TRANSFUSED AND PHARMACOLOGICAL IRON: RELATIONSHIP OF OVERLOAD TO HLA ANTIGENS

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Summary

Forty-eight haemodialysis patients were divided in two groups according to presence or absence of haemochromatosis alleles (HA). Serum ferritin concentrations were determined and analysed according to blood transfusion history. Furthermore 20 patients were given iron saccharate and post treatment ferritin concentrations were determined at 15 and 30 days.

Following blood transfusion, only HA+ patients increased ferritin concentrations, while intravenous iron administration produced increased ferritin both in HA+ and HA− patients. Therefore it is advisable to minimise transfusions in HA+ patients, while intravenous iron administration should be avoided regardless of HA status.

Introduction

Blood transfusion and the administration of parenteral iron are used frequently to correct chronic anaemia in haemodialysis patients (HD). However, this often causes iron overload [1]. It has also been shown that ‘haemochromatosis alleles’ HLA A3, B7, B14 predispose to iron overload [2].

To evaluate the importance of blood transfusion and parenteral iron administration in causing iron overload, we have studied patients undergoing chronic haemodialysis, dividing them according to the presence or absence of ‘haemochromatosis alleles’ (HA). As ferritin is considered a reliable marker of total body iron [3,4], we have used this to determine iron overload.

Material and methods

Forty-eight patients (27 males and 21 females, mean age 45 yr, range 7–74) on chronic haemodialysis four hours thrice weekly, for a mean of 44 months duration (range 6–139) for end-stage renal disease of various aetiology, have
been studied. None of the patients had evidence of active liver disease or ongoing 
infection and there were no apparent differences in dietary iron intake. None of 
the patients had received iron supplements during the three months preceding 
the study. None had received any blood transfusion in the same period; furthermore 
a group of patients (n = 28) had received no transfusions for at least 15 
months prior to the study (non-transfused), the others (n = 20) had received at 
least one unit of blood between three and 15 months prior to the study (trans-
fused).

HLA typing was determined by a standard microlymphocytotoxicity technique 
[5]. Haematological parameters and serum iron were determined using standard 
techniques. Serum ferritin was assayed by a RIA method [6] using a commercial 
kit (Becton-Dickinson, Milano, Italy); the 95 per cent confidence limit for both 
sexes in our laboratory was: 18–185 ng/ml. A patient was said to have ‘iron 
overload’ when the serum ferritin concentration exceeded 400 ng/ml.

For the purpose of the study, the patients were divided into two groups 
according to the presence (HA+, n = 15) or absence (HA−, n = 33) of at least one 
of A3, B7, and B14 antigens. Age, sex, red blood cell count, haemoglobin concen-
tration, serum iron, duration and frequency of dialysis and transfusion 
regimen did not differ significantly in the two groups (Table I).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HLA+ (15 patients)</th>
<th>HLA− (33 patients)</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.0 ± 4.60</td>
<td>42.0 ± 2.93</td>
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</tr>
<tr>
<td>Time on dialysis (months)</td>
<td>51.0 ± 9.55</td>
<td>38.0 ± 6.09</td>
<td></td>
</tr>
<tr>
<td>Red blood cell count (x 10^6/L)</td>
<td>2874.17 ± 188.23</td>
<td>2800.69 ± 91.33</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>8.71 ± 0.57</td>
<td>8.77 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>Serum iron (g/dl)</td>
<td>87.0 ± 9.80</td>
<td>71.06 ± 4.22</td>
<td></td>
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<tr>
<td>Patients transfused</td>
<td>8/15</td>
<td>12/33</td>
<td></td>
</tr>
<tr>
<td>Units of blood/patient*</td>
<td>3.5 ± 1.23</td>
<td>2.08 ± 0.35</td>
<td></td>
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</tbody>
</table>

Entries: X ± SEM
NS: not significant
*: in the last 15 months

Furthermore, from the total number of patients, 20 patients (7 HA+ and 
13 HA−) with normal baseline ferritin values were selected and treated with 
200 mg x 5 i.v. iron saccharate.

Statistical analysis was carried out using Fisher’s exact test, p > 0.05 was 
considered non significant. In the text and tables the data are expressed by the 
average values and the Standard Error of the Mean (SEM).

All the patients gave informed consent to the study.

Results

Mean serum ferritin concentrations were significantly higher in HA+ patients 
compared to HA− patients: 439.21 ± 178.47 ng/ml vs 166.22 ± 33.79 ng/ml
(p<0.05). The difference between serum ferritin values was statistically significant in HA+ patients who had received blood transfusions within 15 months prior to the study in comparison to those who had not (760.37 ± 296.16ng/ml vs 70.31 ± 24.66ng/ml, p<0.05). Previous transfusions did not influence serum ferritin concentrations in HA− patients (190.16 ± 52.52ng/ml, transfused vs 152.54 ± 44.48ng/ml, non transfused). In addition, serum ferritin concentrations were significantly higher in transfused HA+ patients in comparison to transfused HA− ones (760.37 ± 296.19ng/ml vs 190.16 ± 52.52ng/ml, p<0.05). The difference between serum ferritin concentration related to HA status is not statistically significant in non-transfused patients (70.31 ± 24.16ng/ml, HA+ vs 152.54 ± 44.48ng/ml, HA−, NS) (Table II).

**TABLE II. Ferritin concentrations in transfused and non-transfused dialysis patients in relation to haemochromatosis alleles**

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Transfused</th>
<th>Non-transfused</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>↓</td>
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</tr>
<tr>
<td>HA+</td>
<td>439.21 ± 178.47 (15)</td>
<td>760.37 ± 296.19 (8)</td>
<td>70.31 ± 24.66 (7)</td>
</tr>
<tr>
<td></td>
<td>¶*</td>
<td>¶*</td>
<td></td>
</tr>
<tr>
<td>HA−</td>
<td>166.22 ± 33.79 (33)</td>
<td>190.16 ± 52.52 (12)</td>
<td>152.54 ± 44.48 (21)</td>
</tr>
</tbody>
</table>

Entries: ng/ml of serum ferritin (X ± SEM)
* p<0.05  ** p<0.005
NS: not significant
In brackets: number of patients

**TABLE III. Effect of parenteral iron on serum ferritin concentrations in relation to the haemochromatosis allele status**

<table>
<thead>
<tr>
<th>Time 0</th>
<th>After 15 days</th>
<th>After 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA+ (7)</td>
<td>109.7 ± 29.28</td>
<td>367.14 ± 82.09</td>
</tr>
<tr>
<td>HA− (13)</td>
<td>86.0 ± 21.94</td>
<td>320.38 ± 42.72</td>
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</tbody>
</table>

For legends see Table II

The intravenous administration of iron saccarate caused a significant increase of ferritin as assayed both 15 and 30 days after iron administration in all 20 treated patients, regardless of the HA status (Table III).
Discussion

Ferritin represents the most important detectable form of iron storage in the body. Interest in ferritin is due to recent studies demonstrating that serum ferritin reflects the quantity of iron in the tissues [4]. Hence serum ferritin has a clinical value in assessing both iron deficiency and iron overload [4]. In haemodialysis patients iron stores depend on a balance between blood loss during dialysis and iron uptake [7]. The latter depends on the absorption of dietary iron and both therapeutic and transfusion iron. Iron overload is a common problem in haemodialysis patients; its prevalence is as high as 70 per cent among patients receiving parenteral iron [2]. It has been suggested that the tendency to iron overload is related to genetic factors, i.e. the presence of at least one of the HA antigens [2]. Our results confirm these observations; in fact in our patients, iron overload was demonstrated only in the HA+ transfused patients. It is of interest that recently [8] a correlation has been demonstrated between serum ferritin concentrations and the number of blood transfusions. In addition our study shows that the increase of serum ferritin following intravenous iron saccharate is independent of the HA status of the patient.

In conclusion, our results suggest that a strict blood transfusion policy should be applied, particularly to the HA+ patients as these patients are at particular risk of transfusional iron overload. The increase in serum ferritin concentrations, regardless of the HA status, following intravenous iron supports the theory that transfused and oral iron follow two different metabolic pathways. The impressive findings of Ali et al [9] that intravenous iron supplements, although increasing serum ferritin concentrations, may not increase iron stores in the bone marrow should lead to a ban of intravenous supplement therapy in the treatment of anaemia in haemodialysis patients regardless of the HA status of the patients.

Acknowledgments

The authors acknowledge the help given by Mrs Gina Del Gallo and the expert secretarial assistance of Mrs Gabriella Valente.

References

3  Eschbach JW, Cook JD. Trans ASAIO 1977; 23: 54
4  Lypsichitz DA, Cook JD, Finch CA. N Engl J Med 1974; 290: 1213
5  Terasaki P, McClelland JD. Nature 1964; 204: 998
Open Discussion

CATTELL (Chairman) Given the iron kinetics and the fact that you measured the serum ferritin immediately on concluding the five week course of intravenous iron is this sufficient time to reach a steady state?

ELLI We performed the determination at 15 and 30 days after completing the administration. The administration was given on a weekly basis, 200mg i.v. once a week, and 15 and 30 days after the last infusion we determined the serum ferritin.

CATTELL But you may not at that stage have reached a steady state in respect to serum ferritin values. Does it then reflect the true iron status of the body?

ELLI The haematologists tend to agree that the steady state for iron balance is reached almost immediately. The totally insignificant difference between the values found at day 15 and day 30 seems to support this view.

COMTY (Minneapolis) We presented similar but less elegant studies in 1978 to the American Society of Nephrology. One of the problems is that the gastroenterologists tell us that serum ferritin is not a good method of measuring iron stores in patients with acute liver disease. I agree with you because we have seen acute iron overload develop in patients as they evolved acute hepatitis and we have seen a very high incidence of what might be considered to be haemochromatosis in our patients with chronic aggressive hepatitis. On the other hand we have seen another phenomenon in the dialysis patients over the years. Certain patients do seem to hyper-absorb dietary iron in the absence of hepatitis, and absence of abnormal liver function tests, and with a normal liver biopsy. I agree with you that there is something different about patients with HBsAg+ but I’m not sure what it is. The question of whether ferritin is a valid measure of iron stores in the hepatitis patient needs evaluation, meanwhile we use serum ferritin as an indicator of iron stores.

ELLI All of our patients had negative hepatitis antigens, and we made sure that our patients had no on-going liver diseases before entering them into the study. In the follow-up it turned out that they actually were not having any liver disease or any infection, that is another cause of possible mistake in ferritin determinations. As far as the intestinal iron absorption is concerned our patients were on a diet which could be considered homogenous. They were not supplemented with oral therapeutic iron; they were just on a free diet.

PECCHINI (Cremona) We have made a similar study but we have given iron only once (you have given the dose four times). Maybe the differences in overload between transfusion and therapeutic iron is related to HLA.

ELLI Theoretically this could be the cause, but we have to take into account the free interval from the last preceding therapeutic iron administration, and
possibly the total amount of iron the patients had received during their life. Our patients had received no therapeutic iron for at least three months prior to the study. Furthermore it is our policy to limit as much as possible iron supplementation to our patients.