Influence of Iron Status in the Response to the Deferoxamine Test

Jorge B. Cannata, José L. Fernández-Martín, Bernardino Díaz-López, Mario Alonso, Inés Olaizola, Gonzalo Acuña, Carlos Caramelo, and Jaime Alvarez-Grande

ABSTRACT

The study presented here was carried out to evaluate the possible relationship between serum iron and iron transferrin saturation with the response to the deferoxamine test in 86 chronic renal failure patients undergoing hemodialysis. The deferoxamine test was performed as a diagnostic tool for aluminum intoxication. Basal serum aluminum levels correlated with: (1) serum aluminum levels after the infusion of deferoxamine \( r = 0.45; P < 0.05 \); (2) serum iron levels \( r = -0.26; P < 0.05 \); and, (3) iron transferrin saturation \( r = -0.33; P < 0.05 \). The increase in serum aluminum levels after deferoxamine administration (DAI) showed a negative relationship with serum iron levels \( r = -0.23; P < 0.05 \) and iron transferrin saturation \( r = -0.26; P < 0.05 \). The correlations improved when analysis of this study included only those patients with high serum iron levels or high serum transferrin saturation \( r = -0.55 \). Patients with low probability of having aluminum overload (serum iron levels < 40 \( \mu \)g/L and DAI < 150 \( \mu \)g/L) had significantly higher values of serum iron, iron transferrin saturation, and serum ferritin levels compared with those patients with a high probability of having aluminum overload (serum aluminum levels > 40 \( \mu \)g/L and DAI > 150 \( \mu \)g/L). The study presented here suggests that patients who have indicators of iron repletion would tend to have lower increases in serum aluminum levels after the challenge with deferoxamine and presumably a higher incidence of false negative results with the deferoxamine test. These findings indicate that iron measurements must be always taken into account when interpreting the deferoxamine test.

Key Words: Aluminum, iron, chelating agents, transferrin

Aluminum and iron share relevant chemical features, are transported by the same serum proteins, and are chelated by the same compounds (1-4). Recent works have suggested that they also share mechanisms of gastrointestinal absorption and cellular uptake (5). In addition, the aluminum overload may induce anemia, increases in the erythropoietin requirements, and also a decrease in intraerythrocytic ferritin in spite of the presence of normal iron stores in the body (6).

Nowadays, the suspicion of aluminum overload is made on the basis of the measurement of serum aluminum levels, parathyroid hormone, and also in the increase in serum aluminum levels observed after a single dose of deferoxamine infusion (deferoxamine test).

Previous studies have shown a negative relationship between basal serum aluminum and iron levels (7-9), suggesting that the amount of either aluminum or iron may influence the transferrin capacity to bind the other element. Similarly, because of the capability of deferoxamine to chelate both metals with a higher affinity for the iron compared with aluminum, we could also speculate that the amount of iron carried by the serum proteins and the magnitude of the iron stores may also modify the probability of aluminum to be carried by deferoxamine; consequently, the status of the iron metabolism may influence the result of the deferoxamine test. Therefore, the aim of this study was to evaluate the possible relationship between serum iron levels and iron transferrin saturation with the response to the deferoxamine test.

PATIENTS AND METHODS

The study was carried out in 86 chronic renal failure patients undergoing dialysis in two different dialysis units, in whom the deferoxamine test was performed between 1990 and 1993 as a diagnostic tool for aluminum intoxication. The deferoxamine test was performed when there was a suspicion of aluminum overload on the basis of our current clinical criteria, which consist in having one or more of the following findings: basal serum aluminum levels higher than 40 \( \mu \)g/L; long-term oral exposure to aluminum-containing phosphorous binders (>6 months); or any sign or symptom suggestive of aluminum-induced bone disease (hypercalcemia, bone pain or fracture). In all centers, the aluminum concentration in the dialysate was maintained under 10 \( \mu \)g/L.
throughout the period of study. Informed consent was obtained. The test was carried out following the classic criteria used until 1993, which consisted of 40 mg/kg of deferoxamine in the intravenous infusion (10), and in a continuous perfusion during 2 h immediately after the end of the dialysis session (2,11). The intake of aluminum-containing phosphorous binders was stopped 1 wk before performing the deferoxamine test.

To evaluate the response to the deferoxamine infusion, we measured serum aluminum levels just before the infusion of deferoxamine (Al1) and before the beginning of the next hemodialysis session (Al2). The increase in serum aluminum levels obtained with the deferoxamine infusion (DAI) was calculated as follows: DAI = Al2-Al1. The determination of serum aluminum levels was carried out following the same methodology published in previous reports, by graphite furnace atomic absorption spectrometry, using a Zeeman-3030 spectrometer, with HGA-600 graphite furnace and an automatic sampler (Perkin Elmer, Germany) (12-14).

Serum iron, transferrin, and ferritin levels, and total iron binding capacity (TIBC) and the iron transferrin saturation were measured in the same dialysis session in which the deferoxamine test was carried out.

Iron was determined by colorimetric methods, and transferrin by an immunoturbidimetric test (Boehringer Mannheim, Germany) and ferritin was measured by using a monoclonal antibody (Enzyme immunoassay, Bio-merieux, France). TIBC was measured with a standard technique that measured the total amount of iron transported by transferrin. This value was used to calculate the iron transferrin saturation.

By using a computer-supporting Sigma (Horus Hardware, Spain), t tests for unpaired data and linear regression analysis were carried out. Multiple regression analysis was performed with a statistical package Systat 5.2 for Macintosh.

RESULTS

Because of the profile of patients studied, the levels of basal serum aluminum observed were in the range of those considered as suspicious of aluminum overload (Table 1). A positive and significant relationship was observed between basal serum aluminum levels and the increase of serum aluminum levels after the infusion of deferoxamine \( r = 0.45; P < 0.05 \). Table 1 shows the values found in the other variables studied.

### Basal Serum Aluminum Levels and Iron Measurements

Basal serum aluminum levels correlated negatively with serum iron levels \( r = -0.26; P < 0.05 \) (Figure 1a) and with iron transferrin saturation \( r = -0.33; P < 0.05 \) (Figure 1b). The multiple regression analysis showed that serum iron levels were correlated best with serum aluminum levels \( P < 0.02 \). The linear regression analysis of basal serum aluminum levels versus serum iron levels improved when we consid-

#### TABLE 1. Mean and SD of the different variables studied

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-DFO Aluminum Level (µg/L) (Al1)</td>
<td>75.3 ± 48.4</td>
<td>9.6-228.3</td>
<td>86</td>
</tr>
<tr>
<td>Post-DFO Aluminum Level (µg/L) (Al2)</td>
<td>215.0 ± 131.6</td>
<td>38.7-720</td>
<td>86</td>
</tr>
<tr>
<td>∆ Aluminum Level (µg/L)</td>
<td>139.7 ± 102.4</td>
<td>17.7-541.8</td>
<td>86</td>
</tr>
<tr>
<td>Serum Iron Level (µg/dL)</td>
<td>101.9 ± 43.2</td>
<td>40-260</td>
<td>86</td>
</tr>
<tr>
<td>TIBC (µg/dL)</td>
<td>324.8 ± 78.1</td>
<td>186-525</td>
<td>65</td>
</tr>
<tr>
<td>Iron Transferrin</td>
<td>34.0 ± 17.5</td>
<td>12-84.7</td>
<td>65</td>
</tr>
<tr>
<td>Saturation (%)</td>
<td>275.2 ± 80.0</td>
<td>99-506</td>
<td>66</td>
</tr>
<tr>
<td>Serum Transferrin Level (mg/dL)</td>
<td>532.0 ± 797.1</td>
<td>7-3895</td>
<td>84</td>
</tr>
<tr>
<td>Serum Ferritin Level (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( ^{a} \text{DFO, deferoxamine; TIBC, total iron binding capacity; } \Delta \text{ aluminum, } \text{Al}_{2} - \text{Al}_{1}. \)

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**Figure 1.** Relationship between serum aluminum levels and iron indicators. (A) Serum iron levels. Solid line, all patients \( r = -0.26, N = 86, P < 0.05 \). Broken line, patients with serum iron levels greater than 100 µg/dL \( r = -0.40, N = 36, P < 0.05 \). (B) Iron transferrin saturation: \( r = -0.33, N = 65, P < 0.05 \).
erred only those patients with high serum iron levels (Figure 1a). The basal serum aluminum and serum ferritin levels did not show any significant relationship.

**Deferoxamine Test and Iron Measurements**

The increase of serum aluminum levels showed also a negative relationship with serum iron levels \( r = -0.23; P < 0.05 \) (Figure 2a) and iron transferrin saturation \( r = -0.26; P < 0.05 \) (Figure 2b). The multiple regression analysis showed that iron transferrin saturation was the variable that correlated better with the increase in serum aluminum levels \( P < 0.04 \). When, according to our hypothesis, we analyzed only those patients with high iron transferrin saturation (>40%), we found a better linear relationship \( r = -0.55 \) (Figure 2b). By contrast, in those patients who had an iron transferrin saturation below 40%, there was no significant correlation between the variables studied \( r = 0.04 \).

When the patients were divided in two different groups, normal and low iron transferrin saturation (<40%) and high iron transferrin saturation (>40%) (Table 2), the group with normal and low iron transferrin saturation showed an increase in serum aluminum levels that was significantly higher than those of the group with high iron transferrin saturation. The same response was seen in those patients with serum iron levels lower than 100 μg/dL, compared with those who had higher serum iron levels (Table 2). As expected, a clear parallel between the results obtained with serum iron levels and iron transferrin saturation was found because of the interdependence between both variables \( r = 0.75; P < 0.05 \). No correlation was found between the increase in serum aluminum levels after deferoxamine infusion and serum ferritin levels \( r = 0.007, P = \) not significant.

**Aluminum Overload and Iron Measurements**

We formed two groups, taking into account the two variables we may use in clinical practice as indirect markers of the degree of aluminum overload (12,15) (Table 3). We observed that those patients with low probability of having aluminum overload (serum aluminum levels lower than 40 μg/dl and DAI lower than 150 μg/dl), had significantly higher values of serum iron, iron transferrin saturation, and serum ferritin compared with those patients with a high probability of having aluminum overload (Table 3).

**DISCUSSION**

The bulk of evidence indicates that the main serum aluminum carrier is plasma transferrin. This protein, partially saturated with iron, has a greater binding capacity for iron \( (\log K_1 = 31) \) than for aluminum \( (\log K_1 = 22) \) (16).

This fact explains why iron may displace aluminum from transferrin, whereas the reverse would be more difficult (6,17–19). The competition between these two metals for the same carrier, together with the difference in binding affinity, may explain the negative relationship observed in the serum basal values of iron and aluminum found in previous works and also found in our study (7–9,19).

A similar situation occurs between deferoxamine and its binding capacity for iron \( (\text{affinity constant, } K = 10^{31}) \) and aluminum \( (\text{affinity constant, } K = 10^{22}) \). The higher binding capacity of deferoxamine for iron may explain the differences observed in the result of the deferoxamine test between patients with different concentrations of serum iron and iron transferrin saturations. In those patients with high serum iron levels and high iron transferrin saturation (>40%), a great proportion of the deferoxamine infused would bind iron (because of the higher iron availability), and this

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**Figure 2. Relationship between DAI (Al₂–Al₁) and iron indicators.**

(A) Serum iron levels: \( r = -0.23, N = 86, P < 0.05 \). (B) Iron transferrin saturation. Solid line, all patients \( r = -0.26, N = 65, P < 0.05 \). Broken line, patients with iron transferrin saturation greater than 40% \( r = -0.55, N = 19, P < 0.05 \).
TABLE 2. Mean ± SD of the aluminum level increase post-DFO (Δ aluminum) at different iron transferrin saturation and serum iron levels*

<table>
<thead>
<tr>
<th>Test</th>
<th>Transf. Sat. &lt;40% (N = 46)</th>
<th>Transf. Sat. &gt;40% (N = 19)</th>
<th>Serum Iron Level &lt;100 μg/dL (N = 50)</th>
<th>Serum Iron Level &gt;100 μg/dL (N = 36)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Aluminum (μg/L)</td>
<td>167.2 ± 124.7</td>
<td>104.9 ± 58.7</td>
<td>164.4 ± 120.1</td>
<td>105.5 ± 56.6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Transf. sat., iron transferrin saturation (%); Δ aluminum = Al₂ - Al₁; DFO, deferoxamine.

TABLE 3. Different iron indicators in the two groups of patients with low and high probability of aluminum intoxication (Al intox)*

<table>
<thead>
<tr>
<th>Low Probability of Al Intoxication</th>
<th>High Probability of Al Intoxication</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Serum Al &lt;40 μg/L; Δ Al &lt;150 μg/L)</td>
<td>(Serum Al &gt;80 μg/L; Δ Al &gt;150 μg/L)</td>
</tr>
<tr>
<td>Serum Iron Levels (μg/dL)</td>
<td>140 ± 56.6</td>
</tr>
<tr>
<td>Transf. Sat. (%)</td>
<td>48.4 ± 21.7</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>1073.0 ± 1002.1</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Transf. sat., iron transferrin saturation; Δ Al, aluminum increment post-DFO.

would decrease the possibility of aluminum being bound by deferoxamine. As a result, independently of the amount of aluminum body burden, iron-repleted patients may have less chance to increase their serum aluminum levels after the infusion of deferoxamine. In addition, and supporting these results observed in patients, recent unpublished experimental data from our lab demonstrated that rats with chronic renal failure, receiving the same amount of chronic aluminum load, have significantly higher serum aluminum levels after deferoxamine infusion when they were iron-depleted, compared with when they were iron-repleted.

According to the results obtained with iron transferrin saturation and ferritin levels, we could raise the hypothesis that a relevant difference in aluminum turnover might have occurred in the patients with elevated iron repletion values, namely, that the higher iron transferrin saturation, the more the iron and the lower the aluminum are driven to the tissues to be deposited.

On the contrary, in those patients with no iron repletion (lower serum iron levels and lower iron transferrin saturation), deferoxamine will have less iron available to bind, thus, there will be more chance to bind aluminum, and more possibilities of having a greater difference in the serum aluminum levels pre- and post-deferoxamine infusion. Even though in our study we did not perform aluminum measurements in tissues that would help us to reinforce our results, and we are aware of the known protective effect of iron against aluminum accumulation, the well-known differences in the binding capacity of deferoxamine for iron and aluminum (affinity constants $10^{31}$ and $10^{22}$ respectively) might be by itself a valid explanation for the results found in this study between patients with different iron statuses. The wide range of variations in the amount of iron available for binding to deferoxamine observed in previous studies may explain some controversial data, primarily the unexplained false-negative results observed after the deferoxamine test (20).

Even though this study has focused the attention on the effect of iron, there are other several causes that might influence the specificity and sensitivity of the deferoxamine test (21,22). In our experience, we found it useful to stop the intake of aluminum-containing phosphorous binders during one week, which according to recent reports still represent a non-negligible source of aluminum in 40 to 60% of hemodialysis patients in some European countries (23,24).

In summary, according to our results, patients with higher serum iron levels, and higher iron transferrin saturation, would tend to have lower increases of serum aluminum levels after deferoxamine infusion, and a higher incidence of false negative results with the test. This lack of sensitivity may be more marked if the dose of deferoxamine used for the test is reduced below 40 mg/kg, as it has been recently advised (21). By using a lower dose of deferoxamine, there would be less of the drug available to bind both metals and therefore, the higher affinity for iron would leave less...
deferoxamine free of iron and capable of binding aluminum. This would increase the difference in the response to the deferoxamine test between patients with and without iron repletion, and consequently, the false-negative results with deferoxamine testing may increase.

These findings will be more evident in locations where the degree of aluminum exposure is still high. On the contrary, in cases in which there is little or almost no change in serum aluminum levels, the changes in serum aluminum levels should be minimal and the discussed effect of iron status will not take place. Although further studies are required, our findings indicate that in patients with suspicion of aluminum overload in whom deferoxamine testing is indicated, iron indicators must be always considered in the interpretation of the response to the test because major interpretative errors may occur in cases in which iron status were not taken into account.

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