Redox active plasma iron in C282Y/C282Y hemochromatosis

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Labile plasma iron (LPI) represents the redox active component of non–transferrin-bound iron (NTBI). Its presence in thalassemic patients has been recently reported. The aim of the present study was to quantify LPI in HFE genetic hemochromatosis (GH) and to characterize the mechanisms accounting for its appearance. We studied 159 subjects subdivided into the following groups: (1) 23 with iron overloaded GH; (2) 14 with iron-depleted GH; (3) 26 with dysmetabolic hepatosiderosis; (4) 33 with alcoholic cirrhosis; (5) 63 healthy controls. Both NTBI and LPI were substantially higher in patients with iron-overloaded GH than in those with iron-depleted GH or in healthy controls. LPI was significantly correlated with serum transaminase increase in this group. LPI was elevated in the alcoholic cirrhosis subgroup of severely affected patients. LPI was found essentially when transferrin saturation exceeded 75%, regardless of the etiologic condition. Transferrin saturation above 75% was related to iron overload in GH and to liver failure in alcoholic cirrhosis. LPI is present in C282Y/C282Y hemochromatosis and may be a marker of toxicity due to its potential for catalyzing the generation of reactive oxygen radicals in vivo. (Blood. 2005;105: 4527-4531)

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Introduction

Under normal conditions, serum transferrin is 20% to 35% saturated with iron. However, the iron-binding capacity of transferrin in the plasma of iron-overloaded patients is often exceeded, leading to the appearance of non–transferrin-bound iron (NTBI). This pool of iron, which was first investigated by Hershko et al,[1] has been linked to the development of visceral iron excess and a toxic role toward the cells.[2]

Experimentally, iron promotes the formation of free hydroxyl radicals via the Haber-Weiss reaction. Reactive oxygen species are in turn able to generate increased lipid peroxidation.[3,4] The accumulation of plasma NTBI has been shown in patients with thalassemia to correlate with the appearance of oxidation products and a decrease in plasma antioxidant capacity.[5] In genetic hemochromatosis, Gutteridge et al[6] reported that NTBI stimulated both the peroxidation of membrane lipids and the formation of hydroxyl radicals.

The chemical form of NTBI remains poorly defined but is likely to be heterogeneous, involving both nonprotein and protein-bound forms that might or might not be chelatable, depending on the degree and source of iron overload.[7] The nonprotein ligands appear to correspond to low-molecular-weight organic compounds such as ascorbate, phosphate, carbonate, organic acids, and amino acids. Grootveld et al[8] proposed that NTBI in the plasma of a patient with genetic hemochromatosis (GH) existed apparently as iron citrate or iron-citrate-acetate ternary complexes, yet mostly in forms that are not directly chelatable by deferrioxamine.[7] The majority of NTBI component in plasma is bound to albumin.[9]

Several methods have been introduced to quantify NTBI levels using different approaches. A 3-step assay system has been proposed, based on mobilization by nitrilotriacetate with inclusion of cobalt salt that blocks endogenous apo-transferrin, ultrafiltration, and high-performance liquid chromatography[10-12] or a fluorescence assay where NTBI is mobilized with oxalate while endogenous apo-transferrin is inhibited by gallium chloride.[13]

Recently, Esposito and colleagues[14] assessed the capacity of serum iron to engage in the formation of reactive oxidant species through a methodology that involves minimum sample manipulation and exogenously added factors. The so-called labile plasma iron (LPI) is an iron-chelatable component of plasma (or serum) that redox cycles when prompted with physiologic concentrations of ascorbate while forming radicals that are detected by oxidant-sensitive fluorescent probes. Its presence in plasma can be considered as the potential source of circulating iron that might evoke tissue overload and ensuing damage. Previous studies have shown the presence of LPI in iron overload (both transfusional and nontransfusional).[15,16] The aim of the present study was to quantify LPI in untreated HFE GH as compared to GH iron-depleted by phlebotomies; dysmetabolic hepatosiderosis (DYSH),[17,18] a condition with mild iron excess but high serum ferritin levels; alcoholic cirrhosis (AC), in which low serum transferrinemia may generate by itself the appearance of NTBI[19]; and healthy controls. In addition, we aimed at characterizing the mechanisms that account for LPI appearance by analyzing the relationship between LPI, serum iron parameters, and liver function tests.

Patients and methods

Patients

A total of 159 male subjects were prospectively included. The main studied group consisted of *HFE* GH patients, including 23 iron-overloaded patients, homozygous for the C282Y mutation in the *HFE* gene, with phenotypic iron excess (ie, transferrin [Tf] saturation > 50% and increased hepatic iron determined by magnetic resonance imaging or biochemically on liver biopsies). The mean hepatic iron concentration was 219 μmol (range, 110 μmol-635 μmol). Only one patient had a cirrhosis on histology. These patients had neither hepatocellular insufficiency (prothrombin index mean, as indicated in Table 1, was 95% [range, 82%-122%], serum albumin mean was 41.5 g/L [range, 34 g/L-50 g/L]) nor cholestasis (gammaglutamyl transferase mean was 37 IU/L [range, 13-173], bilirubin mean was 11 μL [range, 5 μL-21 μL; normal values 2 μM-18 μM]. Transaminase increase was present as compared with controls and iron-depleted GH. None of the patients had previously gone through venesection therapy or blood donation. The group also included 14 iron-depleted HFE1-GH patients who had completed their iron depletion (depletion criteria were serum ferritin < 50 μg/L and normal serum iron and Tf saturation) and were under maintenance therapy by regular phlebotomies.

The comparative groups were subdivided into healthy controls and subjects with abnormal serum iron parameters. There were 63 white volunteers who constituted the healthy control group. They were included while undergoing a systematic medical examination in a health appraisal center of the Caisse Primaire d'Assurance Maladie d'Ille-et-Vilaine. Strict inclusion criteria were applied: normal serum iron status; absence of excessive alcoholic consumption according to the World Health Organization (WHO) criteria of less than 3 U/d and/or less than 21 U/wk; body mass index less than 27 kg/m²; and no known acute or chronic disease. The second group included subjects with abnormal serum iron parameters. There were 26 patients with dysmetabolic hepatosiderosis (DYSH) characterized by high serum ferritin, insulin-resistance syndrome (defined by the presence of one or more of the following findings: body mass index > 25, abnormal glucose metabolism, hyperlipidemia), and confirmed hepatosiderosis (hepatic iron concentration determined by magnetic resonance imaging or biochemically on liver biopsy > 36 μmol/g). These patients did not carry homozygous C282Y *HFE* mutation. There were 33 patients with alcoholic cirrhosis, with one or more antecedents of clinical decompensated cirrhosis (ascites, oesophageal variceal bleeding, portal systemic encephalopathy), and an absence of other known cause of chronic liver disease. According to the Child-Pugh classification,[20] 4 patients were Child A, 14 were Child B, and 15 were Child C. 26 patients (79%) were included in the study during their hospitalization for a pre–liver transplantation check-up and had been therefore alcohol abstinent for at least 6 months.

All the volunteers and patients signed an informed consent to participate. This study was approved by the local Comité Consultatif de Protection des Personnes se prêtant à la Recherche Biomédicale (CCPPRB; University Hospital Pontchaillou, Rennes, France).

Laboratory procedures

Peripheral venous blood was obtained from all patients after an overnight fasting. Iron status (serum iron, transferrin, transferrin saturation, ferritin), liver function tests (serum albumin, alanine aminotransferase [ALT] and aspartate aminotransferase [AST], gammaglutamyl transpeptidase, prothrombin time), serum protein electrophoresis, C-reactive protein (CRP), and blood cell count were measured on fresh samples in the Biochemistry and Haematology laboratories of our hospital. Serum aliquots were stored at −80°C until determination of NTBI and LPI. Serum iron was measured by a ferene-S method (Sera-pak; Bayer, Puteaux, France) using a synchron CX7 delta (Beckman, Villepinte, France). Serum transferrin was determined by immunonephelometry (BNII analyzer; Dade Behring, Paris, France). Transferrin saturation values were calculated using the formula: % transferrin saturation = 4 × (serum iron concentration in μM / serum transferrin concentration in g/L). Ferritin was determined by chemiluminescence using an ACS 180 (Bayer). HFE-1 gene mutation was assessed as described elsewhere.[21] Hepatic iron concentration was measured either by quantitative liver biopsy analysis using Barry and Sherlock's method,[22] or by magnetic resonance imaging as previously described.[23,24]

NTBI and LPI were determined in all subjects on frozen serum samples in the Department of Biological Chemistry, Institute of Life Sciences, Hebrew University of Jerusalem. NTBI was measured by the fluorescence-based one-step assay based on fluoresceinated transferrin.[13]

LPI component of NTBI

The method for determining LPI is based on the conversion of the nonfluorescent dihydrorhodamine (DHR) to the fluorescent form by various oxidants, such that the generation of reactive oxygen species can be followed as an increase in DHR fluorescence. In the LPI assay (Aferrix, Rehovot, Israel), each serum sample is tested under 2 conditions: with 40 μM ascorbate alone and with 40 μM ascorbate in the presence of 50 μM iron chelator deferiprone (L1). The difference in the rate of oxidation of DHR in the presence and absence of chelator represents the component of plasma NTBI that is redox-active. The slopes (r) of DHR fluorescence intensity with time were calculated from measurements taken between 15 and 40 minutes. To calculate LPI, duplicate values of r in the presence (r_{L1}) and absence (r) of L1 were averaged and the LPI concentration (μM) was determined by comparing the difference in slopes of the sample ($\Delta r = r-r_{L1}$) with the difference in the slope corresponding to 1 μM free iron (Fe) (Δr_{st}), as obtained with known Fe concentration standards. LPI was calculated from $\Delta r/\Delta r_{st}$. The Fe concentration standards (0-5 μM) were prepared in Hanks balanced salt solution (HBS) containing 20 mg/mL bovine serum albumin, and were assayed for LPI similarly to serum samples.[14]

Table 1. Bioclinical features obtained in the different groups of patients

	Controls N = 63	GH, overloaded N = 23	P*	GH, depleted N = 14	P*	DYSH N = 26	P*	Cirrhosis N = 33	P*
Age, y	51 ± 12	44 ± 10	NS	56 ± 11	NS	55 ± 11	NS	52 ± 10	NS
BMI, kg/m²	24.2 ± 2.3	25.4 ± 3.9	NS	25.9 ± 2.3	NS	29.3 ± 4.4	< .001	25.3 ± 5	NS
Albumin, g/L N: 35-50	42 ± 4	41.5 ± 4	NS	41 ± 3	NS	40 ± 4	NS	29 ± 6	< .001
AST, IU/L N: 0-50	20 ± 5	30 ± 15	< .05	22 ± 9	NS	27 ± 18	NS	60 ± 29	< .001
ALT, IU/L N: 0-60	24 ± 9	51 ± 27	< .01	29 ± 13	NS	44 ± 25	< .05	38 ± 21	< .05
Serum iron, μM N: 12.5-25	18.7 ± 5.7	33.9 ± 6	< .0001	21 ± 5.8	NS	23.4 ± 5.7	.01	19.7 ± 10.5	NS
Transferrin, g/L N: 1.7-3.5	2.14 ± 0.3	1.57 ± 0.3	< .0001	2.1 ± 0.4	NS	2.05 ± 0.4	NS	1.75 ± 0.8	.05
Transferrin saturation, % N: 23-45	35 ± 11	87 ± 16	< .0001	40 ± 14	NS	47 ± 14	< .01	56 ± 34	< .05
Ferritin, μg/L N: 55-345	192 ± 104	1362 ± 1145	< .001	48 ± 36	< .001	767 ± 494	< .001	651 ± 812	< .05
NTBI, μM	0.154 ± 0.328	0.761 ± 0.504	< .001	0.221 ± 0.559	NS	0.381 ± 0.381	NS	0.385 ± 0.560	NS
LPI, μM	0.038 ± 0.07	0.250 ± 0.289	< .01	0.019 ± 0.127	NS	0.018 ± 0.03	NS	0.095 ± 0.4	NS

Results are expressed as the mean plus or minus SD. N indicates normal values; GH, genetic hemochromatosis; DYSH, dysmetabolic hepatosiderosis; NS, nonsignificant.
*Comparison between groups and controls with Dunnett test.

Statistical analysis

SPSS 10.0 for Windows (SPSS, Chicago, IL) was used for statistical analysis. Statistical analysis was performed using the Kruskall Wallis, Mann-Whitney, and Spearman tests, as appropriate. Each group was compared with the control group using Dunnett posttest analyses. Results are expressed as mean plus or minus the standard deviation (SD). A P value of less than .05 was considered significant.

Results

Descriptive data

Bioclinical findings in the different groups are indicated in Table 1 and NTBI and LPI levels for the 5 studied groups are presented in Figure 1.

In the iron-overloaded GH group, NTBI and LPI were significantly increased as compared with controls ($P < .01$) and with the iron-depleted GH group ($P < .05$). In the latter group, NTBI and LPI values were similar to values obtained in the control group. The DYSH group had no significant increase versus control group for both LPI and NTBI. In the whole cirrhosis group, the increase of both NTBI and LPI did not reach significance as compared with controls. However, when subdividing this group into 2 subgroups according to the Child-Pugh classification,[20] LPI was significantly enhanced ($P < .05$) in the most severely affected patients (ie, in those with Child C cirrhosis).

Correlation studies

In order to approach the possible causal or secondary factors related to LPI levels, correlation studies were performed in the whole population and in the various studied groups.

In the whole population, the correlation between NTBI and LPI was not strong (Rho = 0.386) but it was statistically significant ($P < .001$). There were no relationships between, on one hand, NTBI or LPI and, on the other hand, age, body mass index, alcohol consumption, tobacco consumption, biologic inflammatory syndrome (C-reactive protein and/or leukocyte cell count), and kidney function (serum creatinine and uremia). NTBI and LPI were significantly correlated with serum iron, transferrin, transferrin saturation, and ferritin. LPI was more significantly correlated with AST than was NTBI with AST (Rho = 0.247, $P = .002$ versus Rho = 0.214, $P = .01$). A very significant correlation ($P < .001$) was found between LPI and transferrin saturation (Rho = 0.411; Figure 2).

In the overloaded GH group, the correlation between LPI and NTBI was significant ($P < .001$, Rho = 0.484). A stronger correla-
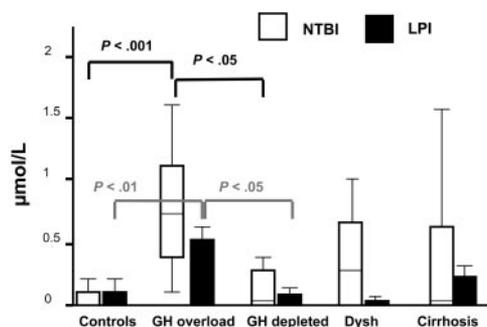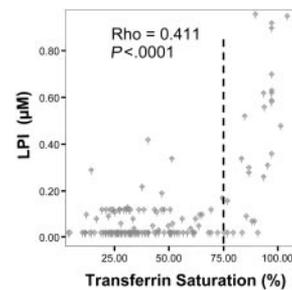

Figure 2. Correlation studies in whole population between LPI and transferrin saturation. Rho = correlation coefficient with Spearman test.

tion was observed between LPI and transferrin saturation (Figure 3A). It is noteworthy that no significant correlation was observed between both LPI or NTBI and transferrin or hepatic iron concentration. NTBI was slightly correlated with serum iron, whereas LPI was not. In addition, LPI was more significantly correlated with AST levels (Figure 3B) than was NTBI with AST ($P = .002$ vs $P = .04$).

In the DYSH group, there was no significant correlation between LPI or NTBI levels and the other tested parameters.

In the alcoholic cirrhosis group, despite the fact that there was no significant difference with controls, we found a significant correlation between NTBI and LPI ($P < .05$, Rho = 0.413) as well as between LPI and transferrin saturation (Figure 4A). In addition, a negative correlation was found between LPI and biochemical indices of hepatocellular failure such as transferrin (Figure 4B) and prothrombin index (Figure 4C).

Discussion

Chronic iron overload leads to the development of multivisceral complications. It is therefore of importance to identify the molecular mechanisms potentially involved. An excess of biochemically reactive iron in serum and/or within the cells is likely to participate in the pathogenesis of the disease. With the advent of the LPI assay for quantitating plasma circulating iron species that are chemically reactive, it became possible to assess the potentially toxic forms of iron that might be responsible for iron-related damage.[2] Relatively high levels of LPI were initially found in thalassemic patients who were strongly iron overloaded and presented transferrin saturation values higher than 85%.[16] These observations prompted us to search for the presence of NTBI and LPI in *HFE*-GH as compared

Figure 1. NTBI and LPI levels in the 5 studied groups. Boxplots represent median, quartiles (ie, 25 to 75 percentile), and spread values (ie, 10 + 90 percentiles) for groups. Only statistically significant differences with Dunnett posttest analyses are indicated. □ indicates NTBI; ■, LPI.
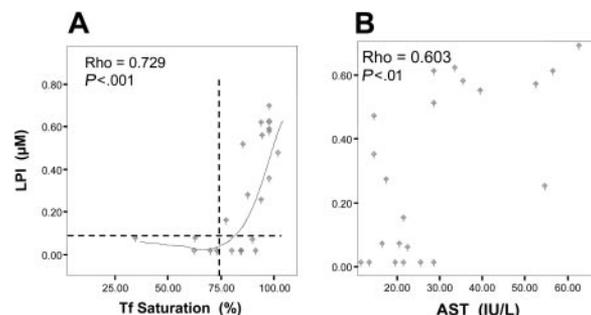

Figure 3. Correlation studies in the iron-overloaded GH group. (A) Correlation between LPI and transferrin saturation, Fitline with local linear regression, normal kernel. (B) Correlation between LPI and AST. Rho = correlation coefficient with Spearman test. Vertical dashed line represents the 75% threshold; horizontal line, 0.10 threshold for LPI.
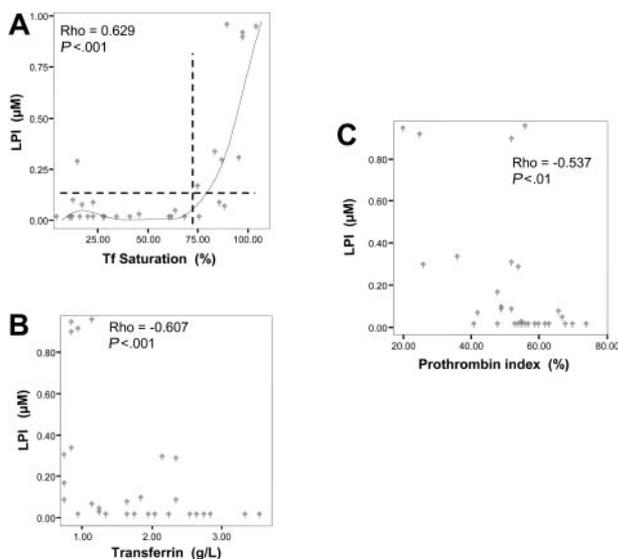
Figure 4. Correlation studies in the cirrhosis group. (A) Correlation between LPI and transferrin saturation, Fitline with local linear regression, normal kernel. (B) Correlation between LPI and transferrin. (C) Correlation between LPI and prothrombin index. Rho = correlation coefficient with Spearman test. Vertical dashed line represents the 75% threshold; horizontal line, 0.10 threshold for LPI.

with DYSH and cirrhosis, 2 situations associated with abnormal iron metabolism.

Our results clearly demonstrated that both LPI and NTBI can be detected in untreated patients with GH. As previously described,[11] using another method NTBI was mainly found when Tf saturation was higher than 45% and disappeared when Tf saturation dropped below 45% during depletive therapy by venesections. LPI also showed a correlation with transferrin saturation but the threshold for LPI appearance was close to 75%. This may explain, at least partly, why the correlation between NTBI and LPI was not a strong one, and supports the fact that NTBI and LPI in this population are likely to correspond to different iron biochemical speciations. It is noteworthy that a small number of patients with Tf saturation below 75% exhibited nevertheless a significant amount of LPI, suggesting that in some cases other environmental and/or genetic factors may favor the appearance of LPI. The LPI assay uses ascorbate, at physiologic concentrations, as a redox prompting agent to engage iron in redox cycling. Variations in serum antioxidant capacity among patients could explain LPI differences despite similar Tf saturation and/or NTBI levels. Antioxidants such as vitamin E and ascorbate have the dual capacity to promote redox cycling at relatively low concentrations and to act as powerful scavengers of radical oxygen species at higher concentrations. Patients with untreated hereditary hemochromatosis are known to have reduced circulating levels of antioxidant vitamins, whereas vitamin C levels return to normal when iron excess has been eliminated by venesection therapy.[25,26]

The relationship between LPI and transferrin saturation is further documented by the results obtained in DYSH and in cirrhotic patients. Indeed, in DYSH where NTBI was detected (but without statistically significant increase), no increase of LPI was found. This could be related to the peculiar profile of this group in terms of iron status. Indeed, DYSH corresponds to mild hepatic iron overload (as shown by hepatic iron concentration data) and is characterized in the serum by the contrast between a relatively moderate increase in serum transferrin saturation and a marked elevation of ferritin levels. In the whole cirrhosis group, the

increase in both serum NTBI and LPI did not reach statistical significance, probably due to important standard deviations, but when considering the most severe patients (ie, those classified as Child C), LPI became significantly increased. Moreover, in the whole cirrhotic group, we found a significant correlation between LPI and transferrin saturation, with LPI appearing most frequently when transferrin saturation exceeded 75%.

Considering LPI in the different patient groups, 2 main observations are of interest. First, LPI usually appeared in the serum when transferrin saturation exceeded 75% whatever the etiologic situation (ie, hemochromatosis or cirrhosis). Second, the mechanisms accounting for LPI appearance were different according to the etiology. Thus, in iron-overloaded genetic hemochromatotic patients, the relationship that we found between LPI and transferrin saturation was linked to the ratio between the 2 parameters used for its calculation and not to a significant correlation to either of these parameters when considered separately. It is known, in this disease, that the combined effect of serum iron increase with slight transferrin decrease forms the basis for the major diagnostic value of transferrin saturation determination, especially in a screening view.[27] In cirrhotic patients the mechanism for LPI appearance was different since transferrin saturation increase (which was correlated with LPI) was solely related to transferrin decrease. Indeed, no correlation was found between LPI and serum iron whereas LPI was significantly correlated to transferrin levels. This transferrin decrease in cirrhosis is due to hepatocellular failure as further illustrated by the significant correlation between LPI and prothrombin test decrease.

This transferrin saturation value of 75% can be considered as the threshold of toxicity for circulating iron and is in full agreement with the data obtained by 2 recent studies. Jensen et al[28] reported, in a population with iron overload due to multiple transfusions, that elevated aminotransferase levels were not seen when transferrin saturation was less than 75%. Pootrakul et al[15] showed, in iron-overloaded beta-thalassemia/HbE patients, that in 97% of serum samples (n = 267), the LPI levels were more than 0.4 μM and the percent of transferrin saturation was more than 85%. Our finding of a stronger correlation between LPI and AST (versus NTBI and AST) underlines the potential role of LPI as a marker of cellular toxicity.

In conclusion, this study of 3 patient populations with iron-related situations compared with strictly defined healthy controls indicates that LPI is (1) present in overloaded genetic hemochromatosis and in severe alcoholic cirrhosis when transferrin saturation exceeds 75%; (2) due to different mechanisms in these 2 diseases in so far as the role of transferrin saturation decrease is critical in cirrhosis but only partial in hemochromatosis; and (3) likely to exert cellular toxicity as suggested by the significant correlation in GH between LPI and transaminase increase. Future studies will further document the bioclinical impact of this circulating iron species in *HFE* hemochromatosis as well as in other genetic iron-overload situations and in liver conditions characterized by severe hepatocellular insufficiency.

Acknowledgments

References

1. Hershko C, Graham G, Bates GW, Rachmilewitz EA. Non-specific serum iron in thalassemia: an abnormal serum iron fraction of potential toxicity. Br J Haematol. 1978;40:255-263.

2. Brissot P, Loréal O. Role of non-transferrin-bound iron in the pathogenesis of iron overload and toxicity. Adv Exp Med Biol. 2002;509:45-53.

3. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. 1990;186:1-85.

4. Stadtman ER. Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. Free Rad Biol Med. 1990;9:315-325.

5. Cighetti G, Duca L, Bortone L, et al. Oxidative status and malondialdehyde in β-thalassemia patients. Eur J Clin Inv. 2002;32:55-60.

6. Gutteridge JMC, Rowley DA, Griffiths E, Halliwell B. Low-molecular-weight iron complexes and oxygen radical reactions in idiopatic haemochromatosis. Clin Sci. 1985;68:463-467.

7. Breuer W, Ermers MJ, Pootrakul P, Abramov A, Hershko C, Cabantchik ZI. Desferrioxamine-chelatable iron, a component of serum non-transferrin-bound iron, used for assessing chelation therapy. Blood. 2001;97:792-798.

8. Grootveld M, Bell JD, Halliwell B, Aruoma OI, Bomford A, Sadler PJ. Non-transferrin-bound iron in plasma or serum from patients with idiopathic haemochromatosis: characterization by high performance liquid chromatography and nuclear resonance spectroscopy. Biol Chem. 1989;264:4417-4422.

9. Hider RC. Nature of nontransferrin-bound iron. Eur J Clin Invest. 2002;32(suppl 1):50-54.

10. Gosriwatana I, Loréal O, Shuli L, Brissot P, Porter J, Hider RC. Quantification of non-transferrin bound iron in the presence of unsaturated transferrin. Anal Biochem. 1999;273:212-220.

11. Loréal O, Gosriwatana I, Guyader D, Porter J, Brissot P, Hider RC. Determination of non-transferrin bound iron in genetic hemochromatosis using a new HPLC-based method. J Hepatol. 2002;32:727-733.

12. Singh S, Hider RC, Porter JB. A direct method for quantification of non-transferrin bound iron. Anal Biochem. 1990;186:320-323.

13. Breuer W, Cabantchik ZI. A fluorescence-based one-step assay for serum non-transferrin-bound-iron. Anal Biochem. 2001;299:194-202.

14. Esposito BP, Breuer W, Sirankapracha P, Pootrakul P, Hershko C, Cabantchik ZI. Labile plasma iron in iron overload: redox activity and susceptibility to chelation. Blood. 2003;102:2670-2677.

15. Pootrakul P, Breuer W, Sametband M, Sirankapracha P, Hershko C, Cabantchik ZI. Labile plasma iron (LPI) as an indicator of chelatable plasma redox activity in iron overloaded beta-thalassaemia/HbE patients treated with an oral chelator. Blood. 2004;104:1504-1510.

16. Esposito BP, Breuer W, Slotki I, Cabantchik ZI. Labile iron in parenteral iron formulations and its potential for generating plasma nontransferrin-bound iron in dialysis patients. Eur J Clin Invest. 2002;32(suppl 1):42-49.

17. Mendler MH, Turlin B, Moirand R, et al. Insulin resistance-associated hepatic iron overload. Gastroenterology. 1999;117:1155-1163.

18. Moirand R, Mortaji A, Loréal O, Paillard F, Brissot P, Deugnier Y. Liver iron overload with normal transferrin saturation: a new syndrome. Lancet. 1997;349:95-97.

19. De Feo TM, Fargion S, Duca L, et al. Non-transferrin-bound iron in alcohol abusers. Alcohol Clin Exp Res. 2001;25:1494-1499.

20. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. British J Surg. 1973;60:646-649.

21. Jouanolle AM, Fergelot P, Gandon G, Yaouang J, Le Gall JY, David V. A candidate gene for hemochromatosis: frequency of the C282Y and H63D mutations. Hum Genet. 1997;100:544-547.

22. Barry M, Sherlock S. Measurement of liver-iron concentration in needle-biopsy specimen. Lancet. 1971;2:100-103.

23. Gandon Y, Guyader D, Heautot JF, et al. Hemochromatosis: diagnosis and quantification of liver iron with gradient-echo MR imaging. Radiology. 1994;193:533-538.

24. Gandon Y, Olivié D, Guyader D, et al. Non-invasive assessment of hepatic iron stores by MRI. Lancet. 2004;363:357-362.

25. Brissot P, Deugnier Y, Le Treut A, Regnouard F, Simon M, Bourel M. Ascorbic acid status in idiopathic haemochromatosis. Digestion. 1978;17:479-487.

26. Young IS, Trouton TG, Torney JJ, McMaster D, Callender ME, Trimble ER. Antioxidant status and lipid peroxidation in hereditary haemochromatosis. Free Radic Biol Med. 1994;16:393-397.

27. Brissot P, Guyader D, Loréal O, et al. Clinical aspects of hemochromatosis. Transfus Sci. 2000;23:193-200.

28. Jensen PD, Jensen FT, Christensen T, Nielsen JL, Ellegaard J. Relationship between hepatocellular injury and transfusional iron overload prior to and during iron chelation with desferrioxamine: a study in adult patients with acquired anemias. Blood. 2003;101:91-96.