Diagnostic Indices of Zinc Deficiency in Children with Renal Diseases*

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ABSTRACT

Abnormal taste acuity and zinc depletion have been reported with end-stage renal disease. In order to determine when altered taste and zinc deficiency begin in the course of chronic progressive renal disease and to assess the various indices of zinc depletion, 14 pediatric patients were studied who were in various degrees of renal failure. They were not yet on dialysis or in need of transplantation. Taste acuity was abnormal in all patients. The mean plasma zinc levels were less than normal, but the differences were not statistically significant. The hair and RBC zinc concentrations, however, were significantly depressed. Analysis of the data indicate that zinc deficiency begins early in the course of chronic renal failure, that hair and RBC zinc measurements are the most reliable indicators of zinc status, and that poor intake of zinc containing foods is the major cause of the zinc depletion.

Introduction

Anorexia, impaired growth, and inadequate kilocalorie (kcal) intake are characteristic features of chronic renal failure in children.7,11 Zinc is an integral component of several enzymes including the alkaline phosphatases, carbonic anhydrases, and deoxyribonucleic acid (DNA) and ribonucleic-acid (RNA) polymerases.15 It is postulated that zinc is also a cofactor in an enzyme that governs taste threshold and that altered taste (dysgeusia, hypogeusia) secondary to zinc deficiency results in poor appetite and anorexia.5,6 Additional symptoms of zinc deficiency include delayed growth and sexual maturation,9 delayed wound healing,3 dermatitis,10 and alopecia.10

Altered taste acuity and zinc depletion have previously been reported in adults14 and children2,12 with end-stage renal dis-
ZINC DEFICIENCY IN CHILDREN WITH RENAL DISEASE

It is not known, however, if these abnormalities might actually begin earlier in the course of chronic progressive kidney disease. There is considerable uncertainty regrading the causes of the zinc deficiency and the reliability of the various clinical and laboratory indices of zinc depletion.

These issues were addressed by studying 14 patients (mean age 10 years, range six months to 19 years) with varying degrees of chronic renal failure who were not yet on dialysis or in need of transplantation. The average (± SD) serum creatinine concentration of the patient population was 4.4 ± 3.7 mg per dl and ranged from 1.2 to 11.2 mg per dl. Zinc levels of the patients were compared to those of age matched control subjects also studied in our laboratory.

Methods and Materials

The study included a dietary history, anthropometric measurements, taste acuity tests, and a determination of hair, plasma, red blood cells, and urinary zinc concentrations.

Diet History

Patients kept a food diary using a free format method for two days preceding each visit. Instructions for recording on the provided form were given and food models of representative portion sizes were shown. Each patient was given a portable food scale for use at home and instructed in its use. Foods were coded according to Handbook 8 of the U.S. Department of Agriculture. A computerized dietary assessment program (based on Handbook 8 and current literature values for zinc) was used to determine nutrient intake.

Anthropometric Measurements

Standard procedures were used for the determination of height, weight, triceps skinfold, and mid-arm muscle circumference at each clinic visit.

Taste Acuity

The method of Henkin et al was used to administer the taste acuity test. The composition of the solutions used is as follows:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl 3.0 g per liter</td>
<td>1000 mg per dl</td>
</tr>
<tr>
<td>5.3 g per liter</td>
<td>1750 mg per dl</td>
</tr>
<tr>
<td>10.0 g per liter</td>
<td>2650 mg per dl</td>
</tr>
<tr>
<td>HCl 0.07 normality</td>
<td>Urea 460 mg per dl</td>
</tr>
<tr>
<td>0.16 normality</td>
<td>860 mg per dl</td>
</tr>
<tr>
<td>0.33 normality</td>
<td>1460 mg per dl</td>
</tr>
</tbody>
</table>

Subject and age-matched normal controls were graded by the same individual on their ability to detect a tastant (detection) and to identify correctly the flavor (recognition). Points were given to compute the detection and recognition scores. One point was given for failure to detect or recognize the least concentrated solution in each flavor category. Two points were given for failure to detect or recognize the middle concentration, and three points for failure to taste correctly the strongest concentration. A total score of one was normal. A score from two to eight indicated mild taste impairment, from eight to 16 indicated moderate taste impairment, and above 17, severe taste impairment.

Sample Collection and Analysis

All glassware and polypropylene bottles and tubes were purchased new and acid washed before use with 6 N. analytical grade nitric acid.

Standards were made from 1000 ppm stock solutions.* Working standards for red blood cell analysis of 0.3, 0.5, 0.6, and 0.8 μg per ml of zinc were made with 0.05 percent Triton-X.† Working standards for plasma analysis of 0.1, 0.3, 0.5, 0.6, and 0.8

† Analysis showed normal saline, TCA, and Triton-X did not contain significant amounts of zinc. Blanks of all diluents used were analyzed, and appropriate calculations were made for each set of samples analyzed.
μg per ml of zinc were made with deionized water. Trichloracetic acid solution was made fresh every three days. Working standards were made up in acid washed glassware and stored in polypropylene bottles. Standards and samples were read immediately after preparation.

Flame techniques on the atomic absorption spectrophotometer were utilized. The instrument setting used for the analysis of hair, plasma, and red blood cell zinc on a spectrophotometer† included: Wave length 213.9 nm; Slit setting, 4 (0.7 nm); Source, 15 mv; and Flame type, air acetylene oxidizing.

Hair. Samples were cut from the nape of the neck with a stainless steel scissor. Hair from within two cm of the scalp was used. The sample was cut into 0.5 to 1.0 cm lengths and washed for 30 minutes in 30 ml of nonionic detergents§ using a mechanical shaker. After rinsing in 250 ml deionized water the hair was further shaken in a cold solution of 20 ml of 0.1 M Ethylene diamine tetracetic acid, di-sodium salt (EDTA) for 15 minutes. After rinsing again in deionized water, the hair was dried at 60°C for 18 hours. Dried hair (0.1 g) was dry ashed at 450°C for 24 hours. Following ashing, five ml of one percent nitric acid were added to each sample. Samples were transferred from crucibles to polyethylene tubes, and analyzed by flame atomic absorption spectrophotometry.

Blood. Samples were collected by venipuncture in a special trace mineral free vacutainer. The vacutainer contained 0.5 ml of sodium heparin per 5.0 ml of whole blood. A blank vacutainer containing 0.5 ml of sodium heparin was made for each lot of heparin used.** Hematocrits were taken at the time of collection. Collected samples were centrifuged for five minutes at 8000 rpm. The plasma fraction was removed with an acid washed Pasteur pipet and introduced into a sterile polypropylene test tube.4 Red blood cells were washed with 0.15 m (0.9 percent) saline by adding the saline, mixing, centrifuging, and removing the saline layer. This process was done twice; a third aliquot of saline was added in a 1:1 ratio and mixed. The hematocrit of the diluted red cells was then taken and recorded. Plasma and red blood cells were frozen until analysis. After plasma samples were thawed to room temperature, 2.0 ml of sample were added to 3.0 ml of 20.0 percent trichloroacetic acid (TCA) to precipitate the protein. The sample was centrifuged and the supernate analyzed. After thawing, 0.5 ml of red blood cells were added to 4.5 ml of 0.5 percent Triton-X to lyse the cells.18

Urine. Twelve hour urine samples were collected in standard plastic urine collection bags. Volume was measured and an

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† Perkin-Elmer Atomic Absorption Spectrophotometer, model 306.
§ Tergitol, 10 ml per L.
alliquot acidified with conc. HCl (3 percent of volume). Urine was stored in polypropylene containers until analysis. Acidified urine was diluted 1:4 with deionized water and analyzed by flame atomic absorption spectrophotometry.

Results

Dietary Intake

Calorie intake was below the Recommended Daily Allowances (RDA per kilogram) in seven of 14 children. Protein intake was below normal in only three patients. Dietary zinc intake was inadequate (< 0.3 mg Zn per kg) in 11 of 14 children.

Anthropometric Measurements

The mean height of our patient was only on the eighth percentile (range 1st to 25th percentile). The weight, triceps skinfold thickness, and mid-upper arm muscle circumference were appropriate for their height, however.

Taste Acuity

Taste acuity (detection, recognition) was altered or impaired in all children (figure 1). Three children who had hydronephrosis had the unusual finding of dysgeusia, and in particular misidentified sour flavor as sweet.

Zinc Values

Hair. Zinc values (mcg per g) were below normal (< 2 SD below normal mean) in each of the nine patients tested (table I). Mean values ± SD were 47 ± 15 (normal 88 ± 5) for the zero to five years old, 87.5 ± 10 (normal 153 ± 5) for the six to 15 years old, and 157 ± 10 (normal 180 ± 4) for the 16 to 20 years old (figure 2). The values were all significantly lower than the normal values (P = < 0.05).

Plasma. Zinc concentrations (mcg per dl) were below normal in 57 percent of the patients, and were 88 ± 22 compared to normal values of 112 ± 12. This difference was not significant (p = 0.20).

RBC. The RBC zinc concentrations, however, (figure 3) were below normal in 64 percent of the patients and averaged 881 ± 235 mcg per dl, significantly lower than the normal control values of 1250 ± 150 mcg per dl (p < 0.05).

Urine. Zinc excretion was measured in nine patients and the mean for the group was 1.9 ± 1.2 mg per l. Excretion was
Clinical and Laboratory Correlates

There was a strong correlation between both kcal and zinc intake ($r = 0.96, p < 0.01$) and protein and zinc intake ($r = 0.73, p < 0.01$).

Hair zinc correlated with both percent ideal body weight ($r = 0.69, p < 0.05$) and height percentile ($r = 0.74, p < 0.05$). A similar correlation was found between RBC zinc and both percent ideal body weight ($r = 0.56, p < 0.05$) and height percentile ($r = 0.61, p < 0.01$). No correlations were found, however, between these anthropometric measurements and plasma zinc concentrations.

Taste acuity detection scores correlated with RBC zinc levels ($r = 0.83, p < 0.05$) but not hair or plasma levels. Taste detection and also recognition correlated with percent ideal body weight ($r = 0.82, p < 0.05$).

Hair zinc (figure 4) correlated with RBC zinc ($r = 0.77, p < 0.01$) but not with plasma zinc. Plasma zinc also failed to correlate with RBC zinc.

Discussion

The traditional laboratory assessment of zinc deficiency has included measurements of hair, plasma or serum, and RBC zinc concentrations.

In collecting blood specimens, great care is needed to avoid zinc contamination. The customary red stoppered vacutainers have been found by us to be highly contaminated with zinc, necessitating the use of polypropylene tubes or trace mineral free vacutainers. In addition, the zinc content of heparin was found to vary considerably, depending upon the lot used.

Hair zinc is an indicator of total body zinc stores, but unless the hair sample is taken close to the scalp, it may not reflect recent body stores. Plasma or serum zinc are less reliable diagnostic tests. Levels can fluctuate, without changes in total body stores, during periods of stress or illness, such as trauma, inflammation, or infection. In addition, shifts may occur between plasma and the RBC's in patients with chronic renal failure, particularly during episodes of acidosis. Shifts predominately affect plasma (or serum) zinc levels, as greater than 80 percent of blood zinc is usually in the red cells, and as the...
normal RBC concentration is approximately 15 times greater than the plasma concentration.4 It is not surprising therefore, that no correlation was found by us between plasma zinc concentrations and percent ideal body weight, percentile height, taste acuity, hair, or RBC zinc levels. In our opinion, plasma (or serum) zinc measurements do not give a reliable indication of patients’ zinc status.

It was found that hair and RBC zinc values correlate well and that both are good indicators of total body zinc stores. The RBC zinc values may be a better measure of the current zinc status, however. In addition, RBC zinc assays are easier to perform than hair measurements.

Our study indicates that zinc deficiency usually begins early in the course of chronic progressive renal disease and that the taste acuity test is a reliable, non-invasive and inexpensive screening procedure. The taste acuity detection component was particularly helpful as it correlated very strongly with RBC zinc levels.

Good correlation was found between the indices of body zinc and those of percent ideal body weight and percentile height. It is our opinion that zinc measurements are also predictive of nutritional status.

The major cause of the zinc deficiency in our renal patients seems to be decreased zinc intake. Zinc intake was below normal in 79 percent of the children, and there were strong correlations between both calories, protein, and zinc intakes.

The zinc deficiency is probably the major cause of the abnormal taste acuity (hypogeusia, dysgeusia), which in all likelihood contributes to the poor dietary intake. Thus, a self-perpetuating cycle is established. In addition, as increased urinary zinc losses were noted in some of the patients, this may also be a contributing factor.

References