Relationships between plasma ferritin and aminotransferase profile in haemodialysis patients with hepatitis C virus

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Abstract

Background. HCV infection is a major complication among patients undergoing dialysis therapy throughout the world. In the years prior to the use of human recombinant erythropoietin (rHuEpo), patients undergoing haemodialysis were subjected to an excessive iron load as a consequence of frequent blood transfusions. Recent data in the non-dialysis population have shown a positive correlation between iron deposits and the severity of HCV hepatitis and between iron deposition and an impaired response to interferon therapy.

Methods. One hundred and five haemodialysis patients were studied. Every patient was screened for HCV infection by ELISA II and HCV RNA. Serum biochemistries were analysed by SMAC20. Ferritin was measured by radioimmunoassay.

Results. The aminotransferase levels for the HCV positive (n = 39) and negative patients (n = 66) were below the normal levels for the general population. The mean values of aminotransferases and plasma ferritin were, however, higher in the HCV-positive patients than in the HCV-negative patients. A positive correlation between aminotransferases and plasma ferritin was evident in HCV-positive patients, which was absent in the HCV-negative individuals. The histological severity of liver disease (n = 7) was, however, not statistically related with the levels of either ferritin or aminotransferases.

Conclusion. HCV infection is a relevant variable when estimating iron deposits by measuring plasma ferritin. Accordingly, a misinterpretation of the actual amount of iron deposits may occur in HCV-positive patients, which should be taken into account at the time of planning their iron reposition therapy. On the other hand, the level of iron deposits might have a significant role in the evolution of HCV-related liver disease.

Key words: aminotransferases; ferritin; haemodialysis; hepatitis C virus

Introduction

In the past decade infection with HCV has been increasingly recognized as a major complication among patients undergoing dialysis therapy throughout the world [1-3]. At the present time the principal screening method for HCV infection is based on the detection of antiviral antibodies by ELISA assays [1,2]. The results of this method are frequently supplemented by the measurement of HCV RNA by PCR [4,5]. Aminotransferases (ASAT and ALAT) are employed as an adjuvant method in the follow-up of antiviral treatments [6]. However, the use of aminotransferase levels to assess the severity of HCV-related illness is limited, because the level and persistence of enzyme elevation and the histological patterns of liver damage are not well correlated [4]. Increase of aminotransferases has been reported as an inconsistent feature of HCV positive dialysis patients [7]. However, in interpreting these findings it is necessary to consider that normal values of aminotransferases are lower in dialysis patients than in the general population [8,9]. The use of aminotransferases as a marker of liver disease in dialysis patients may be plausible if the threshold of normal level for dialysis patients is shifted lower.

The magnitude of the HCV infection problem makes it prudent to consider HCV-related chronic liver disease in the differential diagnosis for liver damage by any other agent, e.g. iron accumulation. The role of iron in liver cell damage is a well-established feature of haemochromatosis; however, it has been less characterized as a pathogenetic mechanism of hepatocellular injury in cases of secondary haemosiderosis. In the years prior to the use of human recombinant erythropoietin (rHuEpo), patients undergoing haemodialysis were subjected to an excessive iron load as a consequence of frequent blood transfusions. Pathology studies have disclosed that the liver is the principal organ for iron deposition in these patients [10]. In recent years it has become apparent that the iron status of the patient must be considered to determine an efficacious rHuEpo dose.
Recent data in the non-dialysis population have shown a positive correlation between iron deposits and the severity of HCV hepatitis and between iron deposition and an impaired response to interferon therapy [6,11,12]. Due to the coexistence of iron accumulation and HCV infection, dialysis patients are susceptible to developing HCV infection/iron interactions. Of additional interest, plasma ferritin, which is the most widely used marker for the routine evaluation of iron deposits, may vary due to HCV disease and this may have relevant consequences in the practical approach to the treatment of dialysis anemia.

We have therefore examined the putative relationships between HCV infection and iron deposition in haemodialysis patients. To address this issue we compared the levels of aminotransferases (ASAT and ALAT) and ferritin in a group of HCV-positive with a group of HCV-negative individuals submitted to regular dialysis treatment in the same dialysis units. Moreover, liver histology samples were obtained to further ascertain the relevance of the ferritin and aminotransferases data in terms of liver disease.

Subjects and methods

One hundred and five patients (50 female and 55 male, mean age 60.3 ± 8.6 years) were studied. Each patient had undergone haemodialysis therapy for at least 12 months in three Units of the University Hospital. Patients were randomly selected and only individuals who remained in the same unit for the 12 months of the study were included. Every patient was screened for HCV infection with an ELISA II (Ortho Diagnostics Inc, Raritan, NJ). Additional, confirmatory diagnostic methods included RIBA II or HCV RNA determinations by nested PCR, as previously described [4].

None of the individuals studied had markers of acute hepatitis, i.e. positive HCV antibodies or appearance of increased aminotransferases within the last 12 months. Patients with at least two positive determinations of HCV antibodies (by ELISA II or RIBA II) and/or HCV RNA were considered to be HCV positive. None of the patients had positive markers for hepatitis B or human immunodeficiency virus infection or a history of excessive alcohol intake or administration of hepatotoxic drugs.

Serum biochemistries were analysed by SMAC20 on a monthly basis; by this method, the normal values of the non-dialysis population were ASAT: 11 to 35 U/l and ALAT: 5 to 43 U/l. Ferritin was measured by radioimmunoassay, as described [13] (Nichols Institute, San Juan Capistrano, CA). For comparative purposes, the mean values of each variable during the last 12 months (1994–95) were used. The role of transfusions in the amount of iron accumulation was analysed by classifying the patients in three categories according to the number of transfusions received: (1) 0–9; (2) 10–20, and (3) more than 20 transfusions. All the patients were treated with a regular dialysis schedule and received the current medications of chronic renal failure. As a part of the therapy, some of them were treated with rHuEpo and oral or parenteral iron supplements. No differences between the HCV-positive and -negative groups existed in the proportion of patients receiving the aforementioned medications (P NS).

Since iron kinetics are influenced by the administration of parenteral iron, no samples were taken for measuring ferritin within 3 weeks of the last intravenous iron administration. To assure the reliability of ferritin measurements done at this time, we assessed the plasma ferritin profile in four patients, in serial samples taken after the administration of one dose of i.v. iron gluconate (62.5 mg iron, Ferrlecit, Rhône-Poulenc Rorer).

In order to further assess the putative role of the recently employed (1991–1995) rHuEpo therapy in the correlations found in the present study, data from a group of HCV-positive patients (33 male, 27 female) corresponding to the pre-rHuEpo period (1989–1990) were retrospectively analysed. These patients were dialysed in the same Units using routine practices similar to the groups analysed in 1994–1995. Only one patient received alpha interferon therapy (3 million units post-haemodialysis, 12 months).

Liver samples were obtained by percutaneous biopsy from seven HCV-positive patients, who were included in a prospective protocol for the putative use of alpha-interferon before kidney transplantation. The remaining HCV-positive patients were not biopsied because of lack of inclusion in transplantation or interferon administration programmes, the existence of comorbid entities or lack of informed consent. All the biopsies were performed after obtaining informed consent and after preparation of the patients according to a previously described protocol [4].

The samples were fixed in formalin, embedded in paraffin and stained with haematoxylin-eosin, Masson trichrome, Wilder, periodic acid–Schiff-diastase, orcein, and Perl's stains. All specimens were examined by a pathologist who was not given access to the clinical data. For statistical purposes the severity of liver damage was quantified by means of the Knodell index [14], necrosis, portal inflammation, and fibrosis. The iron content was estimated as the amount of haemosiderin, and classified in four categories as follows: (1) no haemosiderin or traces, (2) spotted haemosiderin accumulation, (3) disseminated haemosiderin accumulation of mild intensity, and (4) severe, global, haemosiderin accumulation.

Values are expressed as mean ± SEM. Unpaired Student's t test, chi-square, and linear regression analyses were applied for statistical comparisons. A P value < 0.05 was considered significant.

Results

The values of both aminotransferases, ASAT and ALAT, and ferritin are shown in Figure 1. The aminotransferases were markedly lower in HCV-negative patients; the mean value was at the lower end of the normal range for the non-dialysis population and all the values fell within a narrow range (ASAT, 5–48 U/l, ALAT, 3–46 U/l). A positive correlation between plasma ferritin and aminotransferases was found in the HCV-positive group (Figure 1 a, b). On the contrary, no significant relationship existed between plasma ferritin and aminotransferases in the HCV-negative group (Figure 2 a, b). To rule out any possible influence on ferritin values by the use of intravenous iron, we found that the values of ferritin became near normal after the third dialysis session following one intravenous dose of iron, as follows: ferritin (μg/l) baseline 260.2 ± 74.8; 36 h after administration 577.5 ± 110.4; post-third haemodialysis 300.2 ± 104.2
Fig. 1. Linear regression analysis of the correlations between (a) ferritin/ASAT, and (b) ferritin/ALAT, in the HCV-positive group. To discount any bias due to unusual individual characteristics the P value was assessed both including and excluding the extremely high values corresponding to one of the patients; the correlations were equally significant (P<0.001) in both cases.

(n=4 patients, P<0.05 between 36 h after and the other two samples).

Ferritin values were significantly higher in the HCV-positive group (Table 1). Furthermore, ferritin values corresponded to the number of transfusions only in the HCV-positive patients (r²=0.229, P<0.001), whereas no consistent relationship was found in the HCV-negative group (r²=0.08; P NS). This finding was related to the different proportion of patients belonging to each transfusional group between the HCV-positive and the HCV-negative individuals (category 1, 38.8 versus 73.5%; category 2, 30.5 versus 22%; category 3, 30.5 versus 4.4% respectively). As can be derived from these numbers, the HCV-positive patients received proportionally more transfusions than the HCV-negative individuals (P=0.012). In this regard, the number of transfusions was statistically proportional to the duration of dialysis (category 1, 3.6±0.4 years; category 2, 6.6±0.8 years; category 3, 10.3±1.5 years, P=0.01 among the three groups). Moreover, the time on dialysis was significantly longer in the HCV-positive (9.08±0.9 years) than in the HCV-negative group (3.4±0.293). Giving additional support to the validity of the correlation between ferritin and aminotransferases values, we observed a clear-cut relationship between these two variables in the HCV-positive patients analysed in the pre-rHuEpo period (x±SD: ALAT 38.1±2.7, ASAT 35.3±3.4, ferritin 391.2±53, r²=0.285 and r²=0.122, P<0.001 and P<0.01 respectively for ALAT/ferritin and ASAT/ ferritin).

The data from the patient who received post-dialysis alpha-interferon also depict aminotransferases and ferritin as related markers. In the year prior to interferon administration, the patient was positive for HCV RNA by PCR, ALAT was 257±76 U/l, and ferritin was 1000±112 mg/l. With 12 months of treatment, HCV RNA was not detected and ALAT and ferritin mean values were 80.7±10 U/l and 598±86 mg/l respectively. Six months after cessation of interferon therapy, ALAT was 77.7±11 U/l and ferritin was 101±16 mg/dl; the patient remained negative for HCV RNA.

The seven histological samples were distributed as follows: three patients had chronic active hepatitis, one had chronic persistent hepatitis, one had generalized haemosiderosis, and two had non-specific reactive changes. The haemosiderin accumulation correlated markedly with plasma ferritin (r=0.851, P<0.01). The severity of the Knodell index did not, however, correlate with the histological accumulation of haemosiderin (r=0.262, P NS) or ALAT levels (r=0.301, P NS).
Discussion

The present study disclosed aspects of HCV infection in dialysis patients which have direct practical consequences. The main finding demonstrates the existence of a significant association between the levels of plasma aminotransferases and ferritin in HCV-positive patients.

In spite of their relative non-specificity, aminotransferases are a commonly used marker to indicate the presence of chronic liver illness.

The present study expands previous data from our group and others, which show a lower level of aminotransferases in dialysis patients without immunological markers of liver disease [4]. Although aminotransferases were higher in HCV-positive patients, in most cases these values were within the normal range for a non-uraemic population and were indeed lower than those found in a non-dialysis HCV-positive population [15]. This latter finding may account for the reported lack of correlation between the increase of aminotransferases and liver pathology in dialysis patients [4,7,16]. In these studies only values higher than 1.5 to 2 times the maximum of normality in non-dialysis individuals were considered abnormally elevated. The present data support the notion that the value at which the aminotransferases are considered abnormally elevated should be decreased in dialysis patients. This will result in a gain in the sensitivity of aminotransferases as a marker of liver injury without leading to a major decrease in specificity.

The relevant relationship between ferritin levels, a marker of liver iron accumulation, and aminotransferases, a marker of hepatocyte damage, can be interpreted in two different ways. According to Di Bisceglie et al. [17], plasma ferritin may be increased as a consequence of intracellular ferritin release from damaged hepatocytes. On the other hand, the linear positive relationship between ferritin and aminotransferases may suggest the existence of a potentiating effect of iron overload on HCV-induced injury. The HCV-positive patients were administered dialysis therapy for a longer period of time and were also exposed to a greater amount of transfusion-related iron than the HCV-negative patients; this fact may account for the greater iron load in the former group. In addition they had a greater exposure time to possible intradialysis infection by HCV. The possible association between iron and HCV in facilitating liver cell damage and conditioning the response to interferon therapy has been recently supported by several studies [6,11,18–20]. This association has a sound mechanistic basis due to the role of iron in reactions producing highly reactive cytotoxic molecules. Iron accumulation is a recognized fact in several liver diseases, but its precise pathogenetic role remains still undefined [21]. Recently, Farinati et al. have found a positive correlation between plasma ferritin, liver iron and free radical activity [22]. Our histological data do not however, support a correlation between liver iron content and the severity of the hepatic damage as assessed by the Knodell index. This result was not unexpected, since in our previous study [4] and in those of other authors [2], the histological severity did not correlate with aminotransferases activity. These histological findings, as well as the data from the interferon-treated patient, highlight the role of ferritin as a complementary marker of hepatocyte injury, although they do not support the value of either ferritin or aminotransferases as predictors of liver pathological progress. A more extensive series of biopsies are necessary to clarify this issue with a higher degree of certainty.

The present results have relevant application to the management of haemodialysis patients. Since plasma ferritin levels are employed as the usual biochemical test for evaluating the amount of iron stores in patients treated with rHuEpo, one should note that the presence of HCV may lead to overestimation of iron stores. This fact may perhaps explain some cases of dialysis-associated anaemia which are iron responsive in spite of normal or high plasma ferritin levels. Recent data from Fishbane and Maesaka suggest the existence of misevaluations of the amount of iron stores in dialysis patients, although they failed in identifying the causative mechanism [23]. In view of the high prevalence of HCV infection in this setting, our data may provide a clue for understanding many of Fishbane’s cases.

The present results indicate that the status of HCV infection should be considered as a relevant variable when estimating iron deposits by measuring plasma ferritin. In addition the association of plasma aminotransferases and ferritin levels should be considered when utilizing aminotransferases as markers of dialysis-associated liver disease.

Acknowledgements. The authors wish to thank the nurses of Fundación Jiménez Díaz and Fundación Igigo Alvarez de Toledo and M. Martina for editorial help. This study was supported in part by the Fundacion para el Estudio de las Enfermedades del Higado and Instituto Reina Sofia de Investigaciones Nefrologicas (IRSIN).

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*Received for publication: 4.9.95
Accepted in revised form: 17.5.96*