Ann Hematol (2007) 86:831–835
DOI 10.1007/s00277-007-0336-0

ORIGINAL ARTICLE

The relationship between iron overload and clinical characteristics in a Spanish cohort of 100 C282Y homozygous hemochromatosis patients

Albert Altes · Angels Ruiz · Clara Martinez · Anna Esteve · Maria Dolores Vela · Angel Francisco Remacha · Pilar Sarda · Vanessa Bach · Montserrat Baiget

Received: 9 February 2007 / Accepted: 18 June 2007 / Published online: 17 July 2007

Abstract We studied the relationship between iron removed by venesection, sex, age, and clinical characteristics in a group of 100 Spanish probands with hereditary hemochromatosis (HH), all C282Y homozygous in the *HFE* gene. Iron overload was higher in men than in women ($P<0.0001$) and increased with age ($P=0.02$). Forty-four patients presented with liver disease (28 had fibrosis–cirrhosis of the liver), 24 with diabetes, 18 with arthropathy, and 13/73 men with impotence. No clinical consequences of hemochromatosis were observed in 43 patients. The number of clinical complications was higher in men ($P=0.01$) and increased with age ($P=0.006$) and with the amount of iron removed ($P<0.0001$). The amount of iron removed was significantly higher by univariate analysis in patients with liver disease ($P<0.0001$), diabetes ($P=0.007$), arthropathy ($P=0.006$), and impotence ($P=0.003$) than in patients without these complications. In the multivariant analysis, only liver disease maintained a significant relationship with the amount of iron removed ($P<0.0001$). Diabetes and arthropathy were closely related with previous liver disease, and impotence appeared mainly in hemochromatosic men with diabetes and alcoholism.

Keywords Hemochromatosis · Liver cirrhosis · Diabetes · Arthropathy · Impotence

Introduction

Hereditary hemochromatosis (HH) is a genetic disease characterized by excessive iron absorption despite iron overload. It is usually associated with the presence of C282Y mutation of the *HFE* gene in homozygosity [13]. Complications such as liver fibrosis/cirrhosis and/or diabetes mellitus are common as a consequence of prolonged iron overload. Clinical complications usually appear during aging because iron accumulates in tissues progressively over time [6]. Nevertheless, in a study with a large number of HH probands, no significant correlation was found between the age of patients and their liver iron concentration [1].

In this work, we studied the relationships between sex, age, and clinical characteristics with the degree of iron overload in a Spanish cohort of 100 proband HH patients, all C282Y homozygous, using univariant and multivariant statistical methods.

A. Altes · A. Ruiz · C. Martinez · A. Esteve
Hematology Department,
Hospital de l'Esperit Sant de Santa Coloma de Gramenet,
Barcelona, Spain

M. D. Vela
Hematology Department, Hospital de Blanes-Calella,
Barcelona, Spain

A. F. Remacha · P. Sarda · V. Bach · M. Baiget
Hematology and Genetics Service, Hospital de Sant Pau,
Barcelona, Spain

A. Altes (✉)
Servei de laboratory,
Hospital de l'Esperit Sant de Santa Coloma de Gramenet,
Avda Mossen Pons i Rabada s/n 08923,
Barcelona, Spain
e-mail: aaltes@hes.scs.es

Materials and methods

All patients selected for this study were referred to the hematologist by biochemical evidences of iron overload with or without symptoms compatible with genetic hemochromatosis. Transferrin saturation and serum ferritin were measured at diagnosis by standard methods in samples obtained after an overnight fast. HFE mutations were analysed using the LightCycler equipment [3] (Roche Diagnostics, Mannheim, Germany) in those patients with a transferrin saturation index ≥55% or a serum ferritin level ≥400 μg/L in two occasions. Finally, 100 of nonrelated homozygous C282Y patients were included in the study. All patients were diagnosed between 1985 and 2005. In all patients, the total amount of iron removed was calculated as the number of phlebotomies (with 450 ml of blood drawn at each session) multiplied by 0.2 (the number of grams of iron removed per session). We considered patients with a severe iron overload as those in whom ≥5 g of iron were removed by phlebotomy (≥25 procedures). In some patients, the hepatic iron concentration and the hepatic iron index were determined as previously described [4].

Definition of clinical complications

Liver damage was diagnosed when AST and ALT enzymes were above normal limits at least twice at intervals of over 3 months, and in patients with histological evidence of liver fibrosis/cirrhosis. Patients considered diabetic were those receiving treatment for diabetes and those with a fasting plasma glucose test of ≥126 mg/dl on two occasions. Arthropathy (in association with HH) was defined as the presence of symmetric, polyarticular involvement of the metacarpophalangeal joints or proximal interphalangeal joints, and arthritis related to calcium pyrophosphate deposition disease crystals. Cardiac disease was based on history and/or clinical signs of congestive heart failure. Clinical data on impotence in male patients was recorded. Alcohol consumption was assessed from patient history and >80 g/day of ethanol was considered excessive. Viral serology data for hepatitis B and C were available in all patients.

Statistical analyses

Univariate relationships between categorical variables were analysed by the chi-square test and differences in means by the Student's *t* test. Multivariate regression models were fitted to study the relationship of clinical features (liver damage, diabetes, arthritis, and impotence) with age at diagnosis, gender, excess alcohol use, and positive hepatitis serology. *P* values of <0.05 were considered statistically significant.

Results

General characteristics of population

Table 1 summarises the biochemical characteristics of the 100 study patients. Seventy-three patients were men and 27 were women with a median age of 45 years (range 11–73). Fourteen patients consumed more than 80 g/day of alcohol and 3 had a positive serology for hepatitis C virus. Forty-four patients presented with liver damage, and a liver biopsy was performed in 33 of them. Fibrosis–cirrhosis of

Table 1 Age and biochemical characteristics of the cohort of 100 homozygous C282Y patients

Parameter	Mean	Median	Range
Age (in years); *N*=100	45	45	11–73
Men (*N*=73)	46	46	11–72
Women (*N*=27)	45	45	11–73
Transferrin saturation; *N*=100	82%	84%	30%–161%
Men (*N*=73)	86%	85%	32%–161%
Women (*N*=27)	73%	70%	30%–116%
Ferritin (μg/l), *N*=100	1,259	861	17–10,000
Men (*N*=73)	1,563	1,093	154–10,000
Women (*N*=27)	435	253	17–2,605
Iron removed (in g); *N*=100	5.5	4.2	0.5–20
Men (*N*=73)	6.7	5.6	1.4–20
Women (*N*=27)	2.3	1.5	0.5–6.6
Liver iron concentration[a] (*N*=22)	221	192	21–456
Men (*N*=19)	240	198	21–456
Women (*N*=3)	107	128	55–138
Hepatic iron index[b] (*N*=22)	4.6	4.5	0.43–8.75
Men (*N*=19)	4.9	4.5	0.43–8.75
Women (*N*=3)	2.7	3	1–4

[a] μMol/g liver dry weight.
[b] (μMol/g liver dry weight)/age in years.

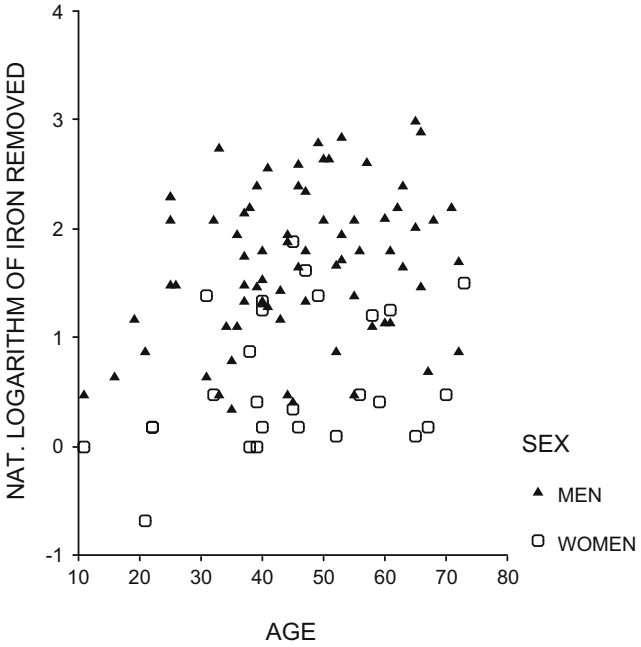

Fig. 1 Relationship between body iron stores as assessed by the natural logarithm of iron removed by venesections and age at diagnosis in patients with hemochromatosis, all C282Y homozygous. (73 men, 27 women, r=0.24, P=0.02)

the liver was finally diagnosed in 28 and liver cancer in 4. Twenty-four of the 100 patients had diabetes, 18 presented with arthropathy and 3 had cardiac insufficiency. Impotence was diagnosed in 13 (18%) of males.

Study of the relationships between sex and age with the body iron burden and the number of clinical complications

The median of iron removed by phlebotomies in men was significantly higher than in women (2.3 vs 6.7 g, P<0.0001) with a median difference of 4.4 g (95% confidence interval difference of 2.7–6.2 g). The correlation coefficient for individual iron removed by phlebotomies compared with age was 0.24 (P=0.02; Fig. 1). The correlation coefficient of iron removed and age was 0.24 in men and 0.23 in women.

Forty-three patients were free of symptoms at diagnosis. Thirty patients suffered 1 clinical complication, 15 had 2 simultaneous complications, 9 had 3 and 3 had 4. The number of complications was narrowly related with the male sex, and increased with age and with the amount of iron removed. The distribution of gender, median age, and iron removed by phlebotomies according to the number of clinical complications is showed in Table 2.

Study of the relationship between clinical complications and the body iron burden

In univariate studies, all four clinical complications were more frequent in patients with more than 5 g of iron removed (Table 3). The amount of iron removed in patients with liver damage was significantly higher than in those without this complication (median of difference=5 g; 95% CI 3.5–6.4 g, P<0.0001). Patients with diabetes also had more iron than those without (median of difference 3 g; 95% CI 1.1–5; P=0.002). Differences were not significant either for arthritis or for impotence (P=0.07 and P=0.14, respectively). Multivariate regression models were used to investigate which of these complications was independently associated with iron burden (Table 4). The presence of liver damage was narrowly related with the amount of iron removed by phlebotomy (P<0.0001), and this clinical complication was also related with alcohol abuse (P=0.006) and diabetes (P=0.045). Diabetes was highly related with the presence of liver damage (P=0.004) and with age (P=0.01), but unexpectedly not with the amount of iron removed (P=0.3). Arthropathy was more frequent in patients who had liver damage (P=0.01), and impotence was significantly related with the presence of diabetes (P=0.002) and alcohol abuse (P=0.01).

Discussion

The aim of this study was to investigate the relationships between sex, age, and clinical characteristics with iron overload in HFE-associated HH by means of multivariate statistics. Two characteristics should be emphasized in this series of 100 proband patients. First, all patients were C282Y homozygous, making it a genetically uniform

Table 2 Distribution of gender, median age and iron removed by phlebotomies according to the number of clinical complications

	Number of clinical complications					
	0	1	2	3	4	*P*
N	43	30	15	9	3	
Men (%)	24 (56%)	24 (80%)	13 (87%)	9 (100%)	3 (100%)	0.011
Median age (range)	38 (11–67)	46 (32–73)	53 (25–70)	47 (33–67)	65 (25–71)	0.006
Iron removed ≥5 g (%)	5 (12%)	15 (50%)	12 (80%)	8 (89%)	3 (100%)	<0.0001

Table 3 Univariate analysis between iron overload and clinical complications associated with hemochromatosis

Variable	Iron removed		
	<5 g; *n* (%)	≥5 g; *n* (%)	*P*
Presence of liver damage			
Yes	10 (23)	34 (77)	<0.0001
No	47 (84)	9 (16)	
Diabetes mellitus			
Yes	8 (33)	16 (67)	0.007
No	49 (65)	27 (35)	
Arthropathy			
Yes	5 (28)	13 (72)	0.006
No	52 (63)	30 (37)	
Impotence			
Yes	1 (8)	12 (92)	0.003
No	31 (52)	29 (48)	

sample. Second, iron burden was measured in all cases by quantifying the amount of iron removed by phlebotomy, probably the most accurate method [7].

The mean and median amount of iron removed in our series of patients was lower than expected (Table 1). The reason is obvious; the diagnosis of genetic hemochromatosis in the pre-HFE era was based on the demonstration of a heavy iron overload (hepatic iron index >1.9 or iron removed by venesection therapy >5 g). The median iron removed in homozygous C282Y patients, even in those referred to a doctor for biochemical data compatible with iron overload, is lower than that found in HH patients in old studies.

Forty-three patients in this series had no clinical complications. There were more women in the asymptomatic group than in the symptomatic one (44% vs 14%, P=0.001). In the asymptomatic group, the individuals were younger (median age 39 vs 50 years, P=0.0001) and with significantly less iron removed by venesections (3.1 vs 7.3 g, P<0.0001). The number of clinical complications depends on sex, age, and amount of iron removed (Table 2). One curious finding in this study was that liver disease was always present when patients had more than one complication.

Table 4 Summary of the significant relationships between clinical features in the 100 genetic hemochromatosis patients with iron removed and other clinical factors (multivariate regression model)

Clinical feature	Related clinical factors	*P*
Liver damage	Iron removed	<0.0001
	Alcoholism	0.006
Diabetes	Liver damage	0.004
	Age	0.01
Arthropathy	Liver damage	0.01
Impotence	Diabetes	0.002
	Alcoholism	0.01

As we expected, iron overload in HH men was significantly higher than in women. One result of particular interest was that the amount of iron removed correlated with the age of the patients. This result agrees with clinical evidence and contradicts the absence of relationship between iron load and age in HH patients previously found by Adams et al. [1]. The cause of this discrepancy may be a higher genetic homogeneity of our cohort (all C282Y homozygotes) than the nongenotyped group studied by Adams et al. [1]. Another explanation may be that the cohort of Adams et al. reached a maximum level of body iron stores without further changes with age.

Four clinical complications in relation with HH could be studied in this work: liver damage, diabetes, arthropathy, and impotence. Only three cases of clinically significant cardiac insufficiency were diagnosed in our series of patients precluding the study of this clinical complication. Liver damage was included in statistical studies rather than fibrosis–cirrhosis because the presence of this complication was determined in all patients and not only in the 33 who had a liver biopsy. Finally, two clinical characteristics, fatigue and skin pigmentation, were not considered in this study; the first because it is a subjective symptom and the second because it is a usual trait in the healthy Spanish population.

In univariant studies, all clinical complications of HH were linked with the amount of iron removed. Nevertheless, when multivariant methods were employed to analyse the same data, only liver disease was significantly related with the body iron burden. Diabetes and arthropathy were related with liver disease (P=0.004 and P=0.01, respectively), but not with the amount of iron removed and, for example, 75% of patients with diabetes (18/24) had simultaneous liver disease (P=0.001). Finally, impotence was more frequent in men with HH that suffered diabetes or alcoholism (P=0.002 and P=0.01, respectively) than in patients without these complications. In univariate analysis, the influence of liver disease (a complication narrowly related with the amount of iron removed) on appearance of diabetes or arthropathy may be confounded with the true effects of the body iron burden. Likewise, in our results, impotence is directly linked with diabetes and alcohol, both narrowly related with liver disease and, in consequence, with iron overload.

In the series of Niederau et al. of 251 patients [14], the prevalence of diabetes in individuals with hepatic cirrhosis was 72%. Nevertheless, diabetes was diagnosed in only 18 of 120 patients (15%) who did not have cirrhosis at the time of diagnosis. In the previously cited study of Adams et al., the authors found that diabetes was more related with cirrhosis (P<0.0001) than with liver iron concentration (P=0.01) [1]. It is interesting to note that in recent large epidemiological studies with selected homozygous C282Y patients, an

association was found between HH and liver disease, but not with diabetes [2, 5]. The decrease in prevalence of diabetes or arthropathy in recent series of HH patients may be explained by a lower prevalence of liver disease in these series, especially important in epidemiological studies of asymptomatic C282Y homozygous individuals.

Can liver disease influence the appearance of other complications like diabetes or arthropathy independently of iron overload? Liver diseases are in general diabetogenic. The prevalence of diabetes in patients with chronic hepatitis C is two to three times higher than would be expected in the general population [8]. Up to 80% of patients with cirrhosis due to any cause may have insulin resistance, and between 20% and 63% will develop diabetes mellitus [9]. Some authors stated that the increased incidence of insulin resistance in diabetes patients with HH was due to the underlying hepatic disease [11]. It is interesting to note that patients with hemochromatosis show hyperinsulinemia, and hence insulin resistance without impaired glucose tolerance in the noncirrhotic stage. Because pancreatic insulin secretion is not impaired in these noncirrhotic patients, iron accumulation in the hepatocytes may be responsible for the impaired insulin effect and may cause impaired hepatic insulin extraction [10]. The liver plays a major role in the regulation of the immune response, and changes in the production and blood levels of a number of cytokines in liver disease may influence the appearance of arthropathy [12].

In conclusion, in patients with HH, the amount of iron removed is higher in men than in women, and increases with age. Although body iron burden is related with all the complications of HH, liver disease seems the most related, facilitating the appearance of further disorders. This timing may explain why in modern series of C282Y homozygous patients, diagnosed in epidemiological studies and with a very low prevalence of liver disease, other clinical complications such as diabetes or arthropathy have the same prevalence that is observed in general non-HH population.

Acknowledgements This work was partially supported by grants from Fondo de Investigaciones Sanitarias (PI-04/1120) and Agencia d'Avaluació de Tecnología i Recerca Mèdica (005/29/2004). This work complies with the current law of Spain inclusive of ethics approval.

References

1. Adams PC, Deugnier Y, Moirand R, Brissot P (1997) The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic haemochromatosis. Hepatology 25:162–166
2. Adams PC, Reboussin DM, Barton JC et al (2005) Haemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 352:1769–1778
3. Altes A, Ruiz A, Barceló MJ et al (2004) Prevalence of C282Y, H63D and S65C mutations of *HFE* gene in 1146 newborns from a region of Northern Spain. Genet Test 8:407–410
4. Barry M, Sherlock SA (1971) Measurements of liver-iron concentration in needle-biopsy specimens. Lancet 1:100–103
5. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T (2002) Penetrance of 845→A (C282Y) HFE hereditary haemochromatosis mutations in USA. Lancet 359:211–218
6. Beutler E (2006) Haemochromatosis: genetics and pathophysiology. Annu Rev Med 57:331–347
7. Brissot P, Bourel M, Herry D et al (1981) Assessment of liver iron content in 271 patients: a reevaluation of direct and indirect methods. Gastroenterology 80:557–565
8. Harrison SA (2006) Liver Disease in patients with diabetes mellitus. J Clin Gastroenterol 40:68–76
9. Holstein A, Hinze S, Thieben E (2002) Clinical implications of hepatogenous diabetes in liver cirrosis. J Gastroenterol Hepatol 17:677–684
10. Niederau C, Berger M, Stremmel W et al (1984) Hyperinsulinaemia in non-cirrhotic haemochromatosis: impaired hepatic insulin degradation? Diabetología 26:441–444
11. Olsen NS, Nuetzel JA (1950) Resistance to small doses of insulin in various clinical conditions. J Clin Invest 29:862–866
12. Parke AL, Parke DV (1994) Hepatic disease, the gastrointestinal tract, and rheumatic disease. Curr Opin Rheumatol 6:85–94
13. Pietrangelo A (2004) Hereditary haemochromatosis—a new look at an old disease. N Engl J Med 350:2383–2397
14. Strohmeyer G, Niederau C (2000) Diabetes mellitus and haemochromatosis. In: Barton JC, Edwards JC (eds) Haemochromatosis. Cambridge University Press, Cambridge, pp 268–277