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## Patients with biochemical iron overload: causes and characteristics of a cohort of 150 cases

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**Abstract** Biochemical iron overload (IO) is a frequent metabolic abnormality. It may be caused by several diseases, and data regarding the relative frequency of these are scant. A single diagnostic protocol including clinical, biochemical, and genetic data was used to diagnose the cause of biochemical IO in a group of 150 patients referred by general practitioners. Severe alterations of the HFE gene (42 patients, 28%), hepatitis C virus infection (33 patients, 22%), and dysmetabolic syndrome with iron overload (DSIO) (22 patients, 15%) emerged as the main causes, and other single causes were found in 20 patients (13%). In 19 patients (13%), multiple causes of IO were found, and in 14 patients no cause was found, 5 of whom had classical criteria of genetic hemochromatosis (GH) without HFE mutations. Transferrin saturation index (TSI) had a very low positive predictive value (0.16) for GH among patients with biochemical IO in this setting. In conclusion, 90% of patients with biochemical IO were diagnosed with a specific disorder. GH, hepatitis C infection, and DSIO were the major causes, and a large group of patients had multiple causes of IO. TSI is not a useful indicator of GH in patients referred by general practitioners.

**Keywords** Iron · Transferrin saturation · Ferritin · Hemochromatosis · Dysmetabolism

### Introduction

Biochemical iron overload (IO) is frequent among adults in developed countries, affecting 13% of 1016 elderly white Americans aged 67–96 in the Framingham Heart Study [5, 7, 13]. Distinct diseases such as genetic hemochromatosis (GH), viral hepatitis, alcoholism, dysmetabolic syndrome with iron overload (DSIO), and rarer disorders share IO as a common phenotypic expression, causing some confusion in the differential diagnosis of patients. Moreover, data about the relative frequency of these disorders are scant.

The main objective of this study was to ascertain the type and characteristics of each of these disorders in an unselected group of 150 patients with biochemical IO consecutively sent by general practitioners to a specialized unit. Based on the results we try to clarify the relative frequency of each of these disorders.

### Materials and methods

Between January 1998 and July 2001, 207 patients were referred by general practitioners and visited our unit for suspicion of IO. Diagnosis of biochemical IO was confirmed in 150 by two fasting measures of transferrin saturation index (TS) above 45% or ferritin levels of more than 350  $\mu\text{g/l}$ . Transferrin saturation was calculated dividing serum iron levels by total binding iron capacity. All these patients were submitted to an extensive medical interview directed at all known causes of biochemical IO, with special emphasis on alcohol abuse (more than 60 g/day), hypertension, blood transfusions, porphyria cutanea tarda, and cataracts. A biochemical screening was done to disclose common metabolic abnormalities [serum cholesterol and triglyceride levels and glucose tolerance test or hemoglobin (Hb) A1c levels]. Serologies for hepatitis B and C were performed in all cases. All patients were tested for GH-related mutations (C282Y and H63D mutations of the HFE gene) by a previously described method [1]. Body mass index was measured and considered to be abnormal when  $>25 \text{ kg/m}^2$ . Concomitant inflammatory diseases potentially capable of causing hyperferritinemia were ruled out on the basis of the absence of clinical signs and a high erythrocyte sedimentation rate (ESR). Ceruloplasmin and  $\alpha_1$ -antitrypsin were measured in patients with liver disease only.

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**Table 1** Age, gender, and metabolic iron data of patients. Continuous variables are shown as median (range). *TSI* transferrin saturation index, *HCV* hepatitis C virus, *DSIO* dysmetabolic syndrome with iron overload

Diagnosis (n)	Age (years)	Gender (M/F)	TSI (%)	TSI(>45%)	Ferritin ( $\mu\text{g/l}$ )	Ferritin (>350 $\mu\text{g/l}$ )
C282Y/C282Y (24)	50 (34–67)	21/3	87 (49–111)	24/24	1342 (351–4000)	24/24
C282Y/H63D (14)	52 (28–71)	7/7	64 (43–88)	13/14	449 (60–1007)	10/14
H63D/H63D (6)	54 (37–64)	5/1	56 (39–67)	5/6	761 (329–1500)	4/6
HCV (33)	55 (34–78)	22/11	69 (42–100)	31/33	1016 (305–2794)	32/33
DSIO (20)	55 (29–76)	16/4	58 (47–96)	20/20	1021 (271–2545)	19/20
Alcohol (7)	54 (30–67)	7/0	58 (51–75)	7/7	488 (59–984)	4/7
Transfusion (6)	38 (22–62)	4/2	82 (57–100)	6/6	3284 (1228–8334)	6/6
Multiple (19)	58 (35–75)	18/1	68 (46–98)	19/19	715 (220–1946)	16/19
Unknown (14)	52 (24–71)	11/3	67 (27–100)	12/14	905 (404–1704)	14/14

#### Statistical methods

Positive and negative predictive values were calculated by usual methods [2]. Differences between continuous variables were measured by Mann-Whitney U tests. The results were considered statistically significant when *P* values were less than 0.05.

## Results

Severe alterations of the HFE gene were the most frequent causes for biochemical IO (44 individuals, 29% of patients). Of these patients, 24 were C282Y homozygous, 14 C282Y/H63D compound heterozygous, and finally 6 were H63D homozygous. In 17 of 24 C282Y homozygous patients, a liver biopsy was done and hepatic iron index (HII) calculated. HII was >2 in 16 of 17 patients. Positive and negative predictive values of TS >45% to diagnose GH in this context were 0.16 and 1, respectively. The second cause of biochemical IO was hepatitis C virus (HCV) (33 patients, 22%): 16 of these 33 patients (48%) were heterozygous for the H63D mutation of the HFE gene. Dysmetabolism was the third cause in this series (20 patients, 13%). In this group a high rate of metabolic pathology was observed as expected: 19 of 20 patients showed glucidic metabolic alterations, and all patients had abnormalities in cholesterol or triglyceride levels. Eighteen were obese and seven had hypertension. All of them had liver steatosis measured by an echographic exam and 16 showed increased transaminases. Eleven patients were H63D heterozygous.

The rest of the causes had a low frequency compared with those described above. Alcohol abuse was the origin of biochemical IO in seven patients (5%), and six patients had malignant diseases and were submitted to repeated blood transfusions (4%). A miscellaneous group of seven patients had well-described but infrequent causes of IO such as sideroblastic anemia (three), thalassemia (two), unstable hemoglobin (one), and hepatocarcinoma due to liver cirrhosis (one).

Interestingly, more than one cause was found in 19 patients (13%). Seven of these were C282Y/H63D compound heterozygous with HCV infection (three), alcoholism (one), thalassemia (one), Wilson's disease (one), and finally one patient who was compound heterozygous, alcoholic, and HCV positive. Six were H63D homozygous with HCV (two), alcoholism (two),

dysmetabolism (one), and alcoholism with HCV (one). HBV and dysmetabolism in one patient, alcoholism and HCV in another, and alcoholism and dysmetabolism in three other patients complete this group.

It was impossible to find a reason for IO in a group of 14 patients (9%). Five of them had classical criteria of hemochromatosis with a hepatic iron index (=ratio of hepatic iron concentration over age) >2, nevertheless without mutations in the HFE gene, and two with a clear familial incidence of disease. Two of the remaining nine patients were C282Y heterozygous. A minor biochemical IO was found in these nine patients. The age, gender, and iron metabolic data of patients in all groups (except miscellaneous) are shown in Table 1.

Increases in metabolic iron parameters were especially severe in the C282Y homozygous group and in transfused patients, both groups with a significant increase of TS and ferritin (*P*<0.05) in comparison with other groups that shared similar parameters with them.

## Discussion

Iron overload can be associated with various pathological conditions. In this study, 150 patients with biochemical signs of IO were studied by clinical and laboratory methods in a specialized hematology consulting unit to ascertain the cause of IO in each case and to clarify the relative importance of each type of IO in our setting. It is important to remember that this study is not applicable to the general population because the paper focuses on preselected patients referred for hematological consultation.

Severe alterations of the HFE gene were the main cause of IO in this group of patients. Most of them were C282Y homozygous and were directly diagnosed with hemochromatosis [12]. In fact, in 16 of 17 patients with hepatic iron index measured from liver biopsy, this parameter was >2. Other patients were compound heterozygous or H63D homozygous with a lower IO, but their IO was only explained by these genetic abnormalities. Some authors consider double heterozygous patients with IO as GH, but H63D homozygous patients are not in general considered GH patients even though they have IO [16]. In view of our results, it seems some people have IO with H63D homozygous as the only

cause. Transferrin saturation index is considered the best single biochemical test to detect GH in the general population, with a positive predictive value (PPV) of 0.64 to 0.92 [3, 8, 19]. The very poor PPV observed in our patients (0.16) can be explained because they were sent from general practitioners and were not representative of the general population. Our cohort of patients had a very high prevalence of confusing diseases causing elevation of TS (in some cases very similar to GH) with a severe decrease in the diagnostic capabilities of the TS test. These data must be taken into account when diagnosis of GH is to be done in selected populations of patients referred to the hospital.

The second cause of biochemical IO was HCV. It is known that this virus can produce biochemical IO by an unknown mechanism [11], not always reflecting a true excess of liver iron [27]. It seems that patients affected by HCV with severe IO have a poorer prognosis than patients without, with a low response rate to interferon treatment [17, 18, 26]. HCV is very frequent in Spain and more common among patients with a high degree of complex pathology. This group of patients represents a problem for diagnosis because it is difficult to differentiate whether biochemical alterations of the liver are only caused by a viral effect or can be in part subsequent to IO [25]. We previously described that IO is not mainly due to HFE mutations in this group of patients [24], but a slight increase of allelic frequency of H63D mutation was observed (28.4%) similar to that found in this group of patients (24%). It is not clear if therapeutic phlebotomies can improve the prognosis of this large group of patients.

Dysmetabolic syndrome with iron overload was defined by Deugnier and co-workers as a clinical constellation of IO and metabolic abnormalities [21]. Initially, a normal TS was required to diagnose this syndrome, to clearly differentiate this acquired metabolic process of GH, but this requirement has been recently removed [20]. The importance of IO in this syndrome is not clear, as happens in other liver diseases such as nonalcoholic steatosis and steatohepatitis (NAS/NASH). These are other processes with high prevalence of metabolic abnormalities and frequent presence of HFE mutations [6]. Twenty of our patients had clinical criteria of DSIO. All of them had two metabolic abnormalities and more than 50% three or more, with a very high frequency of obesity. All of them presented echographic signs of liver steatosis. Recent data demonstrate that association of hyperferritinemia and multiple metabolic abnormalities identifies patients at risk for NASH [10]. Of 20 patients with DSIO, 16 had increased transaminasemia and probably NASH. NASH is associated with hepatitis, liver steatosis, and usually multiple metabolic alterations. The presence of hyperferritinemia and iron overload is frequent in this disorder and has been associated with a faster progression to liver fibrosis or cirrhosis [15] than NASH without IO. Prior studies are not clear about the prevalence of DSIO and NASH, but our results show that these conditions are frequent among patients with IO. Some relationship exists between DSIO, NASH, and HFE

mutations as Deugnier and co-workers stated [20]. Those data were similar to those found in our patients who had an allelic frequency of H63D mutation (20%) higher than that previously found by us in Spanish blood donors [4].

A long list of pathologies related with IO completed the single causes of increased biochemical iron parameters in this group of patients. Interestingly, more than one cause was discovered in a big group of 19 patients. The high frequency of some of these pathologies, such as HFE mutations, HCV infection, polytransfusion, or alcoholism, explains this phenomenon and they are probably not related. Curiously, even though more than one cause was present, iron increase was not more severe in these patients in comparison with the others.

Finally, 9% of patients had IO without a well-defined cause. In one-third of these (five patients), a classical diagnosis of GH that was not HFE related could be made (by HII >2) with a clear familial presentation in two cases. The rest remain undiagnosed.

A criticism of this study could be that we defined IO as a permanent increase of ferritin and/or transferrin saturation when there are more accurate methods to measure iron load. Nevertheless, methods such as liver biopsy with biochemical measure of iron, SQUID magnetometers, and magnetic resonance are invasive and dangerous (in the case of liver biopsy) or expensive and difficult to do in clinical practice [3, 9, 14, 22]. These considerations explain why ferritin and TS remain the most usual and useful methods to diagnose and follow patients with IO in clinical practice [23]. Classification of this kind of frequent disease should be based on practical methods and not on those used in medical literature only.

In conclusion, based on medical history and the results of standard biochemical and genetic tests, the cause of IO can be determined in 90% of all patients. Three causes (severe alterations of the HFE gene, HCV, and DSIO) account for 65% of cases. Multiple causes are frequently involved and some patients can suffer from GH not related to HFE.

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