Copper-related blood indexes in kidney dialysis patients

Nancy J Emenaker, Robert A DiSilvestro, N Stanley Nahman Jr, and Susan Percival

ABSTRACT

Previous work has suggested that kidney hemodialysis patients could be at risk for either moderate copper deficiency or copper toxicity. The present study examined copper-related blood indexes in subjects undergoing hemodialysis treatments with membranes that are not copper-based, in subjects undergoing chronic ambulatory peritoneal dialysis (CAPD), and in control subjects. Both dialysis groups had low plasma copper and ceruloplasmin activities. This occurred despite high plasma interleukin 6 concentrations, a situation that usually elevates plasma ceruloplasmin and copper values. CAPD and hemodialysis subjects had low ratios of ceruloplasmin activity to immunoreactive protein, and low ratios of plasma copper to ceruloplasmin protein. Both are signs of copper deficiency. In contrast, copper-containing erythrocyte superoxide dismutase (SOD) activities were high in hemodialysis subjects and showed a nonsignificant trend toward high values in CAPD subjects. Blood mononuclear cell copper contents were highly variable within each group, and there were no significant differences between groups. In conclusion, ceruloplasmin-related indexes in kidney dialysis patients not dialyzed with copper-based membranes suggested a tendency toward moderate copper deficiency. However, this contention could not be confirmed by erythrocyte SOD activity or mononuclear cell copper measurements. Am J Clin Nutr 1996;64:757-60.

KEY WORDS
Copper, ceruloplasmin, dialysis, kidney, superoxide dismutase

INTRODUCTION

Several previous studies examined copper-related blood measurements in kidney dialysis patients (1-8). The results have been very conflicting. Some of these studies suggest that dialysis patients are moderately copper deficient. Signs of copper deficiency have been low values for plasma copper as well as for plasma concentrations of ceruloplasmin, a protein normally containing nearly all the copper in serum (9), and for activities of the erythrocyte copper enzyme superoxide dismutase (SOD). However, other studies report normal or even high values for these same indexes. In one of these studies (3), dialysis patients had high plasma copper but normal ceruloplasmin concentrations. This result could be indicative of potentially toxic amounts of nonceruloplasmin copper in plasma. Thus, depending on which study is cited, hemodialysis patients may be prone to copper deficiency or copper toxicity, or have normal copper status.

This diversity of results may be due to the different locales for the studies. The different subject groups may have had differences in mean dietary copper intakes, dialysis procedures, drug intakes, and the prevalence of other health problems. One especially big difference may be whether or not copper-based membranes were used for dialysis.

The present study reexamined the issue of copper-related blood indexes in dialysis patients. None of the subjects used copper-based membranes, which are no longer a popular choice in this country. Both hemodialysis and chronic ambulatory peritoneal dialysis (CAPD) patients were examined. This allowed distinctions to be made between effects of kidney disease, dialysis in general, and hemodialysis treatments. Unlike previous work, this study provided the first assessment of ceruloplasmin activities, plasma copper, and erythrocyte SOD activities in a single group of dialysis patients. Another new feature of the present study was evaluation of two copper-related indexes not previously measured in dialysis patients: the ratio of ceruloplasmin activity to protein and mononuclear cell copper contents. The former has been proposed to be an indicator of copper status even when ceruloplasmin synthesis is elevated by stress (10, 11). Mononuclear cell copper, although it has received limited testing as a copper status indicator, is proposed to be a very sensitive indicator of marginal copper deficiency (12).

SUBJECTS AND METHODS

Subjects

The human subjects protocol was approved by The Ohio State University Human Subjects Review Committee. Male and female hemodialysis subjects (n = 23; age range 28-77 y, mean age 50 y) and CAPD subjects (n = 26; age range 24-79 y, mean age 55 y) were recruited from the outpatient dialysis unit at The Ohio State University Hospitals. Only one of the females received estrogen therapy. None reported use of copper-containing mineral supplements, and none were anemic based on hematocrit values. Control subjects (n = 23) were free of any known major health problems, and were sex- and age-

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age-matched to the hemodialysis patients. The CAPD patients had virtually the same sex distribution as the hemodialysis patients, and their ages were all within 1–4 y of at least one of the hemodialysis patients studied. Hemodialysis patients were dialyzed on either cellulose acetate, saponified cellulose esters, or polysulfone-based membranes. Ten milliliters of blood were collected from each subject into low–trace element, EDTA-containing tubes (Venoject; Terumo Medical Corp, Elkton, MD). Blood was collected after an overnight fast just before dialysis treatment.

Assays

Ceruloplasmin activity was determined by oxidation of p-phenylenediamine (13). Units were defined as the change in absorbance at 540 nm/1.5 min. Ceruloplasmin protein was assessed by radial immunodiffusion using plates and standards from Behring Diagnostics (Sommerville, NJ). SOD activities were determined in ethanol:chloroform extracts of erythrocytes by a modified pyrogallol assay (14) with conditions described earlier (15). Activity was expressed as units per gram protein in the cell extracts. Cell protein was quantitated by the BioRad protein assay (BioRad Laboratories, Rockville Centre, NY). Plasma and mononuclear cell copper was measured by atomic absorbance spectrometry (flame for plasma, flameless for cells). Mononuclear cells were separated on Nycoprep (Nycomed Pharma, Oslo) according to the manufacturer’s instructions. Cell protein contents were determined by the BioRad assay. For ceruloplasmin protein and plasma copper values, normal ranges were those derived from Mayo Clinic data by Boosalis et al. (16).

Plasma interleukin 6 was measured by enzyme-linked immunoadsorbent assay with a kit from R&D Systems (Minneapolis). Blood urea nitrogen, creatinine, and albumin were assayed spectrophotometrically using plates from Sigma Chemical Co (St Louis). Normal ranges for these indexes were assumed to be those given in the Sigma kit instructions.

**TABLE 1**

Renal function indexes measured in dialysis and control patients

<table>
<thead>
<tr>
<th>Assay</th>
<th>Hemodialysis</th>
<th>CAPD (n = 26)</th>
<th>Control (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea nitrogen (mmol/L)</td>
<td>24.5 ± 4.8 [23]1</td>
<td>16.8 ± 4.5 [22]2</td>
<td>6.1 ± 2.5 [30]</td>
</tr>
<tr>
<td>Plasma creatinine (µmol/L)</td>
<td></td>
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</table>

1 ± SD; n in brackets. CAPD, continuous ambulatory peritoneal dialysis.
2 Significantly different from control, P < 0.05 (ANOVA + Tukey-Kramer honestly significant difference test).

**Statistical analysis**

Data were expressed as means ± SDs, and were compared by analysis of variance followed by Tukey-Kramer multiple-comparison test (honestly significant difference test) using the Statistical Analysis System (SAS, Cary, NC). Significance was set at P < 0.05.

**RESULTS**

As shown in Table 1, hemodialysis and CAPD patients had elevated plasma blood urea nitrogen and creatinine, and somewhat depressed albumin values, which are signs of renal disease. Means for control subjects were within the normal ranges (albumin, 565–739 µmol/L; creatinine, 71–124 µmol/L; and urea nitrogen, 2.5–6.4 µmol/L). Plasma copper and ceruloplasmin activity values were below control values for both hemodialysis and CAPD patients (Table 2). All three subject group means were within the normal range for copper (11.8–19.7 µmol/L), although the hemodialysis subjects’ mean was just barely inside the range. Male and female control subjects had significantly different plasma copper and ceruloplasmin activities (data not shown). The low values for ceruloplasmin activities and plasma copper for hemodialysis and CAPD patients, relative to this study’s control values, occurred despite high plasma interleukin 6 concentrations (Figure 1). Elevated interleukin 6 would generally be expected to produce high plasma copper and ceruloplasmin values (17). Ceruloplasmin protein concentrations were elevated in CAPD patients relative to this study’s control values and the normal range (200–600 mg/L).

The ratios of ceruloplasmin activity to immunoreactive protein and of plasma copper to ceruloplasmin immunoreactive protein were each low in both the hemodialysis and CAPD patients (Table 2). There were no differences in these ratios between males and females. For hemodialysis patients, the

**TABLE 2**

Plasma indicators of copper status in hemodialysis and CAPD patients

<table>
<thead>
<tr>
<th>Assay</th>
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<th>Control (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma copper (µmol/L)</td>
<td>11.9 ± 2.4 [20]2</td>
<td>15.2 ± 3.423</td>
<td>18.3 ± 5.0</td>
</tr>
<tr>
<td>Plasma ceruloplasmin activity (U/L)</td>
<td>79.13 ± 29.30 [23]2</td>
<td>100.81 ± 38.662</td>
<td>135.17 ± 62.51</td>
</tr>
<tr>
<td>Plasma ceruloplasmin concentration (mg/L)</td>
<td>453 ± 165 [23]2</td>
<td>660 ± 21823</td>
<td>370 ± 151</td>
</tr>
<tr>
<td>Plasma ceruloplasmin activity: concentration (U/g)</td>
<td>180 ± 40 [23]2</td>
<td>160 ± 502</td>
<td>370 ± 60</td>
</tr>
<tr>
<td>Plasma copper: ceruloplasmin concentration (µmol/g)</td>
<td>29 ± 8 [20]2</td>
<td>25 ± 72</td>
<td>53 ± 2</td>
</tr>
</tbody>
</table>

1 ± SD; n in brackets. CAPD, continuous ambulatory peritoneal dialysis.
2 Significantly different from control, P < 0.05 (ANOVA + Tukey-Kramer honestly significant difference test).
3 Significantly different from hemodialysis, P < 0.05 (ANOVA + Tukey-Kramer honestly significant difference test).
mean for the ratio of ceruloplasmin activity to immunoreactive protein, and the mean for the ratio of plasma copper to ceruloplasmin immunoreactive protein, were each about the same percentage of the control mean (49% compared with 54%). The same was also true for CAPD patients (43% compared with 47%).

Erythrocyte SOD activities were significantly higher in hemodialysis patients than in control subjects (Table 3). CAPD subjects had a nonsignificant trend toward high values. Mononuclear cell copper concentrations varied greatly within the groups (Table 3). As a result, no significant differences were found between groups. There were no sex differences for SOD or mononuclear cell copper values. Subdividing hemodialysis or CAPD patients by antacid use, diabetes mellitus, or established cardiovascular disease generally produced no significant differences for copper-related indexes from those found in the total groups (data not shown). Exceptions were slightly higher values for ceruloplasmin protein for cardiovascular disease and for diabetes, compared with the other hemodialysis or CAPD patients. The small differences did not carry over to ceruloplasmin activities or to activity-protein ratios.

**DISCUSSION**

The purpose of this study was to determine whether dialysis patients show signs of copper deficiency or toxicity. The best previous evidence for toxicity was the observation of high serum ratios of copper to ceruloplasmin in a group of hemodialysis patients (3). This was not found in the present study. In fact, the ratios were below normal. Possibly, the difference between studies was caused by the fact that none of the subjects in the present study used copper-based dialysis membranes.

In the hemodialysis and CAPD subjects, low ceruloplasmin activity and plasma copper values suggested moderate copper deficiency. If poor copper status was responsible for the low ceruloplasmin and plasma copper values, the deficiency would have to be strong enough to overcome a tendency to raise ceruloplasmin protein concentrations. In rats, copper deficiency has to be fairly severe to produce low ceruloplasmin activities when combined with a tendency to produce high ceruloplasmin protein concentrations (15, 18). Such a tendency occurs during many types of physiologic stress states, especially those associated with high secretion rates of inflammatory cytokines (9, 17). The hemodialysis and CAPD patients studied here generally showed high plasma contents of the inflammatory cytokine interleukin 6 (Figure 1), a result consistent with a previous study of hemodialysis patients (19).

The concept that poor copper status contributed to the low plasma copper and ceruloplasmin values was supported by the low ratios of ceruloplasmin activity to immunoreactive protein. Low ratios occur in rats and humans during severe copper deficiency (10, 20), and in humans with rheumatoid arthritis (11). Some evidence suggests that the low ratios in the arthritis patients resulted from a moderate copper deficiency (11). Low ratios have also been noted in Wilson disease (20), and in a group of humans given high-dose vitamin C supplements (21). The Wilson disease and vitamin C studies probably have no bearing on the present study. There is no evidence that any Wilson disease-like effects occur in dialysis patients. There was also no indication that a large number of the present study’s subjects were taking high doses of vitamin C. Thus, a possible explanation for low ratios of ceruloplasmin activity to protein in the dialysis patients is moderate copper deficiency.

Another possible reason for low ratios of ceruloplasmin activity to protein in the dialysis patients is inactivation of ceruloplasmin enzyme function by a copper-independent mechanism. However, this seems doubtful when comparing plasma copper and ceruloplasmin results. The ratio of ceruloplasmin activity to ceruloplasmin protein, for either CAPD or hemodialysis patients, is about the same percentage of control values as is the ratio of plasma copper to ceruloplasmin protein. Thus, the amount of copper associated with ceruloplasmin appeared to determine the amount of activity.

Although the ceruloplasmin and plasma copper data suggest that the hemodialysis and CAPD patients showed moderate copper deficiency, this conclusion was not supported by erythrocyte SOD results. Previous work has produced mixed results for SOD activities in dialysis patients (2, 5–8). The present

**TABLE 3**

<table>
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<th>CAPD</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td>Erythrocyte SOD with extracted protein (U/g)</td>
<td>1 918 800 ± 813 500 [23]</td>
<td>1 235 500 ± 515 200 [21]</td>
<td>991 300 ± 397 300 [30]</td>
</tr>
<tr>
<td>Mononuclear cell copper (μg Cu/g protein)</td>
<td>25.3 ± 10.0 [22]</td>
<td>30.8 ± 19.3 [24]</td>
<td>32.3 ± 22.3 [28]</td>
</tr>
</tbody>
</table>

1 SD ± SD; n in brackets. CAPD, continuous ambulatory peritoneal dialysis. SOD, superoxide dismutase.

2 Significantly different from control, P < 0.05 (ANOVA + Tukey-Kramer honestly significant-difference test).

3 Significantly different from hemodialysis, P < 0.05 (ANOVA + Tukey-Kramer honestly significant-difference test).

**FIGURE 1.** Plasma interleukin 6 concentrations in men and women on hemodialysis and chronic ambulatory peritoneal dialysis (CAPD) patients. *p < 0.05 (ANOVA and Tukey-Kramer honestly significant difference test).
study found high erythrocyte SOD activities in the hemodialysis patients and a nonsignificant trend toward high values in the CAPD patients. This does not necessarily rule out poor copper status. Possibly, dialysis patients have high erythrocyte SOD protein concentrations. Conceivably, such high concentrations could produce above-normal SOD activities even in the presence of moderate copper deficiency.

In summary, values for some copper-related indexes in dialysis patients differed from values in control subjects. The relation between these findings and copper requirements in dialysis patients requires further clarification.

REFERENCES