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Original article

Relationship between copper doses in parenteral nutrition mixtures, serum copper, erythrocyte copper levels, ceruloplasmin and C-reactive protein, in critically ill patients

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SUMMARY

Background and aims: There is no consensus on the recommended amounts of copper to be administered to critically ill patients on parenteral nutrition, as requirements are variable and very difficult to determine in different disease states. The objective of this study was to assess copper status of critically ill patients on total parenteral nutrition in order to prevent inadequate copper administration.
Methods: The study comprised adult patients (20 males and 10 females) requiring total parenteral nutrition for 4–21 days, because of pancreatitis ($n = 5$) or after major abdominal surgery ($n = 25$). Parenteral nutrition was discontinued when the patient tolerated enteral or oral feeding. The following parameters were determined throughout the study (4–21 days): total copper administered by parenteral nutrition, serum copper, erythrocyte copper (Atomic Absorption Spectrometry); serum ceruloplasmin (Ferroxidase activity) and C-reactive protein levels (Immunoturbidimetry, Latex HS).
Results: Total copper administered in parenteral nutrition ranged between 0.03 and 3.8 mg/d, and was higher than prescribed amounts due to copper contamination of individual components. The amount of copper given in parenteral nutrition correlated with changes in erythrocyte copper, but not with changes in serum copper, ceruloplasmin, or C-reactive protein.
Conclusions: Variations in erythrocyte copper levels showed significant correlation with the amount of copper administered daily in parenteral nutrition mixtures, and this biochemical indicator could be useful to monitor copper deficiency or excess in patients on parenteral nutrition. It is noteworthy that copper delivery above 1.2 mg/d was frequent and prompts such monitoring.

1. Introduction

Copper (Cu) is an essential mineral micronutrient which has different biological functions. It is a component of many metalloenzymes, including oxidases, hydroxylases, and superoxide dismutase, and it is also closely linked to iron utilization. Besides, copper is part of the structure of Ceruloplasmin (Cp), and is essential for its function. Cu deficiency is therefore characterized by anemia, pancytopenia, poor wound healing, osteoporosis, aching joints, petechial hemorrhage, achromatism, and kinky hair.[1] It was first described in an infant on long-term copper free total Parenteral Nutrition (TPN), which resulted in skeletal osteoporosis and delayed bone growth.[2] The clinical effects of severe Cu deficiency, which include anemia, neutropenia, thrombocytopenia, heart failure, and even death, have been shown in patients after 19 days on TPN without added Cu.[3,4] It has been reported that Cu derangement

Non standard abbreviations: SCu, serum Cu; ECu, erythrocyte Cu.

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in humans is more relevant during injury or infection when TPN mixtures are not supplemented with Cu. It is largely excreted in the bile, and excessive losses through biliary drainage or high output stomas may quickly lead to Cu deficiency.[5]

Conversely, biliary obstruction together with long term high copper intake can result in high levels of Cu, leading to hepatic necrosis, renal failure, coma, and death. There is evidence regarding the adverse effects of excessive amounts of Cu, which exacerbate inflammatory acute-phase response, and include abdominal cramping pain, nausea, vomiting, diarrhea and liver damage.[6] Therefore, patients with cholestasis on TPN should receive a lower amount of Cu to avoid the possible adverse effects of excessive intake.[7,8]

Hence the importance of preventing both Cu deficiency and excess in critically-ill patients on TPN, and of finding a reliable biochemical indicator to assess the amount of Cu required in different disease states and by each individual patient throughout treatment, in order to maintain adequate copper nutritional status.[9,10]

Nevertheless, it is difficult to establish Cu requirements in humans. Some biological markers can be used to estimate the risk of Cu deficiency or excess, and although a great number of Cu functions are known, there is no ideal indicator of copper nutritional status. The selection of risk indicators to be used will depend on both the specific objectives of the assessment and the available resources. Significant advances in the methods for measuring Cu concentrations in body fluids and tissues have been made. Although serum Cu concentration is the most widely used laboratory indicator of Cu status, serum Ceruloplasmin (CP) has been proposed as an indicator to assess Cu nutritional status.[7–9]

In addition, TPN component solutions are often contaminated with varying concentrations of different trace elements, including copper, depending on the primary components, manufacturer, batch/lot, manufacturing date, packaging, and so forth. Such a contamination could result in the patients receiving a larger amount of Cu than that prescribed.[11,12] Therefore, when TPN is the patient's only source of nutrition, it is crucial to supply the adequate amount of Cu to meet the patient's requirements, and thus avoid deficiency and excess. There is no consensus regarding the recommended amounts of Cu to be administered in TPN mixtures to critically ill patients, because patient requirements vary and are very difficult to determine in different disease states.[4,13]

Thus, the objective of this study was to assess Cu status of critically ill patients on TPN in order to prevent inadequate Cu administration. The biochemical changes in serum Cu (SCu), erythrocyte Cu (ECu), C-reactive protein (C-RP), and serum Ceruloplasmin (Cp) levels and their relationship with total copper content of TPN mixtures were studied.

2. Material and methods

A prospective observational trial was conducted in keeping with the ESPEN guidelines on Parenteral Nutrition for patients with a non-functional gut.[15] The study was approved by the Ethics Committees of the Institutions. Patients or their relatives gave their informed consent to participate in the study.

2.1. Patients

The study comprised 30 critically-ill adult patients (20 males and 10 females), requiring TPN because of pancreatitis ($n = 5$) or after major abdominal surgery ($n = 25$). Cu was determined in TPN mixtures and in patient blood samples upon initiation and discontinuation of TPN. Tolerance to oral feeding was monitored and recorded by the attending physician, and TPN was discontinued when the patient could tolerate enteral or oral feeding (4–21 days).

The characteristics of the studied population are shown in Table 1.

2.2. Total parenteral nutrition mixtures

Total parenteral nutrition mixtures (TPN) were prescribed by the attending physician and prepared by pharmacists at the Nutrition Assistance Unit (*Unidad de* Asistencia Nutricional-UNANUT), Buenos Aires, Argentina, following Good Manufacturing Practices (GMP, WHO, 1992/2002 & ANMAT [National Administration for Drugs, Food and Medical Technology] regulations 2592/2003).[16,17] The bag mixture typically contained 70% dextrose: 250–350 g; 10% amino acids for adults: 60–100 g; 20% lipids (MCT/LCT): 50–80 g; potassium (chloride): 2340–3510 mg (60–90 mM); sodium (chloride): 1411–2117 mg (61–92 mM); magnesium (sulfate): 85–146 mg (3.5–6 mM); phosphorus (sodium phosphate): 576–636 mg (18.5–20.5 mM); calcium (Gluconate): 92–184 mg (2.3–4.6 mM). Trace elements were added to the mixture separately according to prescription, as follows: copper (sulfate): 0–1.5 mg; zinc (sulfate): 5–12 mg; manganese (sulfate): 80–100 μg; chromium (chloride): 4–10 μg; selenium (selenious acid): 40–60 μg; molybdenum (ammonium molybdate): 50–60 μg. Bags for patients with hepatic alterations were prepared without added copper, as prescribed by the physician. In addition, the amount of some components and the administered volume were adjusted by the physician according to the changes in the patient's laboratory/clinical status.

The TPN bag-mixtures were prepared in a sterile area under a laminar flow hood using an Automated Nutrition Compounder System, and a sample was obtained using a sterile syringe without a needle before closing the bags, for copper content determination. The number of bags per patient varied according to length of TPN (4–21 days depending on the clinical course of the patient). Cu content of the mixture was determined in a maximum of 3 TPN bags per patient, and results were averaged to calculate mean Cu content of the mixture administered to each patient.

2.3. Laboratory determinations

Patient blood samples were obtained for determination of hemoglobin (Hb) (Cyanmethemoglobin method), serum Cu (SCu) and

Table 1
Characteristics of studied individuals.

Disorder	Intestinal fistula	Acute pancreatitis	Intestinal obstruction	Cancer of the pancreas	Cancer of the colon	Cholestasis
No. of patients	4	6	8	7	4	1
Sex (M/F)	2/2	4/2	4/4	5/2	4/0	1
Mean values ± SD						
Age (years)	67 ± 8	61 ± 10	67 ± 16	69 ± 5	65 ± 6	66
BMI Kg/m^2	28.5 ± 1.5	26.8 ± 2.0	25.9 ± 3.8	25.2 ± 4.5	23.8 ± 4.6	25.4

Cu in erythrocytes (previously washed with isotonic solution, and hemolyzed with ultrapure water). Cu levels were also determined in TPN mixtures, both at the beginning and at the end of the study, after mineralization using a microwave laboratory system.[18] Samples were run in triplicate simultaneously with a reference material in order to validate each batch. Agreement between the means of triplicate determinations and the reference material within 5% of the certified value and a relative standard deviation below 5% for the triplicates was required for data acceptance. Cu levels in TPN mixtures, serum, and erythrocytes were determined by Atomic Absorption Spectrometry (AAS) with air–acetylene flame, using a Varian Spectrophotometer SpectrAA-20.[19] Laboratory materials were previously washed with nitric acid (20%) and deionized water. C-RP was measured using an immunoturbidimetric method (C-RP Latex HS). Ceruloplasmin activity was measured in a Hitachi 917 autoanalyzer, following the method described by Erel.[20] This colorimetric method is based on the enzymatic oxidation of ferrous ion to ferric ion. Results are expressed in international units (I/U). All CP activity determinations were performed within the same assay; within-run precision (CV) was 0.7%.[21]

Results are expressed as mean ± standard deviation (mean ± SD). Differences were tested for significance using one-way analysis of variance (ANOVA) (INSTAT). When ANOVA showed statistical differences, intergroup comparisons were performed using the Student–Newman–Keuls multiple comparisons test (95% significance level).

3. Results

3.1. Clinical outcome

The attending physician monitored tolerance to oral/enteral feeding to discontinue TPN (4–21 days). Tolerance to oral/enteral feeding was good in 28 patients. Two patients died as a consequence of their critical disease status.

3.2. Cu status assessment

Mean ± SD copper level of the mixtures was 0.94 ± 0.66 μg/ml and Cu concentration in TPN mixtures ranged from 0.03 to 1.9 μg/mL. The total amount of Cu administered to each patient was calculated and compared with the prescribed amounts, which ranged from 0 to 1.5 mg/d. The results showed that the amount of Cu given to the patients studied herein was higher than that prescribed by the physician (Table 2).

The values of the studied biochemical markers of Cu status obtained upon initiation and discontinuation of TPN are shown in Table 3. The results showed that SCu, ECu and CP levels varied greatly among patients. Moreover, and as expected in acute critically ill patients, high levels of C-RP, the acute phase response protein, were observed.

SCu levels were within the normal range[23] in 45% and above normal range in 55% of patients at the beginning of treatment. None of the patients showed values below normal range at either studied time point.

Table 2
Comparison between Cu content determined in TPN mixtures and values prescribed by physicians (Mean value ± SD and ranges, in brackets).

Determined Cu (mg/L)	TPN volume	Total Cu administered (mg/d)	Total Cu prescribed (mg/d)	Administered/ prescribed as a Cu percentage
0.94 ± 0.66 (0.03–1.9)	1968 ± 152 (1500–2200)	1.7 ± 0.9 (0.06–3.8)	0.8 ± 0.4 (0–1.5)	272 ± 192 (15–750)

Table 3
Biochemical markers in studied patients.

Mean values ± standard deviation and ranges (in brackets)				
$n = 30$	Serum Cu μM/L[a]	Erythrocyte Cu μM/L[a]	CP (IU/L)	CRP (mg/L)
Beginning of treatment	20.3 ± 6.9 (9.8–37.5)	11.8 ± 5.2 (3.5–28.8)	603 ± 340 (193–1863)	82 ± 77 (2–312)
End of treatment	19.4 ± 6.6 (9.3–36.7)	11.0 ± 4.5 (2.7–21.1)	699 ± 405 (171–1862)	76 ± 75 (0.3–333)
Reference values	7.08–19.52	5.7–12.3	424–796	<5

[a] μg/dL = μM/Lx6.353.

ECu levels at the beginning of treatment were within normal ranges in 35% of patients, above normal range in 54%, and below normal range in 11%. ECu levels at the end of treatment were within the normal range in 32% of patients, above normal range in 42%, and below normal range in 26% of patients. As shown by the results, the percentage of patients with above normal range ECu levels decreased, whereas the percentage of patients with below normal range ECu levels increased at discontinuation of TPN.

CP values were above normal reference values (800 IU/L)[22] in 10% of patients and below normal (400 IU/L) in 29% of patients at initiation of TPN. At the end of treatment, CP levels were above normal in 26% of patients and below normal in 19% of subjects.

CRP levels were higher than normal in 97% of patients. Only one patient had normal levels (<5 mg/L) at the beginning of treatment, and only one patient had normal levels at the end of treatment.

CP did not correlate with CRP, despite the fact that they are both acute phase proteins, because the kinetics of CP and CRP differ enormously.

CP levels correlated with SCu levels at the beginning and at the end of treatment but did not correlate with ECu levels.

Fig. 1 shows changes in ECu levels as a function of total amount of copper administered in TPN mixture to each patient. A positive correlation between the amount of Cu in the TPN mixture and the changes in ECu levels (0.44; $p = 0.016$) was observed. Based on the changes in ECu, expressed as a percentage of the level observed at the beginning of treatment, the cut off point for Cu content in TPN mixtures was found to be 0.9 mg/d. As shown in Fig. 1, any amount higher than 1.2 mg/d caused a significant increase in erythrocyte Cu levels.

4. Discussion

The main finding of our study is that the patients on TPN received a significantly larger amount of copper than that prescribed, due to contamination of the TPN solutions. The higher Cu

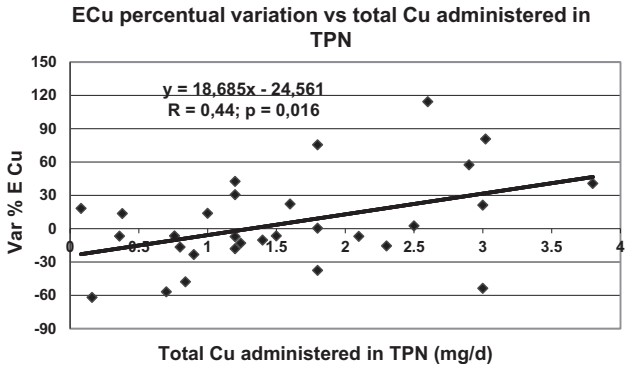

Fig. 1. ECu percentual variation vs total Cu administered in TPN.

values found in TPN mixtures are consistent with previous reports showing that individual components used to prepare TPN may be contaminated with several trace elements, including Cu.[11,12] This contamination is related to the type of primary components, manufacturer, batch/lot, manufacturing date, packaging, and so forth, and can result in the patient receiving a larger amount of copper than that prescribed by the physician.[12,23]

Hence, the present study sought to evaluate changes in SCu, ECu, CRP and CP levels in this group of critically ill patients on TPN.[6,10,9]

The dose of copper prescribed by the physician (Table 4) was in keeping with copper recommendations for adult patients on TPN of 0.3–0.5 mg/day, as established by international scientific associations (Table 4).[24–27] It must be pointed out, however, that the recommended amounts are approximate, and will vary according to the requirement of each patient. Up to 1.3 mg/day of Cu should be administered only to patients with excessive gastrointestinal losses and major burns.[28]

Determination of Cu content in the TPN mixtures showed that the amount of Cu given to each patient ranged from 0.06 to 3.8 mg/day. These values are 260 ± 191% (mean ± SD) higher than the amounts prescribed by the physician. Two patients had liver failure and were therefore not prescribed Cu supplementation. Patients who stopped receiving Cu during treatment maintained normal SCu and ECu values and high Cp and CRP values. It is of note, however, that because copper is an essential trace element, decreasing the amount of copper may be advisable, but discontinuance over long periods of time is not recommended, in order to avoid deficiency and its health consequences.

It is most likely that Cu contamination of TPN mixtures resulted in the patients receiving an increased amount of Cu, with the ensuing increased risk of adverse effects.

Similar levels of contamination in the bags were observed in a previous study.[12] Therefore, we determined Cu levels in 59 individual solutions/emulsions of 14 commercial products available in Argentina (both locally made and imported) and used as components in the bag mixtures. Copper in varying amounts was found as a contaminant in the same components from different manufacturers, different lots/batches, and with different expiration dates, as follows: (range, μg/ml) in: dextrose (0–4.8), amino acids (0–0.58), Calcium gluconate (0–0.71), zinc sulfate solutions (0–0.3)-and lipid emulsions (0.18–1.16). Dextrose and lipid emulsion presented the highest amount of copper. Therefore, most of the total parenteral mixtures prepared with the analyzed solutions had an excess of copper regarding the prescription. In fact, mean copper content of the mixtures was 75% higher than that prescribed, and 3 fold the prescribed amount in several cases. It must be kept in mind, however, that the bags for patients with hepatic alterations were prepared without added copper, as indicated on the prescription, and therefore had the lowest copper levels (0.03 mg/L). Furthermore, the physicians adjusted the composition and the administered volume on a regular basis so as to meet the changing requirements of each patient.

Despite the biological functions of Cu being well known, it remains difficult to assess Cu nutritional status and to establish the requirements of both healthy individuals and critically ill patients. It is therefore important to find reliable biochemical indicators that allow establishing the optimal dose of Cu for critically ill patients on TPN.

SCu concentration is the most frequently used laboratory measure for assessing Cu status. However, serum Cu levels are regulated by strong homeostatic mechanisms, which are influenced by several factors, such as infectious and inflammatory processes, resulting in increased Cu levels and not reflecting the true Cu status.[27]The high SCu levels observed herein are consistent with the acute inflammatory response in critically ill patients. None of the patients studied here had Cu levels below reference values on initiation, discontinuation, or throughout the course of treatment, and all patients but one with high SCu levels returned to normal range.

Table 4
Parenteral Cu requirements in adults according to Scientific Societies.

Scientific Societies	Cu (mg/d)
AMA, 1979 (Refs. 24,25)	0.5–1.5
ESPEN, 2004 (Ref. 26)	0.3–1.3
ASPEN, 2012 (Ref. 14)	0.3–0.5
Prelack O, 2001 (Ref. 27)	Lower than 0.5 mg/d

Erythrocyte copper has been proposed as a biochemical nutritional indicator but has not been used frequently to assess nutritional copper status and has not been studied as a reflection of tissue copper concentrations.[7] Assessing its effectiveness as an indicator of tissue copper levels was beyond the scope of the present study. Nevertheless, it is known that red-cell SOD activity is erythrocuprein copper dependent and is an indicator of copper nutritional status, which falls rapidly in copper deficiency, correlating with the severity of the copper deficiency.[9] In addition, Reiser et al., 1985 found that erythrocyte SOD activity was depressed in copper depletion, despite there being no detectable decrease in serum copper or ceruloplasmin, but was not affected by zinc status.[8] Hence we posited that erythrocyte copper levels could serve as an indicator of the amount of copper to be administered in TPN, and thus would allow monitoring more accurately the amount of copper required by a critically ill adult patient.

Ceruloplasmin (CP) is an alpha-2-serum glycoprotein that carries Cu from the liver to the tissues, containing about 95% of the Cu found in plasma. Each molecule of ceruloplasmin binds six copper atoms, which are associated with oxidase activity; the latter constitutes the basis for a method used to determine serum ceruloplasmin levels.[9,20] Serum CP levels have been proposed as an indicator of Cu nutritional status. Thus, we hypothesized that CP levels might be associated with the copper content in the TPN mixture. However, during the acute phase response, ceruloplasmin increases independently of copper intake,[30] though the response is not immediate. This metabolic delay therefore explains the observed lack of correlation between intake and same-day ceruloplasmin levels.

Ceruloplasmin is an acute phase reactant that increases in inflammatory processes, malignancies, and other pathologies, regardless of Cu intake.[30]

In addition, it has been suggested that the specific enzymatic activity of ceruloplasmin, defined as the ratio of the enzyme activity to the immunoreactive protein, is a better indicator of copper status than either the enzyme activity or the immunoreactive protein alone. Only the specific enzymatic activity of ceruloplasmin was determined in the present study, and was found to be low in 40% of patients on initiation and in 37% of patients on discontinuation of TPN, despite their having normal or high serum Cu levels. These results are consistent with reports showing that CP responses are variable in instances of marginal deficiency and in short-term studies, as is the case of the present research work.

Apo CP (devoid of Cu) is synthesized in the liver, and the predominance of Cp in the circulation is thus holo CP (with Cu). Serum determination of the total amount of CP protein may not reflect CP enzyme activity, since this method determines both the functional holo CP and non-functional apo CP, and the immunologic assay may therefore result in an overestimation of the true amount of functional CP. Serum CP activity is drastically reduced during Cu deprivation, probably because most CP synthesized during Cu

deficiency is apo CP, which is quickly catabolized. According to the literature, ferroxidase activity is enhanced when hepatic Cu loading occurs and liver copper levels correlate significantly with CP protein expression and its enzymatic activity.[29] Therefore, it would be possible that the amount of Cu in TPN might be the limiting factor in CP protein metallation. However, although correlation between serum Cu and ceruloplasmin ($r = 0.5121$, $p = 0.0032$) was observed at the beginning of the present study, the correlation disappeared during the course of TPN treatment.

Significant correlation between changes in Ecu levels and the amount of Cu in TPN mixtures was observed ($r = 0.44$; $p = 0.016$) (Fig. 1), suggesting the usefulness of ECu levels to assess the amount of Cu provided in parenteral nutrition mixtures, according to patient needs. Some of the patients showed a decrease in SCu and ECu levels when they received less than 1.2 mg/day of Cu in the TPN mixture, indicating that they might have increased requirements. These high requirements may be due to increased urinary losses, which are usually very low[5] since SCu is not excreted through the kidneys for it is bound to CP, which is a high molecular weight protein. However, we found high urinary excretion of copper in most patients (mean value: 170 ± 140 μg/d; range: 8–540 μg/d). Although there is no information regarding the amount of copper excreted in the urine during the acute-phase response, some authors have suggested that such amount is higher than normal.[6] It is likely that turnover of ceruloplasmin copper was more rapid in the critically ill patients studied herein (undergoing stress, acute trauma, inflammatory processes, fever, etc) as a consequence of its short half-life.

A decrease in the amount of copper administered through TPN should be considered in patients with cholestasis or hepatobiliary disease. In these cases, the physician prescribes TPN with no added Cu. This was the case of the patient with cholestasis included in the present study. It must be pointed out however, that elimination of copper from TPN could have negative clinical and biochemical consequences, associated with Cu deficiency. In these cases, the amount of Cu in the TPN mixture should be adequate to compensate for Cu losses.[5]

Therefore, in order to avoid the adverse effects of overdosing, it is advisable to measure ECu levels in patients with severe liver disease or on long-term TPN.

5. Conclusions

The results of this study show that:

In agreement with previous reports, TPN mixtures showed a higher Cu content as compared to the physician's prescription due to copper contamination of individual components. SCu levels did not show significant correlation with the amount of Cu administered in TPN. Variations in ECu levels showed a significant correlation with amount of Cu administered daily in TPN and this biochemical indicator could be useful to monitor copper deficiency or excess in patients on TPN. It is noteworthy that copper delivery above 1.2 mg/d was frequent and prompts such monitoring.

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