oxidation generates hydrogen peroxide. In the presence of excess iron, hydrogen peroxide is converted to hydroxyl radicals, thus leading to oxidative stress and release of various proinflammatory and fibrogenic products. There are studies both favoring and refuting the role of iron in the pathogenesis of NASH. Fifty-eight percent patients in a series by Bacon et al. had elevated iron indices, while some patients had stannable iron in their liver biopsy specimens and an elevated hepatic iron concentration. Another study showed the presence of hyperferritinemia with normal transferrin saturation in patients with NASH and suggested that a simultaneous disorder of iron and glucose and/or lipid metabolism in most cases associated with insulin resistance was responsible for persistent hyperferritinemia. On the other hand, Younossi et al. studied 15 patients with NAFLD including NASH and found that all patients had a normal HII and there was no correlation between the degree of iron accumulation and different degrees of clinically aggressive forms of NAFLD and NASH. The relationship between the iron overload and NASH was further complicated by not finding changes of NASH in patients who were homozygotes for hereditary hemochromatosis.
and had massive iron accumulation. Iron abnormalities in patients with NASH may occur as a secondary phenomenon to insulin resistance syndrome and non-hereditary hemochromatosis iron overload may be one of the components of insulin resistance syndrome or as suggested by Mendler et al. the “insulin resistance-associated hepatic iron overload” syndrome.

Earlier studies have shown the prevalence of homozygosity and heterozygosity for either of the C282Y or H63D mutations to be significantly higher (69%) in patients with NASH than controls (40%) [22]. In one study, the presence of C282Y mutation in patients with NASH was significantly associated with the degree of iron staining. HIC and the degree of fibrosis on liver biopsy specimens. Since the C282Y HFE gene mutation was observed almost exclusively among Caucasian subjects of Northern European (Anglo-Celtic) ancestry, its influence on the progression of disease was dependent on the population studied. H63D gene mutation on the other hand is more widely distributed but has a minor role in the pathogenesis of iron overload. As expected, none of our patients was heterozygotes for the C282Y gene mutation and the finding that four patients were heterozygotes for H63D mutation might not be significant. Chitturi et al. addressed this issue of ethnicity in studying the iron abnormalities and HFE mutation in patients with NASH. They found that the frequency of C282Y heterozygosity was increased only in Anglo-Celtic patients with NASH compared to ethnic blood donors. There were no C282Y homozygotes in NASH group and the frequency of compound C282Y/H63D and H63D heterozygotes were not increased in NASH. In addition, they found that hepatic iron was not a factor linked to the hepatic fibrogenesis in patients with NASH [9]. We studied 58 normal controls and 154 individuals with thalassemia trait and found that no subject was either heterozygous or homozygous for the C282Y mutation in the past few years [23]. However, the prevalence of H63D mutation was 16.5% in these 212 subjects, with an allele frequency of 8.73% with two patients being homozygous for the mutation. Even in patients with hereditary hemochromatosis, both of these mutations were negative in eight patients and one patient was heterozygous for H63D mutation suggesting that ethnic background has influences on the frequency of C282Y HFE gene mutation and H63D mutation can be found in normal individuals and non-liver disease patients.

It seems that iron contributes to the pathogenesis of NASH only in individuals with excess serum and hepatic iron. In patients with NASH, who had no serum and hepatic iron abnormalities, other factors might play a part in the pathogenesis of NASH. Since C282Y HFE gene mutation has been found almost exclusively in Caucasians of North-European descent, it is not an important contributor in patients with NASH in other populations including Asian Indians. On the other hand, H63D HFE gene mutation is less specific and its presence in patients with NASH may not be significant.

In conclusion, our study does not favor iron overload and HFE gene mutations as major factors in the pathogenesis of NASH in Asian Indians, but would require further studies to confirm the findings.

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