Iron overload as cardiovascular risk factor in children and adolescents with renal disease

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Abstract

Background. Iron overload can affect cardiac structure and function by the production of free radicals in addition to iron deposits in heart muscle. The purpose of this study was to compare traditional and non-traditional cardiovascular risk factors (CVRF) in children and adolescents on renal replacement with and without iron overload. Also, we evaluated the relationships between iron overload and left ventricular mass (LVM).

Methods. First, in a cross-sectional study, we evaluated traditional and non-traditional CVRF in 143 children and adolescents, 48 on peritoneal dialysis (PD), 53 on hemodialysis (HD) and 42 after renal transplantation according to iron overload. In a second phase with a case–control study, we measured LVM in 12 case patients and 12 matched controls.

Results. Iron overload was identified in 15 patients (10.5%), 11 in HD and 4 in PD (P = 0.002). The group with iron overload had lower body mass index (17 versus 19; P = 0.01), total cholesterol (132 versus 165 mg/dL; P = 0.03) and hemoglobin (8.5 versus 10.6 g/dL; P = 0.003) but higher interleukin (IL)-6 levels (4.8 versus 3.6 ng/L; P = 0.04) and hypertension diagnosis (79 versus 48%; P < 0.001) than those without iron overload. Ferritin showed a positive correlation with C-reactive protein (CRP) and IL-6 levels. In a subgroup of 24 patients (12 with and 12 without iron overload), LVM was not different. However, ferritin levels showed a borderline positive correlation (r = 0.44, P = 0.05) with LVM.

Conclusion. Children and adolescents with iron overload show more CVRFs, especially if they received replacement therapy with HD. Ferritin is related to CRP and IL-6 levels.

Keywords: children; dialysis; ferritin; ventricular function

Introduction

Chronic renal failure (CRF) is a risk factor for cardiovascular disease. Moderate decrease in renal function (<60%) increases 50–70% the risk of myocardial infarction in adults [1] and coronary heart disease in children [2]. Left ventricular hypertrophy (LVH) is the most common cardiac abnormality observed in patients with CRF (~80%) and persists up to 50% in children and young adults after renal transplantation [3].

Traditional and non-traditional cardiovascular risk factors (CVRF) have been associated with endothelial dysfunction, atherosclerosis and cardiac myofibril damage with the subsequent ventricular dysfunction [1,4,5,6].

The increased survival of patients on peritoneal dialysis (PD) has shown other complications such as iron overload [7,8]. Iron overload affects cardiac structure and function by the production of free radicals and iron deposits in the heart muscle. It also promotes the development of atherosclerosis [9,10,11,12]. Iron overload in Mexican children on hemodialysis (HD) varies from 19 to 70% [13] and in adults has been reported between 12 and 73% [14], especially in nephropathies associated with proteinuria [15]. Also, there is evidence that patients may exhibit iron overload long after its suspension and even after an adequate post-transplantation erythropoiesis [16]. Furthermore, this stage has been associated with mutations in the gene for hemochromatosis [17].

However, no studies have reported whether iron overload in pediatric patients with CRF is associated with left ventricular dysfunction and traditional or non-traditional CVRFs. The purpose of this study was to compare number of traditional and non-traditional CVRFs in children on renal replacement with and without iron overload. In addition, we evaluated the relationship between iron overload and left ventricular mass (LVM).

Materials and methods

We included patients under 18 years of age with a diagnosis of CRF in different renal replacement programs. The study was performed in two phases: (i) a cross sectional study to identify the frequency of iron overload and to compare number of CVRFs in those patients with and without this condition; (ii) in a second phase we selected cases from those patients with...
Iron overload who were interested in participating and matched them to control patients without iron overload in a 1:1 ratio, according to age (within 1 year), gender, and type of renal replacement, to compare LVM and to better understand the association between ferritin levels and LVM. None of these patients had received a renal transplantation. In the pre-transplant cases, the parameters of dialysis adequacy were in normal ranges (Kt/V >2 per week in PD and >1.2 in HD). We did not include patients who were using digoxin, with diagnosis of hereditary hemochromatosis, congenital heart malformations, previous cardiovascular surgery, chelation therapy, blood transfusions during the previous month, active infections or diabetes mellitus.

By chart review, we identified age at diagnosis of CRF, etiology of the disease, type of replacement therapy, number of transfusions, dose and time of administration of erythropoietin, route, dose and time of administration of iron and diagnosis of hypertension based on percentiles for age and height [18]. In our hospital, the guidelines of iron administration support the use of oral iron in patients with PD and intravenous iron in patients with HD.

After signed informed consent by children and at least one of their parents, the subjects were included and blood samples with a previous fasting period of 8 h were taken for determination of biochemical variables. Iron overload was considered in case of transferrin saturation >50% and/or ferritin >800 ng/mL.

Lipid profile, high blood pressure levels and body mass index (BMI) were considered as traditional CVRFs, whereas albumin <3.5 g/dL, hemoglobin <12 g/dL, calcium × phosphorus product >50, C-reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor (TNF)-α, hypocholesterolemia <100 mg/dL, malnutrition and hemoglobin <12 g as non-traditional CVRFs.

Laboratory measurements

Fasting blood samples were obtained between 7:00 and 10:00 h in the case of patients on PD and those transplanted. For patients on HD, blood was taken through the central catheter or puncture in the case of arteriovenous fistulae at the beginning of the dialysis session. The volume obtained in all cases was 10 mL, and at the time of blood sampling, children had no central or peripheral edema.

Lipid profile reference values were according to the National Kidney Foundation Disease Outcomes Quality Initiative [5].

Assays

Ferritin, TNF-α and high-sensitivity C-reactive protein (hsCRP) were measured by chemiluminescent assay, IL-6 by immunometric and transferrin saturation by the colorimetric method. We used the VITRO 250 analyzer for the determination of glucose, urea, creatinine, albumin, ferritin, calcium, phosphorus, total cholesterol, triglycerides and lipoproteins of high, low and very low density. Blood cytometry was determined with the SYSMEX XT-1800 device.

Echocardiographic measurements

In a second phase of the study, the echocardiographic study was performed by two blinded observers using a device model MyLab 30 (Genova, Italy) with linear transducers of 3.5 and 5 MHz, for imaging. Echocardiographic studies were performed in the parasternal long- and short-axis, apical 2-chamber and apical 4-chamber, according to the specifications of the American Society of Cardiology [19]. LVEF was obtained by the method of Simpson [20] and LVM according to the formula of Devereux [21]. The index of LVM was calculated dividing the LVM between the height in meters to diminish the effect of weight. Left ventricle hypertrophy was defined in the case of an LVM >38.6 g/m², the 95th value for normotensive healthy children [22]. Each study was recorded on video CD by two blinded observers using a device model MyLab 30 (Genova, Italy). Descriptive statistics was used to determine the distribution of each variable. The Mann–Whitney U-test or unpaired Student’s t-test were performed to evaluate differences between patients with and without iron overload for variables displaying non-normal or normal distribution, respectively. Chi-square test was used for categorical variables. Spearman correlation analysis was performed between iron parameters with each of the variables that showed differences between the groups. All data were analyzed using the NCSS 2004 software.

Ethical considerations

The protocol was approved by the local ethical committee in the University of Guanajuato and in the National Scientific Research Committee of the Instituto Mexicano del Seguro Social.

Results

We studied 143 children and adolescents (72 females) with a median age of 13 years (range 4–17). The etiology of renal failure was unknown in 106 (74%) patients, and the diagnosis was hypoplastic kidneys in 13 (9%), chronic glomerulonephritis in 10 (7%), family nephritis in 7 (5%) and obstructive uropathy in 7 (5%). CRF replacement program was 48 on PD, 53 on HD and 42 on post-transplantation. Iron overload was identified in 15 (10.5%) patients, none of the transplanted group, 4 (8.3%) on PD and 11 (21%) on HD (P = 0.002). These patients had more time in renal replacement program, number of transfusions and higher levels of BMI, IL-6, TNF-α, but lower total cholesterol, HDL cholesterol, hemoglobin and calcium than those without iron overload (Table 1).

The group on HD had more time of disease progression than those in PD (3 versus 1 year, P = 0.016) and had received more transfusions. Transplanted patients did not receive iron or erythropoietin at the time of the measurements. All patients on PD received iron by oral administration, while 89% of patients on HD received it per oral and 11% by intravenous administration. The median dose of iron was 2 mg/kg/day (range 0.6–5), and erythropoietin dose was 146 U/kg/week (range 0–429), and the time of administration for both was 10 months (0–120).

The group with iron overloads showed a greater proportion of malnutrition, hypertension, hemoglobin <12 g/dL, CRP ≥3 mg/L but lower HDL cholesterol levels and proportion of Ca × P product ≥50. Also, the total number of CVRFs was higher in this group [median 6 (3–9) versus 4 (0–8); P < 0.001] compared with patients without iron overload (Table 2).

Ferritin levels in the entire group showed a simple correlation with HDL cholesterol (r = −0.4; P < 0.001), hemoglobin (r = −0.5; P < 0.001), CRP (r = 0.3; P = 0.001) and IL-6 (r = 0.3; P = 0.001) levels.

By treatment modality, we observed a higher proportion of anemia in patients on PD (80 versus 76 and 10% for PD, HD and renal transplantation (RT), respectively; P < 0.001) and a lower proportion of total cholesterol >95th in patients on HD (17 versus 50 and 55% for HD, PD and RT, respectively; P < 0.001). The highest proportion of overweight (BMI > 90th) was observed in transplanted patients (30 versus 2 and 9% for RT, HD and PD, respectively; P = 0.001) and also this group had lower TNF-α levels (14.0 versus 26.0 and 26.3 ng/L for RT, HD and PD, respectively; P < 0.001) and the lowest proportion of IL-6 ≥ 3 ng/L (51 versus 79 and 67% for RT, HD and PD, respectively; P = 0.002).

All patients with iron overload and half of those without iron overload showed three or more CVRFs.

Clinical and demographic characteristics of the subgroup of patients who underwent echocardiography evaluation were similar for age, BMI, hypertension prevalence, lipid
profile, hemoglobin, CRP, IL-6 and TNF-α levels (data not shown), but those with iron overload had higher albumin levels (4.0 versus 3.5 g/dL; \(P = 0.03\)) than patients without iron overload. No significant differences were found in LVEF, LVM and LVM indexed to height in patients with and without iron overload (Table 3).

**Discussion**

We found that the frequency of iron overload (10.5%) was similar to that reported previously [13, 14] and no cases of iron overload were found in transplanted patients, probably due to the recovery in the ability to metabolize iron in the hematopoietic system [17]. In general, the repetitive administration of parenteral iron or blood in attempt to maintain an optimum response to erythropoietin therapy may increase the risk for higher ferritin levels in patients with HD. Iron depot promotes the development of atherosclerosis [9,10,11,12] and our study confirms the presence of more traditional and non-traditional CVRFs in pediatric patients with CRF and iron overload. It was also noted that the frequency of dyslipidemia associated with CRF in children and adolescents was similar to that previously reported in adults [23] and that it was more severe in patients with PD. Shurraw explains this phenomenon by the stimulation of protein synthesis secondary to liver glucose uptake from the dialysate fluid, hyperinsulinemia and selective loss of protein such as in the nephrotic syndrome [24]. Saland and Ginsberg [25] reported recently that the profile of dyslipidemia in children with CRF shows a moderate hypertriglyceridemia, reduced levels of HDL cholesterol and normal or slightly elevated low density lipoprotein (LDL) cholesterol levels. In our study, hypertriglyceridemia was mainly observed in the PD group, while lower levels of HDL and all fractions of cholesterol were observed in the HD group. Oi et al. [26] have explained the lower levels of LDL in HD patients because of having low activity of hepatic triglyceride lipase whose function is to promote the conversion of intermediate density lipoprotein to LDL. It has also been reported that iron dextran in animal models decreases the hepatic synthesis of cholesterol [27, 28], which may explain its levels in patients with iron overload. However, most studies have analyzed subjects with iron levels <800 ng/mL, but not other CVRF.

We found more traditional and non-traditional CVRFs in those patients on HD and iron overload including an increased inflammatory state evidenced by higher levels of IL-6 and TNF-α. It could be secondary to the frequent exhibition of the blood to filters for haemofiltration that stimulates the liberation of inflammatory mediators. This results in a decrease in the expression of the transferring receptors at the cell surface, causing a state of functional iron deficiency although iron overload exists [29, 30, 31]. Nanami et al. [27] demonstrated in cultured endothelial cells that TNF-α promotes the sequestration of intracellular iron suggesting this mechanism as the explanation of their involvement in the pathophysiology of atherosclerosis and cardiovascular disease.

### Table 1. General features in children and adolescents with renal chronic failure according to iron load

| Variables                        | With iron overload (\(n = 15\)) | Without iron overload (\(n = 128\)) | \(P\) |
|----------------------------------|---------------------------------|-------------------------------------|------|
| Age (years)                     | 14 (11–15)                      | 13 (12–15)                          | NS   |
| Age at diagnosis (years)        | 10 (9–11)                       | 10 (9–12)                           | NS   |
| PD (%)                          | 8                               | 92                                  | 0.002|
| HD (%)                          | 21                              | 79                                  |      |
| Renal transplantation (%)       | 0                               | 100                                 |      |
| Months of treatment             | 26 (12–52)                      | 13 (5–32)                           | 0.04 |
| BMI (kg/m²)                     | 17 ± 0.7                        | 19 ± 0.4                            | 0.01 |
| Iron doses (mg/kg/day)          | 2.1 ± 0.2                       | 1.5 ± 0.1                           | 0.003|
| Erythropoietin doses (U/kg/week)| 167 (106–189)                   | 143 (0–152)                         | 0.003|
| Transfusions, \(n\) (%)         | 2 (1–8)                         | 0 (0–2)                             | <0.001|
| Total cholesterol (mg/dL)       | 132 (124–191)                   | 165 (140–197)                       | 0.03 |
| HDL cholesterol (mg/dL)         | 38 (34–48)                      | 47 (41–64)                          | 0.03 |
| LDL cholesterol (mg/dL)         | 70 (55–89)                      | 79 (60–96)                          | NS   |
| Triglycerides (mg/dL)           | 155 (126–229)                   | 143 (112–220)                       | NS   |
| Ferritin (ng/mL)                | 1132 (596–1490)                 | 81 (44–200)                         | <0.001|
| Iron (mcg/dL)                   | 53 (40–103)                     | 57 (44–73)                          | NS   |
| Transferrin saturation (%)      | 25 (20–53)                      | 21 (15–27)                          | 0.003|
| Albumin (g/dL)                  | 3.7 ± 0.2                       | 3.6 ± 0.1                           | NS   |
| Hemoglobin (g/dL)               | 8.5 ± 0.7                       | 10.6 ± 0.3                          | 0.003|
| Ca × P product                  | 42 ± 15                         | 50 ± 15                             | 0.04 |
| IL-6 (ng/L)                     | 4.8 (3–14)                      | 3.6 (2.4–5.9)                       | 0.04 |
| TNF-α (ng/L)                    | 26 (22–33)                      | 22 (15–32)                          | NS   |
| CRP (mg/L)                      | 2 (0.6–5.2)                     | 0.6 (0.2–1.9)                       | 0.01 |

*aIron overload was defined as transferrin saturation >50% and/or ferritin >800 ng/mL. Data are show as mean ± standard error or as median (interquartile range) according to the variable distribution.*
In adult patients on HD, Rasic-Milutinovic et al. [32] demonstrated the association between iron levels, metabolic syndrome and inflammation. Also in post-menopausal women with type 2 diabetes, Qi Lu et al. [33] reported, high iron intake as risk factor for coronary disease. Consistent with our results, high levels of CRP were detected in a group of Brazilian nephropathic children on HD and PD [34], and those patients on HD showed a higher number of CVRFs associated with increased time on this therapeutic modality. Also, in this study, patients with iron overload and increased inflammation had lower BMI, probably related to the malnutrition-inflammation syndrome that has been described in adults [35].

A negative correlation between ferritin and hemoglobin levels was observed, which closes the cycle of anemia, high iron requirements, need for transfusions, iron overload and perpetuation of anemia. Cueto-Manzano et al. [36] reported an inverse correlation between CRP and albumin levels, but a direct correlation between TNF-α and IL-6 in a group of kidney transplanted Mexican subjects which are in accordance with our results.

In patients with cardiovascular disease, a correlation between ferritin and CRP levels has been reported as in our patients, but there was no difference between ferritin levels in groups with and without cardiovascular disease. However, the highest ferritin level in this study was 114.9 ng/mL [37] compared with >2000 ng/mL in our study. The only human model that can reach these levels is homozygous hemochromatosis. This pathology shows cardiovascular damage by myocardial iron depot and accelerating development of atherosclerosis [11]. In these patients, the atherogenic effect of iron has only been demonstrated in the presence of hyperlipidemia [38], which emphasizes the importance of preventing it.

Recently, haptoglobin participation in this process has been demonstrated. This protein is abundant in serum and its primary function is to link the hemoglobin to attenuate the inflammatory and oxidative potential of iron containing and also promotes the elimination of hemoglobin extracorpuscular through the CD163 receptor on macrophages. In animal models, the Hp 2-2 haptoglobin genotype plays a crucial role together with iron overload, lipid peroxidation and iron macrophages accumulation in the inflammatory response to produce bleeding inside the atherome plaque [39].

In our study, patients with iron overload had a higher frequency of at least three CVRFs with different combinations of traditional and non-traditional, depending on the modality of renal replacement. The most frequent traditional CVRF was hypertension which plays a major role in cardiac damage in chronic kidney disease via LVH induction [40]. Furthermore, it has been shown that fibroblasts respond to the presence of iron-loaded myocytes with increased proliferative capacity, even in patients without arterial hypertension [41]. Both mechanisms produce increased LVM, which reduces the coronary reserve and capillary density that occurs in patients with end-stage renal disease exposed to coronary ischemia [42]. Although iron has proven its utility in acute inflammation preventing oxidative stress, it has a deleterious effect in chronic inflammation [43]. Also, iron depot in the myocardium [44] may

| Variables | With iron overload (n = 15) | Without iron overload (n = 128) | P |
|-----------|----------------------------|-------------------------------|---|
| Hypercholesterolemia (≥95th for age) | 21% | 44% | <0.001 |
| Hypertriglyceridemia (≥95th for age) | 64% | 58% | NS |
| HDL ≤ 5th for age | 45% | 20% | <0.001 |
| LDL ≥ 95th for age | 23% | 11% | 0.04 |
| Systolic or diastolic blood hypertension (≥95th for height) | 79% | 48% | <0.001 |
| Overweight (BMI ≥ 90th for age) | 8% | 15% | NS |
| Malnutrition: BMI < 5th for age | 31% | 14% | 0.007 |
| Hypocholesterolemia (<5th for age) | 21% | 11% | NS |
| Albumin < 3.6 g/dL | 47% | 48% | NS |
| Hemoglobin <12 g/dL | 79% | 57% | 0.001 |
| Calcium × phosphorus product ≥ 50 | 20% | 40% | 0.003 |
| CRP ≥ 3 mg/L | 33% | 19% | 0.04 |
| IL-6 ≥ 3 ng/L | 73% | 66% | NS |
| TNF-α ng/L | 26 (22–33) | 22 (15–32) | NS |

| Variables | With iron overload (n = 12) | Without iron overload (n = 12) | P | Power |
|-----------|----------------------------|-------------------------------|---|-------|
| Ejection fraction (%) | 61 ± 4 | 65 ± 3 | NS | 17% |
| LVM (g) | 251 ± 38 | 213 ± 25 | NS | 21% |
| Ventricular mass index (g/m²) | 110 ± 45 | 89 ± 36 | NS | 38% |
favor the development of fibrosis and induces the formation of angiotensin II in the neointima [45], such as in the model of hemochromatosis [22].

We found that the LVM index in both groups was higher than the normal value in the adolescent and adult population [22]. A borderline correlation with ferritin levels was shown in the case–control study which may be related to the small sample size. All our patients had LVH, in contrast with only 70% in nephropathic Turkish children [23]. This could be related to the higher stage of disease at diagnosis and more time spent on dialysis awaiting a kidney transplant.

The importance of monitoring the cardiovascular state in nephropathic children [4] has been sufficiently demonstrated. In addition, Chesney et al. [46] published the list of priorities for clinical research in children with chronic renal disease and considered iron overload [13] referring to our previous report. Patients with iron overload should be considered for preventive measures and early treatment of cardiovascular disease. Moreover, the behavior of the lipid profile, markers of inflammation and myocardial structural alterations requires a further follow-up study of patients throughout their different dialytic stages because each one dominates different factors that add up to deleterious cardiovascular effects.

Conclusions

Patients with iron overload showed higher traditional and non-traditional CVRFs, especially if they were on HD. A borderline correlation between ferritin levels and LVM was found.

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Conflict of interest statement. None declared.

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