Serum ferritin and iron stores in patients on maintenance dialysis

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SUMMARY

The levels of serum ferritin of 51 patients on maintenance hemodialysis and 16 on peritoneal dialysis were compared to the hematological status and the levels of iron stores. Serum ferritin was correlated with serum iron (r = 0.60), transferrin saturation (r = 0.71), the iron staining in bone marrow (r = 0.52) and the number of sideroblasts (r = 0.64). Despite an oral supplement of 116 mg/d iron, in 74 % of patients, the iron stores in the bone marrow were absent or decreased. After discontinuation of oral iron suplements for four weeks, the geometric mean levels of serum ferritin fell significantly from 95 µg/l to 68 µg/l ($p < 0.001$) with a decrease of 0.99 g/dl of hemoglobin.

These results suggest: a) serum ferritin is a reliable index of iron available for erythropoiesis in patients on maintenance dialysis; b) in the population studied, an oral iron supplement of 116 mg/d was not sufficient to maintain adequate iron stores.

Key words: Serum ferritin. Iron stores. Anemia. Dialysis. Transferrin saturation.

RESUMEN

Los niveles de ferritina sérica en 51 pacientes en hemodiálisis y 16 en diálisis peritoneal de mantenimiento han sido comparados con los valores hematológicos y los niveles de los depósitos de hierro. La ferritina sérica se correlaciona con la sideremia (r = 0,60), la saturación de la transferrina (r = 0,71), los depósitos de hierro en la médula ósea (r = 0,52) y el número de sideroblastos (r = 0,64). a pesar de un suplemento de hierro oral de 116 mg/d., en el 74 % de los pacientes los depósitos de hierro en la médula ósea estaban ausentes o disminuidos. Tras interrumpir el suplemento de hierro oral durante cuatro semanas, los valores de la media geométrica de ferritina sérica disminuyeron desde 95 µg/l. a 68 µg/l. ($p < 0,001$), acompañándose de una disminución de la hemoglobina de 0,99 g/dl.

Estos resultados sugieren: a) la ferritina sérica es un buen índice del hierro disponible para la eritropoyesis en los pacienes en diálisis de mantenimiento; b) en la población estudiada, un suplemento de hierro oral de 116 mg/d. no fue suficiente para mantener un nivel adecuado de los depósitos de hierro.

Palabras clave: Ferritina sérica. Depósitos de hierro. Anemia. Diálisis. Saturación de la transferrina.

INTRODUCTION

Almost invariably dialyzed patients have anemia of moderate to severe degree. Its pathogenesis is complex, and includes both decreased red cell survival and decreased red cell production.. For the latter, an impaired synthesis of erythropoietin and impaired response to erythropoietin is usually believed to be the primary cause of anemia. However, an iron deficiency can also contribute to decreased red cell production in dialyzed patients [1,2]. The origin of iron deficiency is related to the blood loss as a result of dialyzer blood loss, blood sampling and occult gastrointestinal blood loss [3,4]. The iron absorption of these patients was not altered and correlated with the levels of iron stores [5-7].

In the present work serum ferritin levels of a population on peritoneal and hemodialysis maintenance were compared with the different iron parameters and the hematological status.

MATERIAL AND METHODS

Subjects. Sixty seven patients from the University Hospital of Malaga were used for this study. The patients' ages varied between 20 and 66 years (43 ± 16 years; Mean ± 1 SD). Average time on dialysis was 21 ± 14 months for the group (range 6 to 68 months). Thirty one males and twenty females were on maintenance hemodialysis for 9 to 30 hours/week with either Gambro-Lundia 17-μ/m^2 or a Cordis Dow 1.3-m^2 dialyzer. Distilled water was used for the production of the dialysate. Eight males and eight females were on maintenance peritoneal dialysis.

All patients had a daily dietary protein intake of approximately 1.0 g/kg of body weight and all were taking phosphate binders and multi-vitamins, including folate and vitamin B 12. During the entire period on dialysis, all patients received iron orally at an average of 116 mg/day of elemental iron (575 mg of ferrous sulfate). None of the patients received parenteral iron therapy or blood transfusions. No patients with liver disease or overt infection were included in the study group.

For each patient the blood losses were calculated on the basis of 5 ml by dialysis [4] and 20 ml each month for the laboratory investigation, multiplied by the total number of times on dialysis as well as the number of months on dialysis treatment. For patients on peritoneal dialysis, only the 20 ml/months for laboratory investigations were taken into account. Renal function has been evaluated by the residual diuresis in the inter-dialysis period and by the levels of creatinine before dialysis.

As a control population, 40 males and 40 females who were not blood donors were used. Informed consent was obtained from all the subjects prior to the study.

Hematological and serological determinations. Hemoglobin, erythrocyte count, hematocrit and red blood cell indices were done with a laser ray counter (Hemac 63OL, Ortho Diagnostics Instruments, New Jersey, USA). Reticulocytes were counted in 2,000 red cells stained with methylene blue and counterstained with Wright's stain.

Bone marrow samples were obtained either by sternal or transiliac bone puncture and were stained for hemosiderin with Prussian blue. Each bone marrow smear was examined «blind» by two observers who had no knowledge of the patient's hematological status and graded on a scale of o to + 3 [8]. Zero was defined as no hemosiderin particles in any of the spicules, representing absent marrow iron stores. 1 + signified only a few hemosiderin particles within the marrow, indicative of depleted marrow iron stores. 2 + represented considerably more hemosiderin than 1 + within the marrow and was considered compatible with normal iron stores. 3 + indicated large quantities of hemosiderin or excessive iron stores. The number of sideroblasts were scored by counting 200 erytroblasts. In grading the marrów for hemosiderin, as well as scoring the number of siderobasts, there was close correlation between the two examiners.

The spectrophotometric method using bathophenanthroline as the indicator reagent was used for measuring serum iron, as is suggested by the International Commission for Standardization in Hematology [9]. Total iron binding capacity was measured by the method described by Ramsay [10].

The serum ferritin level was measured by a commercial radioimmunoassay (Ramco Laboratories, Houston, Tx, USA) based on a two-step method. Briefly, polystyrene beads coated with rabbit antiserum to human spleen ferritin were reacted with patients serum to bind the patient ferritin. Radio-iodinated antihuman ferritin was then added, incubated, centrifuged and the radioactivity of the solid phase complex measured.

Statistical analysis. For all calculations, non-parametric tests were used, the umpaired values were analyzed with the Mann Whitney, the paired values with the Wilcoxon and the correlation were done with the Spearman rank method. Nonparametric tests were chosen because the serum ferritin concentrations were found to follow a skewed distribution.

RESULTS

The hematological data of dialysis patients are shown in Table I. Both groups, male and female, on hemodialysis or peritoneal dialysis were anemic. No differences in the levels of hemoglobin, hematocrit or red blood cells were found between the male and female groups or between patients on hemodialysis and peritoneal dialysis. Slight difrerences in the red blood cell indices were found in the different groups. The reticulocyte counts were similar in patients and controls. Serum iron and transferrin saturation were decreased only in the male patients. Of the fifty one bone marrow examined, twenty nine (57 %) had no iron staining in the bone marrow (grade 0), nine (17 %) had decreased (+ 1), ten (19 %) had normal (+ 2) and three (6 %) had increased iron stores (+ 3). There is a good correlation between the amount of iron staining in the bone marrow and the number of sideroblasts ($r = 0,72$; $p < 0.001$).

For the control group, normal values of serum ferritin were greater than 40 µg/l for male and greater than 20 µg/l for female. In relation to the controls, the levels of ferritin were decreased in the group of males on hemodialysis (figure 1). However, hemodialyzed females or patients on peritoneal dialysis showed no differences in the ferritin levels relative to the corresponding controls. Also, no differences were found when the levels of ferritin were compared between patients on hemodialysis and peritoneal dialysis.

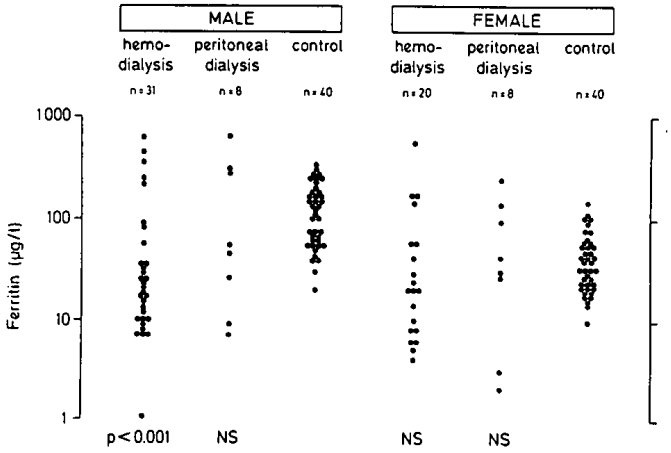

Fig. 1.—*Serum ferritin in dialysis patients.*

In dialysis patients, a small degree of correlation was found between serum ferritin and the levels of hemoglobin ($r = 0.34$; $p < 0.05$), reticulocyte count ($r = 0.35$; $p < 0.05$), MCV ($r = 0.24$); MCH ($r = 0.08$) and MCHC ($r - 0.19$). There is a good correlation between serum ferritin and the other parameters of iron metabolism: serum iron ($r = 0.60$; $p < 0.001$), total iron binding capacity ($r = 0.49$; $p < 0.001$), transferrin saturation ($r = 0.71$; $p < 0.001$) (Figure 2), the iron staining in the bone marrow ($r = 0.52$; $p < 0.001$) and the number of sideroblasts ($r = 0.64$; $p < 0.001$). Patients having either absent or decreased staining marrow iron ($n = 38$) had a geometric mean of serum ferritin of 24 µg/l, whereas the value for

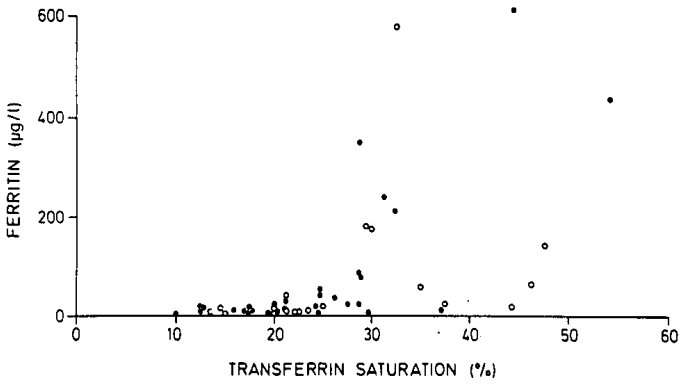

Fig. 2.—*Correlation between the ferritin levels and transferrin saturation in hemodialysis patients (• Males, o Females). Spearman's rank correlation ± 0.71, p < 0.001.*

patients with normal or increased iron stores (n = 13) was 215 µg/l (p < 0.001). The discriminant function analysis provided a mean of estimating the bone marrow category given the serum ferritin value. Table II compares the levels of iron deposits in the bone marrow with the decision criteria of serum ferritin. Only one patients with iron deficient (+ 1) was misclassified as normal and one with normal iron deposits (+ 2) as iron deficient. No correlation was found between ferritin levels with the time on dialysis (r = 0.12), the calculated blood loses (r = 0.10), the residual diuresis (r = 0.14) or the creatinine levels (r = − 0.06).

Transferrin saturation was less well correlated with staining iron in the bone marrow (r = 0.29; p < 0.05) or with the number of sideroblasts (r = 0.54; p < 0.001). However, of the 38 patients with absent or decreased staining marrow iron, e patients showed a transferrin saturation above 25 % and of the 13 patients with normal or increased deposits, one showed a transferrin saturation under 25 %.

After discontinuation of the oral iron suplement (116 mg/d) for four weeks, the levels of serum ferritin fell significantly from 95 µg/l (geometric mean) to 68 µg/l (p < 0.001). The levels of hemoglobin also fell from 8.13 ± 2.26 g/dl to 7.25 ± 2.32 g/dl (p < 0.01).

DISCUSSION

Serum ferritin levels have been used to assess the iron stores of patients on maintenance hemodialysis. A possitive correlation has been found with iron staining in the bone marrow [7,11-18]. In this study serum ferritin was the best predictor of iron storage levels, showing a good correlation with iron staining in the bone marrow as well as with the number of sideroblasts or transferrin saturation levels, which are also reflections of the iron stores. The correlation found in this study between serum ferritin and iron stores in the bone marrow is lower than that reported in other reports [13,18]. This diference may be due to the measurement of iron stores by a semi-quantitative method. Similar correlations of transferrin saturation and derum ferritin have been previously reported [16-18]. Ferritin estimations are expensive and the concentration can be influenced by the presence of liver disease [19]. Gokal et al. [7] have proposed that serum feritin can be replaced by the red blood cell indices MCH and MCV. However, in this study, as in that of Paterson et al. [20], no correlation was found between the MCH or the MCV and ferritin levels. If ferritin assays are not available routinely, the measurement of transferrin saturation can be used as an indicative measure of iron deposits. Our results show that values of transferrin saturation under 25 % correspond with absent or decreased staining marrow iron, as well as with low values of serum ferritin (Figure 2).

Contrary to some publications [7,19,20], in our study, serum ferritin was not correlated with the duration of dialysis treatment. This difrerence may be related to the different iron therapies given to patients on dialysis. Also, no correlations were found with the calculated blood losses, suggesting that blood losses from other origins than the dialyzer or the samples for laboratory investigations may play a role in the iron balance of dialyzed patients.

There is a slight correlation between the levels of serum ferritin and the hemoglobin concentration or reticulocyte number. These results suggest that the iron available for erythropoiesis does not play a major role in the anemia of

TABLE I

HEMATOLOGICAL VALUES OF DIALYSIS PATIENTS AND CONTROLS

	MALE			FEMALE		
	Control (n = 40)[a]	Hemodialysis (n = 31)	Peritoneal dialysis (n = 8)	Control (n = 40	Hemodialysis (n = 20)	Peritoneal dialysis (n = 8)
Red blood cells (10^{12}/l.)	5.41 ± 0.36[b]	2.82 ± 0.83[e]	2.64 ± 0.50[e]	4.44 ± 0.28	2.14 ± 0.30[e]	2.93 ± 0.70[e]
Hemoglobin (g/dl.)	15.49 ± 1.66	8.46 ± 4.74[e]	6.72 ± 1.64[e]	13.44 ± 0.88	6.35 ± 0.91[e]	7.75 ± 2.53[e]
Hematocrit (%)	47.63 ± 2.93	24.62 ± 7.07[e]	24.12 ± 4.52[e]	39.54 ± 2.49	20.82 ± 2.37[e]	25.78 ± 6.43[e]
MCV (fl.)	88.05 ± 3.42	87.98 ± 8.54[c]	91.18 ± 2.78[d]	89.14 ± 3.59	97.74 ± 9.03[e]	87.54 ± 3.90[c]
MCH (pg.)	28.69 ± 3.13	30.65 ± 1.93[c]	25.20 ± 1.80[e]	30.30 ± 1.56	29.75 ± 3.11[c]	25.93 ± 3.67[e]
MCHC (g/dl.)	32.50 ± 3.25	35.38 ± 2.52[d]	27.60 ± 2.40[e]	33.91 ± 0.83	30.42 ± 2.85[e]	29.50 ± 3.61[e]
Reticulocytes (10^{12}/l.)	0.49 ± 0.12	0.55 ± 0.32[c]	0.67 ± 0.53[c]	0.46 ± 0.11	0.50 .006	0.25[c] 0.55 ± 0.33[c]
Serum iron (µmol/l.)	20.56 ± 6.16	14.34 ± 4.66[e]	12.93 ± 2.99[d]	15.08 ± 5.45	15.26 ± 5.725c	12.81 ± 3.92[c]
Transferrin saturation (%)	32.55 ± 6.60	23.31 ± 9.67[e]	21.69 ± 7.53[d]	25.38 ± 9.23	27.60 ± 10.31[c]	23.40 ± 6.98[c]

[a]Number of cases; [b]mean ± 1 SD; [c]Not significant (p > 0.05); [d]p < 0.01; [e]p < 0.001.

TABLE II

COMPARISON OF BONE MARROW IRON STORES TO FERRITIN LEVELS

Bone marrow iron stores	Ferritin			
	Male (n = 31)[a]		Female (n = 20)	
	< 88 µg/l.[b]	> 88 µg/l.	< 40 µg/l.	> 40 µg/l.
0	19	0	10	0
+ 1	4	1	4	0
+ 2	0	5	1	4
+ 3	0	2	0	1

[a] Number of cases; [b] limit between the iron deficient groups (0, + 1) and for normal or excesive iron stores groups (+ 2, + 3) obtained by the discriminant function analysis.

the patients studied. In this study it was found that both males and females on hemodialysis had similar iron stores. In 77 % of males and 70 % of females absent or decreased iron staining was seen. The geometric mean of serum ferritin levels in both groups were similar. However, with the discriminant function analysis performed on the data in our study, taking account the bone marrow iron status, we found that the normal limits in males (88 µg/l) are increased in relation to females (40 µg/l). In both cases, the normal values for dyalisis patients are twice that for normal controls of both sexes. These data confirm the previous observation of Aljania et al. [15].

We found that only male patients exhibit decreased levels of ferritin relative to their control population. This could be due to the fact that the ferritin levels of the female control population were lower than those of the male controls. In this group of female there were some cases of subclinical iron deficiency. Unfortunately, these cases could not be excluded since marrow examination was not done [21], but for ethical reasons this test was not performed. In addition, the normal limits for hemodialysis patients are higher that normal population.

The decrease of iron stores in the patients studied suggests that the daily oral iron supplement of 116 mg is not sufficient to maintain adequate iron stores. From the average fall in serum ferritin concentrarion of 0.90 µg/,/day and the relationship that 1 µg/l of serum ferritin is approximately equivalent to 8 mg of storage iron [22], it is posible to calculate the average daily requirement to be 7 mg/day. Taking into account that iron absorption in subjects with normal iron stores is about 3-5 % [5], the oral iron dose needed in dialysis patients to maintain iron stores is 140-230 mg/day. This estimation is slightly increased compared to that proposed by Lynn et al. [19] or Cotterill et al. [23].

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