Acta Derm Venereol 2008; 88: 341–345

INVESTIGATIVE REPORT

Measurement of Liver Iron Content by Magnetic Resonance Imaging in 20 Patients with Overt Porphyria Cutanea Tarda before Phlebotomy Therapy: A Prospective Study

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Liver iron content was evaluated by a magnetic resonance imaging-based method in 20 consecutive patients with either sporadic or familial porphyria cutanea tarda. Serum ferritin, hepatitis C infection and the presence of the 2 main mutations of the hemochromatosis gene were also investigated. All patients showed good clinical response to phlebotomy. Initial liver iron content was normal (< 40 µmol/g) in 9 cases, slightly increased (40–59 µmol/g) in 3 cases, moderately increased (60–99 µmol/g) in 6 cases or markedly increased (100–199 µmol/g) in 2 cases). The ferritin level was raised (> 400 ng/ml) in 14/20 patients and there was no obvious relationship with liver iron. Increased liver iron content was observed more frequently in patients with hemochromatosis mutation and less frequent in those with hepatitis C infection. Clinical response to phlebotomies was slightly better in patients with increased liver iron content even slightly, but patients with normal liver iron content also responded well, which suggests that iron depletion is an outstanding treatment independent of liver iron content. This study shows that increased liver iron content is not a constant finding in patients with porphyria cutanea tarda, especially in women, and that it is not a prerequisite for the efficiency of phlebotomy. ***Key words: porphyria cutanea tarda; liver iron content; magnetic resonance imaging; phlebotomies.***

(Accepted February, 14, 2008.)

Acta Derm Venereol 2008; 88: 341–345.

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Porphyria cutanea tarda (PCT), the most frequent subset of porphyria in Western countries, is linked to decreased activity of uroporphyrinogen decarboxylase (URO-D). The complex pathological factors leading to overt PCT are well known and have been described elsewhere (1–6). Among these factors, the peculiar relevancy of iron overload is highly suggested by the high efficiency of iron depletion by repeated phlebotomies and by independent report of an association between genetic haemochromatosis and PCT in the same patients (7, 8). Iron overload may inhibit URO-D activity in different ways, including the generation of reactive oxygen species, which can interact directly with the enzymatic activity and/or lead to the direct oxidation of uroporphyrinogen to uroporphyrin, which is not a substrate for URO-D (9); moreover, iron may play a role in photosensitization by uroporphyrin (10). Although iron overload is usually considered to be a hallmark of the disease, present in most, if not all, patients with overt PCT (4–6, 11–18), its pathomechanisms are not fully established to date; however, abnormalities of iron metabolism-regulating genes are likely to be significantly involved, for example HFE gene mutations, as demonstrated by a number of previous studies (19, 20). Despite these widely accepted data, only a few investigations assessing iron content in the liver have been conducted in series of patients with PCT over the last 20 years, probably because its accurate measurement requires complex methods such as mass spectrometry. During the last 20 years non-invasive methods have been developed for accurate evaluation of the iron content of tissues such as the liver, methods based mainly on magnetic resonance imaging (MRI) using either transverse relaxation time or liver-to-muscle gradient-echo T2*-weighted ratio, which have both proved reliable and accurate methods that can be used for routine clinical purposes (21–24). Accordingly, a study was designed to investigate liver iron content (LIC) in 20 PCT patients using an MRI-based method. The data obtained were compared with serum ferritin concentration, hepatitis C infection, the presence of 2 main HFE gene mutations, and with the clinical effect of iron depletion by a series of phlebotomies.

PATIENTS AND METHODS

Study design, selection and description of patients

A total of 20 patients diagnosed with either familial or sporadic PCT between April 2004 and April 2006 were included in this study; 12 men and 8 women aged 37–63 years (mean age 47 years) (Table I). The diagnosis of PCT was established on the basis of the usual clinical and biochemical data, including photosensitivity, facial "metallic" hyperpigmentation, blisters and milia on the dorsum of the hands, skin fragility on sun-exposed areas and high excretion rates of urinary porphyrins with dominance of uro- and heptacarboxy-porphyrins (at least 85% of total urinary porphyrins). Triggering factors usually

 ISSN 0001-5555
doi: 10.2340/00015555-0472

Table I. *Demographic, biological and magnetic resonance imaging (MRI) data from patients with porphyria cutanea tarda*

Patient (gender, age at diagnosis (years))	MRI LIC initial/ post-treatment (if applicable) (μmol/g)	Initial ferritin level (ng/ml)	HFE gene status	HCV infection	Number of phlebotomies and approximative total volume (litres)	ALAT and γGT status at diagnosis	Other relevant triggering factors
1 F, 37*	<40	75	wt/wt	+	5/2.5	ALAT: 2.5×UNL γGT: N	Oestrogen therapy
2 F, 39	<40	645	wt/C282Y	+	6/2.8	ALAT: 2.3×UNL γGT: N	None
3 F, 41*	<40	435	H63D/H63D	–	5/2.2	ALAT: 3.5×UNL γGT: 1.8×UNL	Oestrogen therapy
4 F, 46	<40	264	wt/wt	+	7/3.2	ALAT: 5.1×UNL γGT: 2.5×UNL	Excessive alcohol consumption
5 F, 47†	60	187	wt/wt	–	5/2.4	ALAT: 2.8×UNL γGT: N	Oestrogen therapy
6 F, 52	<40	431	wt/H63D	–	6/2.6	ALAT: 1.75×UNL γGT: 1.3×UNL	Excessive alcohol consumption; nicotine addiction
7 F, 60	50/<40	232	wt/wt	–	4/2	ALAT: 2.6×UNL γGT: 1.4×UNL	None
8 F, 63†	70	1879	C282Y/C282Y	–	6/2.1	ALAT: 3.8×UNL γGT: N	None
9 M, 37	<40	214	wt/H63D	+	6/2.4	ALAT: 4.3×UNL γGT: 2.8×UNL	Excessive alcohol consumption
10 M, 39	50/50	292	wt/wt	+	5/2.5	ALAT: 3.5×UNL γGT: 1.8×UNL	Excessive alcohol consumption; nicotine addiction
11 M, 40†	70	614	wt/wt	–	5/2.2	ALAT: 2.7×UNL γGT: 3.5×UNL	Benzodiazepine therapy; excessive alcohol consumption
12 M, 41	50	2965	wt/H63D	+	6/2.4	ALAT: 3.4×UNL γGT: 5×UNL	Excessive alcohol consumption; nicotine addiction
13 M, 41	90	1458	wt/H63D	+	6/2.8	ALAT: 7.8×UNL γGT: 6.2×UNL	Excessive alcohol consumption
14 M, 44*	<40	787	wt/wt	+	6/2.7	ALAT: 3.9×UNL γGT: 1.6×UNL	None
15 M, 45	<40	423	wt/wt	+	7/2.4	ALAT: 2.7×UNL γGT: 1.7×UNL	Excessive alcohol consumption
16 M, 48	180/75	935	H63D/C282Y	-	6/2.4	ALAT: 3.2×UNL γGT: N	None
17 M, 49†	75	1260	wt/C282Y	+	7/2.8	ALAT: 3.7×UNL γGT: 2.2×UNL	Nicotine and hashish addiction
18 M, 52†	100	763	wt/wt	–	6/2.4	ALAT: 3.6×UNL γGT: 5.2×UNL	Excessive alcohol consumption
19 M, 55	60	411	wt/H63D	–	6/2.4	ALAT: 2.9×UNL γGT: 6.8×UNL	Excessive alcohol consumption
20 M, 63	<40	600	Wt/H63D	+	8/3.2	ALAT: 4.7×UNL γGT: 2.8×UNL	Excessive alcohol consumption

*Presence of a low URO-D activity in red cells (consistent with familial porphyria cutanea tarda). †URO-D activity in red cells not performed.
wt: wild type HFE genotype; M: C282Y mutation of HFE gene; m: H63D mutation of HFE gene; LIC: liver iron content (<40 μmol/g: normal; 40–59 μmol/g: slightly increased; 60–99 μmol/g: moderately increased; 100–199 μmol/g: markedly increased; 200 μmol/g and above: importantly increased); ALAT: alanine aminotransferase; γGT: gamma-glutamyltranspeptidase; UNL: upper normal limit; N: normal; HCV: hepatitis C virus; URO-D: uroporphyrinogen decarboxylase.

associated with overt PCT were systematically analysed in all patients. Erythrocyte URO-D was measured in 15 patients (Laboratoire Mérieux, Lyon, France). Decreased URO-D activity, about 50% of normal, compatible with the diagnosis of familial PCT was found in 3 patients. Those showing normal activity were classified as a sporadic form of PCT (Table I). Liver function tests were abnormal in all patients (Table I) and kidney function was normal in all cases (data not shown).

After the diagnosis was established, all patients were treated with phlebotomies (usually 400 ml per course for 5–8 courses) along with the correction of any relevant triggering factor, such as excessive alcoholic consumption, active hepatitis C virus (HCV) infection (defined by positive serological tests and a viral load above 14 IU/ml according to the polymerase chain reaction (PCR)-based test) or oestrogen therapy (Table I). The clinical results were mainly evaluated on the resolution of blistering and the disappearance of skin fragility.

Biochemical blood tests

Serum ferritin concentration was determined in all patients before the start of phlebotomy therapy.

HFE gene analysis

HFE gene analysis was performed after obtaining informed consent from all the patients. Genomic DNA was extracted from peripheral blood leukocytes and analysed for the 2 main mutations of the HFE gene (C282Y and H63D) using PCR and restriction analysis of the amplified fragments, as described previously (20).

HCV status analysis

A first-step serological test was performed using an enzyme-linked immunoassay (ELISA) (HCV 3.0 ELISA*-test, Ortho-Clinical Diagnosis, Johnson & Johnson, France) for all patients. In patients with a positive result, confirmation was obtained by a chemoluminescence-derived method (Abbot-IMX HCV 3.0* test, Abbot France) followed by an evaluation of viral load in peripheral blood (Cobas Tacqman* test, Roche Diagnostics, France).

MRI-based assessment of liver iron content

Determination of iron content in the tissue was performed with a gradient echo sequences-based method with long Echo Time (TE) sequences (T2* ponderation), allowing the detection of minor iron overload compared with spin echo-based analysis. Using these sequences, a liver with normal iron content provides a hypersignal compared with muscle and LIC is calculated in patients from the signal ratio between liver and muscle, with the hypothesis that no iron overload is present in muscle independently of the LIC. This T2*-weighted gradient echo sequence is considered as highly specific, with 89% sensitivity and 80% specificity in the validation group allowing the detection of all clinically relevant liver iron overload greater than 60 μmol/g (21). The final result was expressed in μmol of iron/g of dry liver tissue and ranked as normal LIC (< 40 μmol/g), slight increase (between 40 and 59 μmol/g), moderate increase (between 60 and 99 μmol/g), marked increase (between 100 and 199 μmol/g) or important increase in LIC (200 μmol/g or more). Measurement of LIC by MRI was performed before any treatment was implemented in all included patients and after completion of phlebotomies and disappearance of relevant clinical lesions in only 3 cases.

RESULTS

According to MRI-calculated data, the initial, pre-therapeutic LIC was normal (9 cases), slightly increased (3 cases), moderately increased (6 cases) or markedly increased (2 cases). Initial ferritin level was high (above 400 ng/ml) in 14/20 patients (ranging from 411 to 2965 ng/ml in this subset). There was no clear relationship between initial high ferritin levels and and increased LIC, since only 8/14 (57%) patients with elevated ferritin displayed elevated LIC vs. 3/6 (50%) in patients with ferritin below 400 ng/ml; conversely, 8/11 (73%) patients with increased LIC showed elevated ferritin levels vs. 6/9 patients (66%) without significant overload. Overall, a discrepancy between these 2 parameters was present in 9/20 patients. Gender appeared to be a significant factor, since the percentage of PCT patients with increased LIC was 66% in men (8/12) vs. only 37.5% (3/8) in women.

HFE gene analysis was conducted in all patients and relevant mutations were identified in 5/20 patients: 1 homozygous C282Y mutation, 2 heterozygous C282Y mutations, 1 heterozygous compound C828Y/H63D mutation and 1 homozygous H63D mutation. Overall, an increased LIC, even slight or moderate, was more frequent in patients with significant mutation of the HFE gene (4/5 including the patient with an important overload who displayed an heterozygous composite mutation: 80%) than in patients with no mutation or heterozygous H63D mutation (7/15: 47%).

The presence of HCV infection was investigated in all patients and provided a positive result that was confirmed by second-line methods in 11/20 patients (Table I). Two patients had active infection with significant viral load and were receiving interferon alpha treatment at diagnosis. Interestingly, no patient was infected by hepatitis B, whereas only one patient was HIV positive. Increased LIC was more frequent when HCV infection was absent (7/9, 78%) than when it was present (4/11, 36%) and this difference was statistically significant using a modified χ^2 test ($p = 0.042$).

Clinical remission after phlebotomies was obtained in all the patients with increased LIC, whereas a disappearance of active clinical lesion was observed in 7 of the 9 patients (78%) with normal LIC. LIC was re-evaluated in 3 patients in clinical remission after phlebotomies, one of them with a marked iron overload (180 μmol/g) and 2 others without significant overload; in the former case, a significant decrease in liver iron load was obtained (a reduction from 180 to 75 μmol/g), while no significant change was noted in the other cases.

MRI-related data, HCV and HFE status are summarized in Table I.

DISCUSSION

This study, using a non-invasive method based on liver MRI to investigate LIC, provided new and quite unexpected results. Indeed, according to MRI-based calculations, 45% of patients displayed normal LIC, whereas most other cases displayed only slightly or moderately increased LIC, with the exception of a single patient with a markedly increased content. This trend was even more obvious in women, of whom 62.5% displayed normal LIC. Owing to the fact that these calculations used a muscle/liver ratio of proton signals, it might be argued that these surprisingly negative results were related to an increase in muscle iron content, resulting in an artificially modified ratio. However, an elevated muscle iron content has not been reported in overt PCT to date, and is unlikely to be significant due to the rarity of iron-storing cells, such as macrophages, in muscle. Accordingly, our study is not consistent with the classically admitted theory that an increased LIC is a prominent data in PCT present in at least 80% of patients, a figure far above our results even if only men are considered (1, 3, 6, 16, 18), since

women usually have lower iron levels, at least before menopause. Possible reasons for this discrepancy may be: the relatively high percentage of women in our series, since iron overload in women was clearly less frequent, which might be related partly to the lower level of alcohol consumption in women; changes in patients' profile with time, with respect to aetiological factors; onset of new triggering factors such as HCV infection; lack of accuracy of previous studies usually relying on semi-quantitative methods, less precise than mass spectrometry. Additionally, evaluation of LIC by an MRI-based method in PCT patients has been reported only in 2 previous studies investigating, respectively, 8 and 20 patients (25, 26); in both studies, MRI-estimated LIC was high in all patients compared with controls. These results appear to be different from our data, but the methods were slightly different (echospin T2* and transverse relaxation time) and these 2 studies have been conducted more than 10 years ago, which is consistent with a shift in aetiological factors since then. Moreover, the increase in LIC was only twice the baseline level in one study, a result actually close to our data for patients with elevated LIC (26).

Regarding the relationship between MRI-based results and the iron metabolism-related parameters, there was a trend toward a correlation between an increased LIC and the presence of significant mutations of the HFE gene. Conversely, in our study, serum ferritin concentration cannot be considered as a reliable predictor of LIC, since a discrepancy between these 2 parameters was present in approximately half of the patients, a figure that contradicts previous reports (24, 27, 28). This lack of reliability of ferritin level as a predictor of LIC is perhaps related to the fact that ferritin rate is influenced by many conditions independent of iron metabolism, including inflammation and liver dysfunctioning, regardless of its origin.

The relatively lower percentage of patients with HCV infection in the group with increased LIC is consistent with previous reports in which a lower prevalence of this infection was observed in patients with significant HFE mutations (20). To date, there is no convincing explanation for this loose tendency, but it can be hypothesized that HCV infection and iron overload are independent triggering factors of PCT that could be relatively exclusive of each other.

As to the clinical implications of the presence/absence of an increased LIC regardless of its level, our results clearly show that phlebotomies can be efficient even when no significant overload is present, although therapeutic results seem slightly better in patients with a higher content. Thus, removal of iron remains an outstanding treatment of PCT, independent of the initial LIC, an unexpected finding reported previously by Lundvall in 1971 (29), which suggests that the correction of increased LIC is probably not its only mechanism of action.

The unexpected results of this study must be confirmed by more extensive reports and emphasize the growing need and use of non-invasive methods for metabolic assessment. In addition to MRI-based studies, other methods may change our current understanding of the actual incidence of iron overload in PCT, such as urinary dosage of hepcidin, which seems to be closely related to the level of iron stores in the liver (30).

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