INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Clinicopathological Analysis of Hematological Disorders in Tube-Fed Patients with Copper Deficiency

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Abstract

Object Anemia and leukopenia caused by copper deficiency are well-documented consequences of long-term total parenteral nutrition. We measured the serum copper levels of bed-ridden patients receiving enteral feeding, and evaluated optical and ultrastructural features of bone marrow before and after copper supplementation.

Patients and Methods Serum samples were obtained from 15 bed-ridden elderly patients receiving tube feeding (TF) and 10 age-matched bed-ridden patients who took food orally (CO), and the copper ceruloplasmin concentration of each sample was measured. Bone marrow samples were obtained from patients who exhibited copper deficiency and leukopenia and/or anemia before and after the copper supplementation, for use in light and electron microscopic analysis.

Results The tube-fed patients had significantly lower mean serum copper and ceruloplasmin concentrations than the control patients. Seven of the 15 tube-fed patients had reduced serum copper concentrations and leukopenia. Six of those 7 patients also had anemia. Copper sulfate was administered to those 7 patients by enteral tube;their copper concentration, anemia and leukopenia improved within 1 month after they were administered copper sulfate. In the bone marrow examination before copper supplementation, light microscopy showed cytoplasmic vacuolization in both myeloid and erythroid precursors, and electron microscopy showed electron-dense deposits in mitochondria and cytoplasm of erythroid and myeloid cells. After copper supplementation, these pathological changes disappeared.

Conclusions Bicytopenia is likely to occur in tube-fed patients with copper deficiency. Copper deficiency appears to be associated with cytoplasmic vacuolization and electron-dense deposits in mitochondria in erythroid and myeloid cells.

Key words: copper deficiency, electron microscopy, tube feeding

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Introduction

Enteral feeding through a naso-gastric tube or gastric fistula has proved to be a safe and effective way of improving the protein-energy nutritional status of patients with brain damage due to cerebral vascular disease (1, 2). Such patients sometimes have trace element deficiencies, and the clinical sequelae of these deficiencies may not be very obvious (3, 4). Some such patients have anemia, often caused by iron deficiency due to gastrointestinal bleeding, but severe copper deficiency usually presents with anemia and or leucopenia (5-7). There have been reports of ringed sideroblasts and vacuolation of erythroid and myeloid precursors in patients with severe copper deficiency (6, 8-15). Although the mechanism of anemia caused by copper deficiency remains unclear, reports indicate that copper deficiency is associated with impaired mitochondrial iron me-

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Table 1 **Copper and Ceruloplasmin Levels in Patients with Tube Feeding and Control Patients**

	Tube feeding (n=15)	Control (n=10)
Leukocyte (4000-9000 /mm^3)	4600+/-2700	5300+/-1500
Hb (12-15 g/dl)	9.3+/-2.2	10.9+/-1.7
Fe (40-162 μg/dl)	45+/-18	46+/-20
UIBC (159-307 μg/dl)	216+/-41	223+/-47
Copper (85-155 μg/dl)	37+/-32 *	107+/-15
Ceruloplasmin (10-40 mg/dl)	11+/-11 *	32+/-4

Values are mean +/-SD. *:$p<0.01$, compared with the control patients.

tabolism (16-18).

The aim of the present study was to measure serum copper levels in bed-ridden patients receiving enteral feeding, and to evaluate optical and ultrastructural features of bone marrow before and after copper supplementation.

Materials and Methods

Patients and sample analysis

The tube-fed subjects (TF group) comprised 15 bed-ridden elderly patients receiving tube feeding at 1 of 5 different hospitals in Nagasaki prefecture (3 men and 12 women; age range, 68 to 93 years). The TF group consisted of 8 patients with cerebral infarction, 5 patients with cerebral hemorrhage, 1 patient with Parkinson's disease, and 1 patient with Alzheimer's disease. The mean duration of tube feeding was 31.3 months (10 to 64 months). The control subjects (CO group) comprised 10 age-matched bed-ridden patients who took food orally (1 man and 9 women; age range, 60 to 93 years). After obtaining informed consent from the subjects or their families, serum samples were collected and preserved at -70℃ until used for analysis. Copper concentration was measured using an atomic absorption spectrophotometer (model Z-8000, Hitachi, Tokyo), and ceruloplasmin concentration was measured using the immunodiffusion method.

Copper supplementation

After obtaining informed consent from the subjects or their families, those subjects who exhibited copper deficiency and leukopenia or anemia were administered copper supplementation:$CuSO_4$ (1000 μg/day) via a tube for 28 days. Serum samples were obtained from the patients within one week after the treatment was completed. Bone marrow samples were collected from some patients before and after the copper supplementation, for use in light and electron microscopic analyses.

Statistical analysis

All data were expressed as mean ± SD. Differences between groups were examined for statistical significance using Student's t-test. A probability value of $p < 0.05$ was considered to indicate statistical significance. Correlation between two defined parameters was determined using Pearson's correlation coefficient test.

Results

Copper level and hematological findings

Table 1 shows the hematological findings and the levels of serum copper and ceruloplasmin in each group. The mean serum copper level of the TF group was significantly lower than that of the CO group (37 +/- 32 μg/dl, 107 +/- 15 μg/dl, respectively; $p < 0.01$). Twelve of the 15 patients in the TF group showed reduced serum copper concentrations (< 70 μg/dl). Serum ceruloplasmin concentrations were significantly lower in the TF group than in the CO group (11 +/- 11 mg/dl, 32 +/- 4 mg/dl, respectively; $p < 0.01$) The WBC count and hemoglobin titer were lower in the TF group than in the CO group, but these differences were not significant. No significant difference was observed in the serum iron level or UBC between TF and CO.

Next, we examined whether or not the copper concentration correlated with hemoglobin titer or WBC count. A significant correlation was observed between copper concentration and hemoglobin titer (Fig. 1A; $r=0.54$, $p < 0.01$) or

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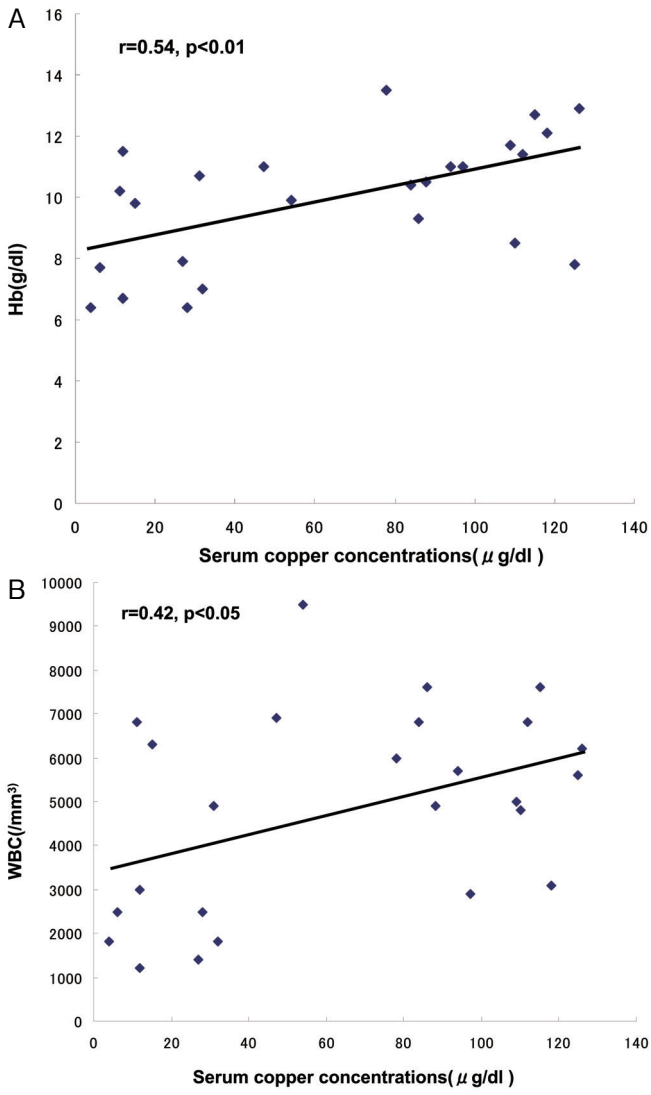

Figure 1. **Correlation between serum copper concentration and hemoglobin titer or WBC count. Correlation was assessed using Pearson's correlation coefficient test. Significant correlation was observed between copper concentration and hemoglobin titer (1A; r=0.54, p<0.01) or WBC count (1B; r=0.42, p<0.05) in all subjects.**

WBC count (Fig. 1B; r=0.42, p < 0.05) in all subjects.

Copper supplementation

Seven of the TF patients had a low copper concentration (< 70 μg/dl) and leukopenia (< 3000/mm^3) accompanied neutropenia (< 1000/mm^3) . Six of those 7 patients also had moderate or severe anemia (hemoglobin < 8.0 g/dl). After informed consent was obtained, those 7 patients were administered copper supplementation via a tube for 28 days.

Copper supplementation therapy resulted in rapid normalization of serum copper and ceruloplasmin concentrations, WBC count and hemoglobin titer (Table 2).

Light microscopic findings of bone marrow

After informed consent was obtained, bone marrow specimens were taken from 3 of the 7 patients who received copper supplementation therapy. Two of the 3 patients were hypocellular, and 1 patient was hypercellular. All 3 patients exhibited vacuolization of erythroid and myeloid cells, and maturation arrest of myeloid cells (Fig. 2A). After copper supplementation, maturation arrest and the vacuolization of erythroid and myeloid cells disappeared (Fig. 2B).

Electron microscopic findings of bone marrow

Before copper supplementation, electron microscopy revealed electron-dense deposits in the mitochondria and cytoplasm of some erythroid and myeloid cells (Fig. 3A). Some myeloid cells had low organelle numbers (Fig. 3B) and nuclear wall hyperchromatosis (Fig. 3C). After copper supplementation, these electron microscopic changes disappeared, and mature neutrophils without deposits were visible (Fig. 3D).

Discussion

Copper deficiency has been observed in adult patients with inflammatory bowel disease (19, 20), total parenteral nutrition (21, 22), massive zinc ingestion (11), and enteral feeding (5-7). The most common laboratory features of hu-

Table 2 **Effects of Copper Supplement Therapy on Hematological Indexes**

	Before therapy	After therapy
Hb (12-15 g/dl)	7.7+/-1.8	11.9+/-1.7 *
Leukocyte (4000-9000 /mm^3)	2000+/-700	5800+/-1200*
Copper (85-155 μg/dl)	17+/-11	104+/-12*
Ceruloplasmin (10-40 mg/dl)	4+/-2	30+/-4*

Values are mean +/-SD. *:p<0.01, compared with the before therapy

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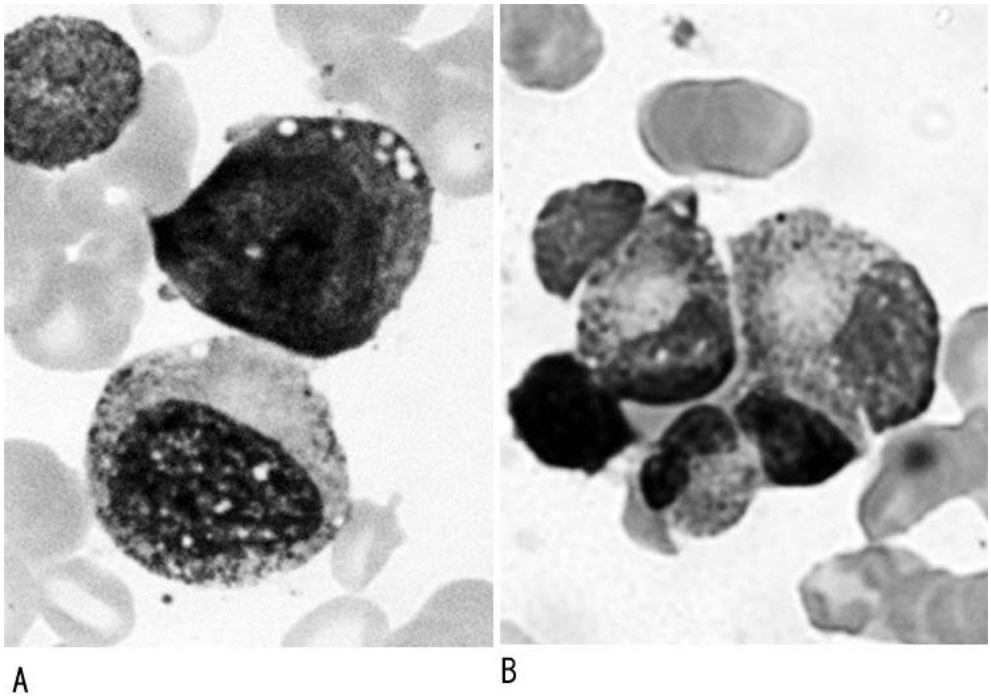

Figure 2. **Microscopic findings of bone marrow aspirate before (2A Wright's stain; magnification, ×1000) and after copper supplementation (2B Wright's stain; magnification, ×1000): Striking cytoplasmic vacuolation is visible in both the myeloid and erythroid precursors (2A). After copper supplementation, the vacuolization of erythroid and myeloid cells disappeared (2B).**

man copper deficiency are anemia and leucopenia; thrombocytopenia is rarely seen. In the present study, we observed copper deficiency in 12 out of 15 tube-fed patients; 6 of those 12 patients exhibited both anemia and leucopenia. All tube-fed patients were fed a commercial enteral diet containing a low amount of copper (10-22 μg/100 kcal) for a long time (mean duration, 31.3 months). All of these cases of anemia and leucopenia improved after copper supplement therapy, suggesting that the anemia and leucopenia were caused by copper deficiency.

The morphology of bone marrow in cases of copper deficiency is mainly characterized by cytoplasmic vacuolation in erythroid and myeloid precursors. There have also been reports of megaloblastic changes, ringed sideroblasts and hemosiderin deposition in plasma cells in cases of copper deficiency (6, 8-15). Cytoplasmic vacuoles have also been found in patients with acute alcoholic intoxication (23), chloramphenicol toxicity (24), pancreatic dysfunction (25) and myeloproliferative syndromes (26). Dunlap et al (8) suggested that cytoplasmic vacuolization in early erythroid precursors is the first erythroid developmental change that occurs in cases of copper deficiency, because many patients with copper deficiency but without anemia exhibit this change. Most of the reported cases of copper deficiency (6, 8-15), and all of the present copper-deficient subjects, exhibited cytoplasmic vacuolization of erythroid and myeloid precursors, which rapidly disappeared after copper supplementation. Although cytoplasmic vacuolization is also associated with other disorders, it may be particularly strongly associated with copper deficiency. In our search of the Pub Med database, we found no reports of electron microscopic examination of copper deficiency in humans. Dallman and Goodman (16) observed that in copper-deficient rats, the mitochondria increased in size, and their findings also suggest an increase in the number of erythroblasts. Electron microscopy of bone marrow erythroblasts from the present copper-deficient patients before copper supplementation showed electron-dense deposits in the mitochondria and cytoplasm of erythroid and myeloid cells, and the mitochondria were not enlarged.

The mechanism of leucopenia and anemia induced by copper deficiency is not clearly understood. There is speculation that the leucopenia observed in cases of copper deficiency is caused by a decrease in neutropenic lifespan with maturation arrest of myeloid cells (27) and antineutrophil antibody (28). It has been proposed that the anemia induced by copper deficiency is caused by a decrease in the activity of copper-dependent enzymes. The enzyme ceruloplasmin ferroxidase catalyzes oxidation of ferrous ions to ferric ions, thereby facilitating the transfer of iron from reticuloendothelial cells to plasma (29). In the present study, serum ceruloplasmin and copper concentrations were significantly lower in the tube-fed patients than in the control patients. Copper/zinc superoxidase activity is also decreased in cases of copper deficiency, and this may accelerate development of cell membrane defects and shorten the survival time of erythrocytes (12). Cytochrome-c oxidase is a copper-dependent enzyme that is necessary for mitochondrial iron utilization and heme synthesis (30). Mitochondria isolated from copper-deficient animals were deficient in cytochrome

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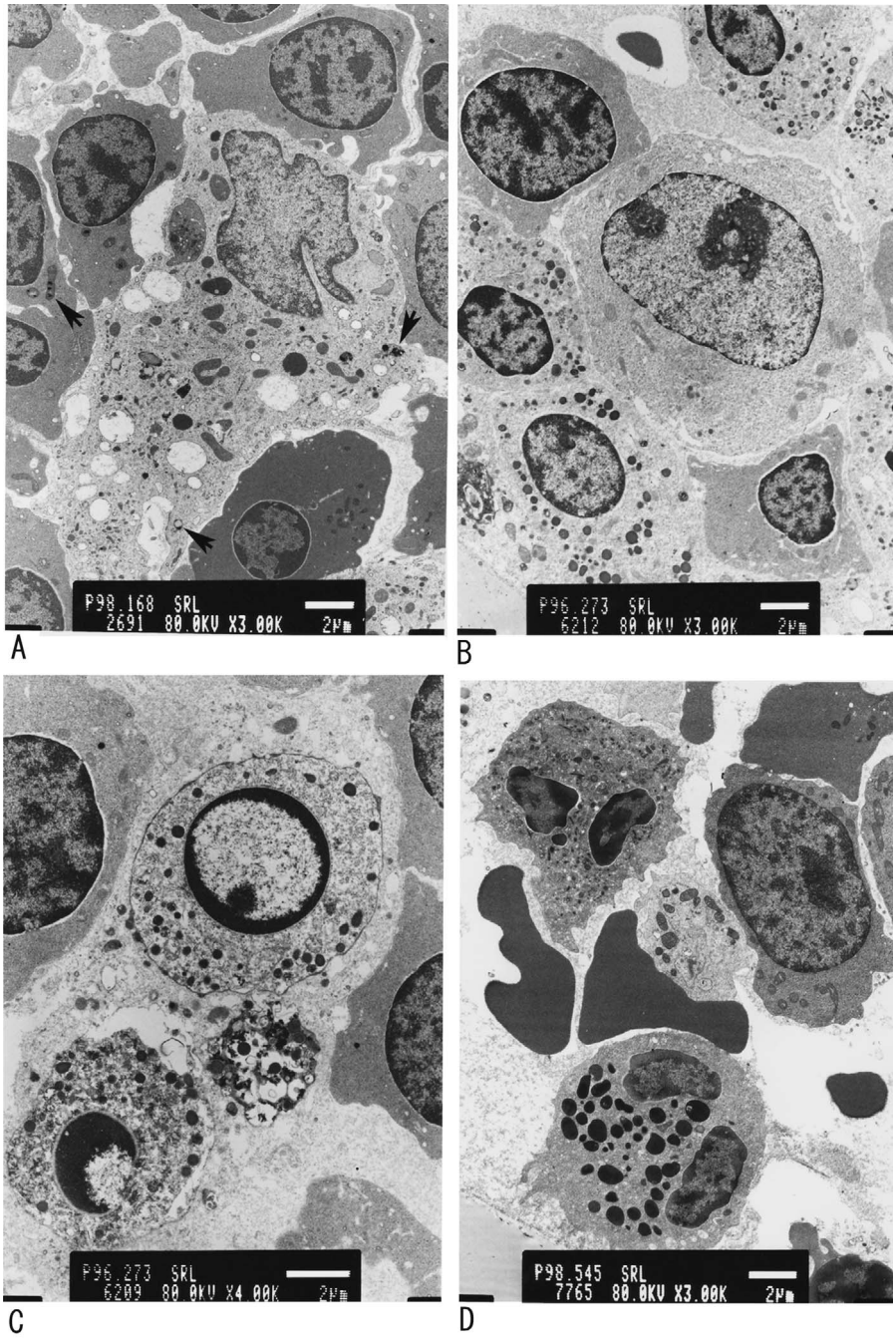

Figure 3. **Electron microscopic findings of bone marrow aspirate before (A-C) and after copper supplementation (D). Electron-dense deposits (arrow) are visible in mitochondria and cytoplasm of erythroid and myeloid cells (3A ×3000). Low organelle numbers (3B ×3000) and nuclear wall hyperchromatosis (3C ×4000) are visible in the myeloid cells. Mature neutrophils without dense deposits are visible (3D ×3000).**

oxidase activity, and failed to synthesize heme from ferric iron and protoporphyrin at the normal rate, perhaps leading to mitochondrial iron accumulation (18). That suggests that the amorphous electron-dense deposits observed in the present electron microscopic images of mitochondria and disappeared after the copper supplementation may consist of iron.

In Japan, many patients with swallowing disturbances are dependent on commercial enteral diets, some of which contain very low amounts of copper. In addition, there are no licensed oral mineral additives in Japan. The results of the present study and previous studies are of limited value due to the small number of patients. However, it appears that there are many patients with latent copper deficiency who are overlooked due to their inability in communicate their symptoms. It is important to carefully monitor copper levels in patients on a long-term enteral diet, and artificial enteral diets containing an adequate amount of copper should be used to feed such patients.

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