SERUM COPPER AND ZINC IN TREATED PATIENTS WITH END STAGE RENAL DISEASE

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Abstract

Uremia is a state manifested by impaired kidney function with the consequences of accumulation of harmful toxins and waste products. In end-stage renal disease, atherosclerosis cardiovascular disease is a major cause of morbidity and mortality. In the present studies included the measurement of serum trace elements Zinc (Zn), Cupper (Cu) in (78) patients aged 16-77 year. They were classified into two main groups according to the type of treatment
1- End-stage renal disease patients on hemodialysis treatment (n=52, age range 16-76 years).
A) Hemodialysis patients with diabetes mellitus (n=20, age range 23-75 years).
B) Hemodialysis patients without diabetes mellitus (n=32, age range 16-67 years).
2- End-stage renal disease patients on conservative treatment (n=26, age range 20-77 years).

The result was compared with those of 25 healthy control groups (age range 16-70 years). The result showed that serum Cu was significantly deceased in patients on hemodialysis treatment but there was a significant increase in patients on conservative treatment. Serum Zn was significantly reduced in all patients studied, however, Cu/Zn ratio was reduced in all chronic renal failure patients on different treatment modalities indicating the influence of renal failure and its treatment on Cu/Zn status. In conclusion; chronic renal failure patients on different modalities of treatment experienced altered Cu, Zn status. The different mechanisms underlying these changes are mentioned.
Key words: Trace elements, renal failure, renal disease.

INTRODUCTION

Renal failure is used primarily to denote failure of the excretory function of the kidneys, leading to retention of nitrogenous waste products of metabolism. Various other aspects of renal function may fail at the same time, including the regulation of fluid and electrolyte status, trace elements (Cu, Zn) and the endocrine function of the kidney. Renal failure can be treated by dialysis (Hemodialysis or peritoneal dialysis) or transplantation (Emil&Jack, 2000). Renal failure is often divided into either acute renal failure (ARF) or chronic renal failure (CRF), depending on the rabidity of onset and the subsequent course of azotemia(an excess of urea or other nitrogenous substances in the blood). CRF is a pathophysiologic process with multiple etiologies, resulting in the attrition of nephron number and function, and frequently leading to end-stage renal disease (ESRD) (Braunwald et al., 2001). The main causes of CRF are glomerulonephritis, polycystic kidney, accelerated hypertension, calculi disease, diabetes mellitus, etc (Buton & Orme, 1978). Uremia is a clinical syndrome that
indicates a condition caused by "Contamination the blood with urine". Many symptoms of Uremia result from accumulation of urea, creatinine, and other nitrogenous end products of amino acid and protein metabolism in blood. (Buton & Orme; 1978). Zinc (Zn) is a trace element that is essential for human nutrition and is one of the most important trace elements, being an essential component of more than 200 metalloenzymes in human includes carbonic anhydrase, alkaline phosphatase, RNA and DNA polymerases, thymidin kinase, (Milne, 1999). Zn is an important element and is second to Fe as the most abundant trace element in the body. The body contains 1-3 gm of Zn, found mainly in the bones, teeth, prostate, muscle, liver, skin, testes and kidney (Milne, 1999). Zn is a cofactor of 200 enzymes, and required for growth development, reproduction, immunity, and metabolism of protein, carbohydrates, lipid, an important antioxidant or free radical scavenger, and has a protective role against membrane peroxidation (Chan et al., 1998). There is a biochemical and clinical evidence for altered Zn metabolism in patients with renal failure. Normal or elevated plasma Zn concentration in patient with kidney failure. Uptake of Zn from the dailysate is thought to be responsible for an increased plasma Zn concentration. Low circulating Zn levels have been reported in patients with uremia. Zn deficiency has been linked to many of the symptoms of renal failure, including loss of appetite, an altered sense of taste and smell. Copper (Cu) is a constituent of certain metalloenzymes and proteins (Milne, 1999). It has a major role in the synthesis of hemoglobin in bone marrow, and required for the synthesis of a number of copper-containing enzymes like ceruloplasmin, lysyl-oxidase, cytochrome C oxidase, uricase, and superoxide dismutase which reduces superoxide radicals to hydrogen peroxidase, which is then reduced to water by the selenium-glutathione peroxidase couple (Chan et al., 1998). Alterations in Cu metabolism in patients with renal failure have been frequently reported. Hypercupremia can occur in predialysis or hemodialysis patient. In hemodialysis patients, high serum Cu levels have been attributed to small amounts of Cu present in cellulosic dialysis membranes because they are manufactured by the cuprammonia method; it has been shown that plasma Cu is elevated despite the type of dialysis (Mitch & Klahr, 1998). Increased urinary losses of Cu and hypocupremia have been associated with the nephritic syndrome. A close correlation exists between urinary Cu excretion and urinary protein losses, indicating that Cu losses are limited to loss of Cu-binding proteins (Mitch & Klahr, 1998).

Materials
1- Chemicals:

   The general laboratory chemicals of highest purity, were used in this study, they are listed in table (1) with their suppliers.

<table>
<thead>
<tr>
<th>chemicals</th>
<th>supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-butanol</td>
<td>Reideldehaen, Germany.</td>
</tr>
<tr>
<td>Thiobarbituric (TBA)</td>
<td>BDH chemicals, Ltd. Pool, England.</td>
</tr>
<tr>
<td>Trichloro acetic acid (TCA)</td>
<td>Randox laboratory, Ltd. England.</td>
</tr>
</tbody>
</table>

2- Instruments:

- Atomic absorption spectrophotometer (shimadzu AA-646), Japan.
- Micropippette 10-20µl, Eppendorf, Germany.

Email: biomgzn.sci@uokufa.edu.iq
- Micropipette 100-1000µl, Slamed, Germany.
- Oven, Mmert, Germany.
- Sensitive balance, A and B Co., Japan.
- Spectrophotometer, Philips, Holland.
- Temp. Controlled centrifuge, Hittich, Germany.
- Vortex mixer, scientific industries INC. Bohemia N.Y., USA.
- Water bath, Schwabch, Germany.

3- Subjects:
1- patients:
This study was conducted during the period from October 2002 until the end of February 2003. Seventy eight patients with chronic renal failure were included in this study. Those patients with acute infection, chronic inflammatory diseases, respiratory disease, hepatic disease, and history of blood transfusion were excluded. The patients in this study were classified into two groups listed in table (2).

Table (2): The number of patients, sex and age range in different CRF patients groups and controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>Female no.</th>
<th>Male no.</th>
<th>total</th>
<th>Age range(years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients on HD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-HD with DM</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>23-75</td>
</tr>
<tr>
<td>-HD without DM</td>
<td>11</td>
<td>21</td>
<td>32</td>
<td>16-67</td>
</tr>
<tr>
<td>Patients on conservative therapy</td>
<td>8</td>
<td>18</td>
<td>26</td>
<td>20-77</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>15</td>
<td>25</td>
<td>16-70</td>
</tr>
</tbody>
</table>

Group 1:
This group consisted of 52 patients (31 males and 21 females). Their age range was (16-75) years (mean ± SD 42.03 ± 13.45 years). The diet of the dialysis patients was not modified from that already prescribed for their end-stage renal disease. The patients received dialysis for 3-4 hours in each session, 2 times weekly with aid of alter touch 100 equipment (Darke willowk, Sweden), using acetate buffer. The blood flow rate was 150-250 ml/min, and the dialysate flow rate was 500 ml/min. this group was classified into two groups:

Group A: classified into two groups:

Diabetic patients undergoing HD: Consisted of 20 patients (10 males and 10 females). They were recruited from the artificial kidney unit in private nursing home hospital and specialized surgeries hospital. Blood samples were taken after an overnight fast before the injection of heparin.

Group B:
Non-diabetic patients undergoing HD: Consisted of 32 patients (21 males and 11 females). Their age range was 16-67 years (mean ± SD 37.93 ± 12.41 years). They were recruited from the artificial kidney unit in private nursing home hospital and specialized surgeries hospital. Blood samples were taken after an overnight fast before the injection of heparin.

Group 2:
Patients with CRF on Conservative treatment:
This group consisted of 26 patients (18 males and 8 females). Their age range was 20-77 years (mean ± SD 50.38 ± 14.82 years). They were collected from outpatients department in private nursing home hospital. Blood samples were taken after an overnight fast.

2- Controls:
Twenty-five apparently healthy subjects were selected as controls group, (15 males and 10 females). Their age range was 16-67 years with (mean ± SD 36.96 ± 16.77 years). None of the controls was diabetic, alcoholic, heavy smokers, or having a history of coronary heart disease, thyroid or other metabolic diseases, on special diet, taking any antioxidants and none of the females were pregnant or on contraceptive pills or had any history of renal problems before taking part in this study.

Blood samples:
Yen milliliters of blood were drown from arteriovenous fistula from patients immediately before HD (pre-HD), after 12 hours fast and 10 ml of venous blood were drawn from controls after 12 hours fast. The sample was transferred into clean plain tube, left at room temperature for 15 minutes for clotting, centrifuged, then serum was separated and kept in more than one Eppendorf tube in the refrigerator at 2-8°C until the time of assay. The samples were not kept for more than 7 days for lipid assay, while serum for MDA analysis was done within 24 hours after sample collection.

Methods
Serum trace elements (Coppers & Zinc):
0.1 ml of serum diluted to total volume of 1ml using 6% n-butanol solutions and was analyzed for their copper and zinc contents using Atomic absorption spectrophotometer (Schimadzu AA-646). Copper and zinc hallow cathode lamps were used at wavelengths of 324.75nm and 213.9nm respectively.

Results
Serum trace elements:
Serum copper (Cu), Zinc (Zn) and Cu/Zn ratio were measured in all patients and the controls subjects.

Serum copper (Cu):
The mean ± SD of serum Cu are shown in table 1 and figure 1&2. In all patient groups on HD treatment the means of serum Cu were significantly lower than in control subjects (P<0.0001).While the means of serum Cu were increased in patients on conservative treatment than in control subjects (P<0.0001). There is also significant difference in serum Cu between HD group and the conservative group (P<0.0001). No significant difference between HD patients with DM and without DM could be found.

Serum Zinc (Zn):
The mean ± SD of serum Zn are shown in table 1 and figure 1&2. In all patient groups the means of serum Zn were significantly lower than in control subjects (P<0.0001).There is also significant difference in serum Zn between the HD group and the conservative group (P<0.0001). No significant difference between HD patients with DM and without DM could be found.

Serum Cu/Zn ratio:
The means ± SD of serum Cu/Zn ratio are shown in table 3 and figure 5&6. In all patient groups the means of serum Zn were lower than in control subjects but it was not significant only in HD group (especially to those with DM).
Table (1): serum Cu, Zn and Cu/Zn ratio (mean ± SD) in different groups of ESRD patients and control group.

<table>
<thead>
<tr>
<th>groups</th>
<th>No.</th>
<th>S. Cu (µmol/L)</th>
<th>S. Zn (µmol/L)</th>
<th>Cu/Zn ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD patients:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- HD with DM</td>
<td>52</td>
<td>10.92 ± 5.19**</td>
<td>8.24 ± 2.31**</td>
<td>1.35 ± 0.51*</td>
</tr>
<tr>
<td>- HD without DM</td>
<td>20</td>
<td>9.87 ± 2.18**</td>
<td>8.03 ± 2.34**</td>
<td>1.26 ± 0.22*</td>
</tr>
<tr>
<td>Conservative patients</td>
<td>26</td>
<td>11.57 ± 6.35**</td>
<td>8.37 ± 2.32**</td>
<td>1.41 ± 0.63</td>
</tr>
<tr>
<td>controls</td>
<td>25</td>
<td>23.96 ± 2.83</td>
<td>18.11 ± 1.92</td>
<td>1.70 ± 0.32</td>
</tr>
</tbody>
</table>

AS compared to the control group
*P<0.01, **P<0.0001

Figure (1): The (mean ±SD) of S.Cu, S.Zn and Cu/Zn ratio in different groups of ESRD patients and control group.
Discussion

**Serum trace elements:**

Serum trace elements (Zn & Cu) concentration was shown in chapter three (table (1&2) and figures (1, 2, 3&4). There have been many studies reading serum trace elements in patients with CRF (especially those undergoing HD treatment).

The results of this study are in agreement with those earlier reports showing that serum Zn and Cu levels are decreased in HD patients, although, there are previous investigations on serum Zn and Cu in uremic patients which had conflicting findings of low (Emin Yilmaz et al., 2000; Karayaylali et al., 1997; Kaminska Galwa et al., 1997; Ongajooth et al., 1996), normal (Avasthi, 1999; Hosokawa, 1997), or high (Dhases & Debroe, 1996; Gallieni et al., 1996; Sondheimer et al., 1988) levels. On the other hand, in these study patients with conservative treatment (predialysis chronic renal failure) have decreased levels in serum Zn but increased levels of serum Cu and these results are in agreement with Dhases & Debroe, 1996; Elizabeth et al., 1994.

A number of factors in renal failure patients' tend to either increase or decrease the body burden of certain trace elements. In uremic patients, the most important factor affecting trace element concentration is the degree of renal failure and modality of renal replacement therapy (Vanholder et al., 2002). Many trace elements are excreted primarily in the urine, and with renal failure they may accumulate. Elements such as Zn and Cu, which are protein bound, may be lost in excessive quantities when there are large urinary protein losses (Gartwright et al., 1995). Excessive uptake or losses of Zn and Cu may also occur during dialysis therapy, depending on their relative concentration in plasma and dialysate and the degree of binding to protein or red cells (Padovese, 1999). Hemodialysis may remove some trace elements if the dialysate concentrations are sufficiently low (e.g. Zn) (Van Renterghem et al., 1999). Because many trace elements are bound avidly to serum proteins, they may be taken up by blood against a concentration gradient when present in even small quantities in dialysate (M anzler & Schreiner, 1990).

Protein-calorie malnutrition, by lowering serum concentration of proteins that bind trace elements, may decrease the serum levels of a number of these elements. Because most zinc circulates bound to albumin, a fall in the zinc concentration should always be interpreted in relation to any change in the albumin concentration.

Restriction of protein, low dietary Zn intake, a specific Zn transport defect, and absence of an intestinal Zn legend, all of these factors may contribute to decreased
serum Zn levels in CRF patients. Otherwise, environmental factors, diet, and the again process may contribute to the trace metal burden in uremia (Lee et al., 2000). On the other hand, for human beings trace elements (especially Zn & Cu) are essential nutrients with a gamut of functions. They are for instance indispensable components of many antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GHPx), so they have some regulatory functions and they may affect immune reactions and influence the equilibrium in the antioxidant defense system and enhance the toxic effect of reactive oxygen molecules (Podracka et al., 1999). Abnormalities of trace elements are primarily the result of uremia, and they may be further modified and sometimes greatly exacerbated by the dialysis procedure (Zima et al., 1999 & Lin et al., 1996). Therefore, altered levels of Cu & Zn in CRF patients (especially those by HD treatment), may increased patients susceptibility to lipid peroxidation in uremia (Lin et al., 1996).

To prevent some complications in chronic HD patients, it is very important to regulate the levels of trace elements by adequate water treatment. Reserve osmosis is able to prevent the accumulation of the majority of trace elements in the patients (Zima et al., 1999).

Copper to zinc ratio is a more variable indicator of the body Zn and Cu status in CRF patients. The finding concerning serum Zn and Cu and Cu/Zn ratio which are decreased significantly in different CRF groups in comparison with controls, support the hypothesis that an imbalance in Zn and Cu status might be involved in human CRF. The result of Cu/Zn ratio may indicate the usefulness of using this ratio more efficiently in the estimation of serum levels of these two highly correlated trace elements, than using each one alone (Widad & Limia, 2003).

References
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