

Zinc supplementation corrects nitrogen retention related complications in cirrhosis patients

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Abstract

Zinc is often deficient in patients with liver cirrhosis, and treatment with zinc provides short-term improvement in protein metabolism. However, the long-term effects of zinc have not been fully clarified. Zinc deficiency is common in cirrhosis and has been involved in the altered nitrogen metabolism. To control the increased BUN the effect of Zn supplementation is studied. The cirrhosis patients were divided in to two groups, the control group was given all routine medicines, but the experimental group was given 3-month oral zinc sulfate supplementation (600 mg/d). Plasma zinc levels were measured in all patients and only those patients were selected for study who had serum Zn was lower than normal. Among patients with type C liver cirrhosis were contacted between June 2015 and January 2016, those with a serum albumin level ≤ 3.5 g/dL and a serum zinc level ≤ 70 μg (1.07 μmol)/dL were selected. The urea clearance was observed significantly increased after Zn supplementation. Psychometric tests improved, as did routine and dynamic liver function tests. Also, the plasma concentration of lipid peroxides was reduced by zinc. No significant changes were observed in the control group. The study indicated that long-term oral zinc speeds up the kinetics of urea formation from amino acids and ammonia. Changes in the hormonal drive and/or the antioxidant activity of zinc might be involved in the general improvement in liver function, whereas the beneficial effects on encephalopathy might stem from decreased ammonia. However, although zinc may play a role in hepatocarcinogenesis, the precise implications remain to be clarified.

Keywords: Type C Cirrhosis; Trace Element; Protein Metabolism, ammonia

Introduction

There is an interest in the potential for micronutrient supplementation, especially Zinc to enhance immune functions and decrease the incidence of infections in all age groups. Many world wide studies found generalized micronutrient deficiency in all age groups. In many double blind, placebo controlled, randomized studies the immunological importance of Zinc was proved. The association between Zinc and immunity was first documented with the discovery of human Zn deficiency by Prasad *et al.* Along with the characteristic hypogonadism and dwarfism; Zn deficient person experienced increased susceptibility to infections. Later a lethal mutation to Holstein-Fresian Cattle was found responsible for the failure to both absorb Zn and develop a thymus. These immuno-deficient cattle could be treated with Zn and symptoms could be prevented. Further evidence for Zn's role in immunity came from the discovery that the inborn error in human metabolism, acrodermatitis enteropathica, was caused by defective absorption of Zn. This was prevented by only Zn supplementation. The first report of Zn deficiency with thymic atrophy and loss of T helper cell was published by Fraker *et al.* The affected person was otherwise normal with normal food intake, with normal growth parameters, but with severe Zn deficiency, that damaged thymus and spleen. The affected spleen and thymus started reduced production of SRBC- a T-dependent antigen. Additional manifestations include disturbed serum immunoglobulin profile due to the deficiency. This specific deficiency also causes reduced antibody production. Repletion with Zn resulted in normalization of plaque response and Zinc is associated with more than 100 metalloenzymes of widely differing function, in which it is generally found located at the active catalytic site of the molecule.[Valle &Galles,1984]. Many deficiency symptoms

of Zn impoverishment can be related directly to it's role in cellular biochemistry, which would include the importance of Zn in the synthesis of the nucleic acids and proteins, the metabolism of carbohydrates and fats, the stabilization of cell membranes and it's emerging involvement in immunocompetence and neurophysiology. Underlying many of these biochemical activities lies the metals highly concentrated electrostatic charge, comparatively small ionic size and large ionization potential. Because of it's highly localized charge and electron affinity Zn is a very effective attacking group, especially in a chemically non selective, but physically constrained manner. It's association with particular proteins ranging from mobile helical molecules as insulin, β -pleated sheet proteins as carbonic anhydrase and mixed proteins as carboxypeptidase's, Lactate dehydrogenate to random structures as metallothionein, confer upon the metalloprotein particular enzymes and kinetic characteristics. [In this way Zn is involved enzymatically in every major catalytic category. It is essential for immuno-competence and membrane stability. It is involved in receptor modulation, in several neuro-endocrine systems and plays a pivotal role in many aspects of DNA replication, protein transcription(Zn chelates with the amino acids cysteine and histidine, forming Zn fingers) and translation factors, and because of its contribution to the structural integrity of the DNA and RNA macromolecules. The recent recognition that Zn acts as a modulator substance for receptor affinity and in cell signalling Now implicates the metal in the fine control of many metabolic processes and thus immunity building. And in neurotransmitters recognition and synaptic transmission.

Cell Mediated immunity is also alerted by the deficiency. Delayed hypersensitivity to skin-test is often compromised in the Zn deficient. The thymic hormone and Thy-1 positive

lymphocytes be lowered in this deficiency. T- Lymphocytes response to a mitogen hemagglutinin was reduced in the patients maintained on diet that did not contain Zn. The T-cell proliferation was markedly increased after Zn supplementation. Zinc is essential for the normal development and maintenance of immune function and when deficient, results in a profound state of acquired immuno-deficiency syndrome. Which may be prolonged even with repletion. The condition is characterized by depressed T-cell mediated immunity and underdevelopment of the thymus gland. Much of the effect possibly relates to a reduced number of available leucocytes, although diminished levels of interleukin-2 and less T-Lymphocyte proliferation may also be important. Recently increasing attention has been drawn to the role of thymulin, a putative peptide hormone of Thymic origin, which requires Zn for functional activity and appears to be involved in the production of interleukin-2.

In several studies with Zinc deficient children suffering from nephritic syndrome and subjects with sickle cell anaemia or voluntary restricted Zn intake, all of whom had reduced Zn status, thymulin level was significantly lowered, but responded to Zn supplementation in vivo or to Zn activation in vitro. Current data suggests that Zn may be required to render thymulin biologically active which may in part explain the depressed immune function which accompany-es Zn depletion.

Liver cirrhosis patients have been shown to have abnormal amino acid balance due to branched chain amino acid (BCAA) deficiency, and replacement of BCAAs has been shown to improve event-free survival and liver function in patients with liver cirrhosis. These findings suggest that abnormal amino acid metabolism may aggravate liver cirrhosis. In addition, administration of BCAAs has been shown to significantly suppress liver carcinogenesis in obese individuals with a body mass index of at least 25. Therefore, metabolic abnormalities associated with liver disease are involved in various pathological conditions; however, their mechanisms have not been fully clarified. Under normal physiological conditions, ammonia is metabolized by the liver, brain, muscle, and kidney. In well-nourished cirrhotic patients, the affected liver has an impaired capacity for removal of ammonia in the form of urea, which may result in increased muscle glutamine synthetase in order to provide an alternative mechanism for ammonia removal as glutamine. Glutamine synthesis also increases to some extent in the brain of these patients. HE may develop as a consequence of increased circulating and cerebral ammonia in well-nourished cirrhotic patients. On the other hand, in malnourished cirrhotic patients, the loss of muscle mass, commonly seen as a consequence of malnutrition, can adversely affect this alternative route of ammonia removal. The brain being the main organ metabolizing ammonia in these conditions, severe HE is commonly diagnosed in malnourished cirrhotic patients. Hepatic encephalopathy is a neuropsychiatric complication of liver disease that affects 20 to 30% of the patients with cirrhosis, reducing health-related quality of life and causing a reversible decline in cognitive function. Previous studies have demonstrated that a reduction in blood ammonia levels improves hepatic encephalopathy, neuropsychological test performance, cognitive function, and health-related quality of life. Lactulose, an ammonia absorption minimizer, has been successfully used to reduce blood ammonia levels in minimal hepatic encephalopathy. However, lactulose has no ammonia detoxification effect,

rendering it ineffective to treat advanced hepatic encephalopathy.

Two major organs are involved in the metabolism of ammonia: the liver, in which ammonia is converted to urea via ornithine transcarbamylase, and the skeletal muscle, where ammonia is metabolized to glutamic acid via glutamine synthetase. Zinc is a critical cofactor in these enzymatic reactions. Animal models have shown zinc deficiency decreases the activity of ornithine transcarbamylase, while zinc supplementation markedly increases hepatic ornithine transcarbamylase activity. Zinc deficiency has also been reported to impair the activity of muscle glutamine synthetase, which leads to hyperammonemia.

Zinc deficiency is observed frequently in patients with cirrhosis and hepatic encephalopathy. Poor nutritional intake caused by a protein-restricted diet, impaired intestinal absorption, and excessive urinary loss are all potential causes of a low serum zinc levels in patients with advanced cirrhosis. Short-term oral zinc supplementation may improve hepatic encephalopathy by correcting the zinc deficiency that compromises the conversion of ammonia to urea. Bresci *et al.* reported better psychometric test performance in a zinc-supplemented group than in a standard therapy group, although the difference was not significant. Similarly, oral zinc supplementation can improve hepatic encephalopathy in patients failing to respond to protein restriction and lactulose. Zinc supplementation, in addition to standard therapies, may increase the hepatic conversion of amino acids into urea, decrease serum ammonia level, and consequently improve health-related quality of life. The effect of long-term oral zinc supplementation in addition to standard therapy on recurrent hepatic encephalopathy has not been established. Despite the low cost and infrequent side effects of zinc supplementation, there is little evidence-based information about the effects of zinc supplementation on hepatic encephalopathy. The aim of this meta-analysis was to assess the effects of oral zinc supplementation in the treatment of hepatic encephalopathy. Zinc, which is a trace metal, is indispensable for growth and differentiation of cells, and is one of the most important nutrients of metabolism in humans. More than 300 proteins possess a zinc-containing region, and these proteins play an important role in the regulation of cell function. Therefore, Zinc may be closely involved in many bodily functions. Homeostasis of zinc in vivo is primarily maintained by a balance between zinc-binding metallothionein protein and the ex-pression of 2 key zinc transporters. Deficiency of zinc can lead to growth disorders, cognitive disorders, and compromised immune function. Further-more, zinc deficiency can accompany liver cirrhosis, leading to abnormal levels of ammonia and other substances due to abnormal protein metabolism, and the onset of hepatic encephalopathy. Zinc replacement has been shown to alleviate hepatic encephalopathy and hyperammonemia and to improve protein metabolism. However, there are very few reports on the long-term efficacy of zinc replacement therapy.

Materials and Methods

Subjects

Among the patients with hepatitis C virus-related liver cirrhosis visiting our facility between June 2015 and January 2016, those satisfying all the following criteria were enrolled in the study: (1) serum albumin levels not higher than 3.5 g/dL;

(2) serum zinc levels no higher than 60 /dL; (3) patients were able to receive periodical outpatient care, and (4) patients provided informed consent to the study.

Protocol

These 18 subjects were randomized into 2 groups: group A (n = 9, zinc supplemented used was 600 mg/day zinc sulfate (containing 136 mg zinc, 2.08 mmol zinc) for patients with a serum zinc level no higher than 60 µg. Group B (n= 9) with no Zinc supplementation. But other routine treatments were same in both the groups.

Methodology

The following biochemical estimations were done

- The serum Zinc status of all the selected patients was analyzed by Nitro-PAPS method developed by Akita Abe. Nitro-PAPS react with Zinc in alkaline solution to form a purple colored complex. The color absorbance was read at 575 nm in spectrophotometer. The diagnostic kit from the ‘Chema’ (Glaxo) company was used for estimation, the kit contained Reagent-A, which had borate buffer 370 mM, pH 8.20, Salicyladoxime 12.5mM, Dimethylgloxime 1.25mM, Surfactants and preservatives. The Reagent-B contained Nitro-PAPS buffer 0-.40ml. The standard solution was Zinc in diluted acid 200 µg/dl. This assays is linear up to 500 µg/dl. The estimations were done in local patho - lab.
- The Zinc was supplemented as ‘Zavit’ to the patients for about a month to one and a half months, in which Zn is present as Zinc Sulfate Monohydrate (41.1 mg in each capsule) [supplementation-2/3 RDA/week]
- The effect of supplementation was estimated again on the serum levels of the Zn.
- The WBC count of the blood samples of all the subjects are calculated by Neubauer’s Hemocytometer, by using WBC diluting fluid, made up of Glacial Acetic Acid-1.5 ml, 1% solution of Gentian Violet in water, distilled water up to 98 ml. A small quantity of Thymol was added to prevent mould growth.
- As urine stagnation due to renal failure is common in all patients, this generally causes infection of urinary tract. The generalized immunodeficiency may be the cause of this infection. So the effect of Zn supplementation was also studied on the UTI.
- The effects of improved Zn levels were noted on the smell, taste and appetite levels of the patients, by ‘sensory evaluation method’.
- The demographic data of all the subjects and controls were collected.
- The blood sugar of all the related persons were analyzed by using NYCOCARD.
- Analysing serum C- Peptide levels- Measuring C-peptide

can help to determine how much of their own natural insulin a person is producing as C-peptide is secreted in equimolar amounts to insulin. C-peptide levels are measured instead of insulin levels because C-peptide can assess a person’s own insulin secretion even if they receive insulin injections, and because the liver metabolizes a large and variable amount of insulin secreted into the portal vein but does not metabolize C-peptide, meaning blood C-peptide may be a better measure of portal insulin secretion than insulin itself. Insulin abnormality is correlated with nitrogen imbalances in some previous studies. A very low C-peptide confirms Type 1 diabetes and insulin dependence and is associated with high glucose variability, hyperglycaemia and increased complications. This estimation was done by Akita Abay Method

- The Blood tests were done to assess the serum level of the following enzymes-
 - Aspartate aminotransferase (AST or SGOT)
 - Alanine aminotransferase (ALT or SGPT)
 - Alkaline phosphatase, 5’ nucleotidase,
 - Gamma-glutamyl transpeptidase (GGT)
 - LDH (Lactate dehydrogenase)
- The AST and ALT readings in such cases are usually between twice the upper limits of normal and several hundred units/liter. One of the most common causes of mild to moderate elevations of these liver tests is a condition referred to as fatty liver (steatohepatitis or hepatic steatosis) and cirrhosis specially.
- Estimation of Coagulation panel (prothrombin time or PT), because in liver malfunctioning, the Prothrombin time is prolonged, because of lesser production of Fibrinogen, Prothrombin and other clotting factors.
 - BUN (Blood Urea Nitrogen) was estimated by using kit of AGAPPE, Liqui CHEK, Kit no-ADI/V 02/110334 by using Urease enzyme.
 - Estimation of Albumin level by using Autoanalyser –Star 21 model. In liver diseases, the production and hence the blood level of Albumin is reduced significantly.
 - Estimation of serum Bilirubin level was done by using Autoanalyser –Star 21 model. In Liver diseases, the conversion and clearance of Mono and Di Bilirubin Glucuronoid hampers significantly, thus serum level of free and conjugated Bilirubin is elevated.
 - Platelet count was done, because in liver diseases due to diminution of platelet factors, specially I & III, the count decreases.
 - Level of Depression was tested by using 8SQ Scale developed by Quarren & Cattle. Because due to higher level of Nitrogen metabolites in blood, deep depression is a common symptom.

Observations & Results

Table 1

Variables	Group B	GroupA
Age (years)	38.2 ± 11.3	48.1 ± 9.5
Sex (male/female)	4/5	8/1
Body Mass Index	20.3 ± 1.0	21.0 ± 2.3
Total Bilirubin (mg/dL)	0.88 ± 1.05	1.19 ± 3.55
Albumin (g/dL)	3.6 ± 1.2	3.1 ± 0.9
Zinc (mg/dL)	51.2 ± 7.8	50.2 ± 6.7
Ammonia (mg/dL)	33.5± 10.9	24.8 ± 24.8

Prothrombin time	72.1 ± 11.2	69.3 ± 03.9
ALT (IU/L)	41.2 ± 27.3	60.5 ± 33.0
WBC (/UL)	4423 ± 1013	3551 ± 826
Hb (g/dL)	10.2 ± 0.7	9.6 ± 1.1
Serum Glucose	129 mg %	211mg %
C-Peptide Level	0.9 ng/mL	2.044 ng/mL
Aspartate aminotransferase	15 units /L	126 Units /L
Alanine aminotransferase	29 /L	243 Units/L
Alkaline phosphatase	56 U/L.	209 U/L
Gamma-glutamyl transpeptidase	29 U/L.	81 U/L
LDH (Lactate dehydrogenase)	122 U/L	218 U/L
Platelets	267,000 /µL	128,000 /µL
Serum urea mg/dL	75.3 mg /dL	56.09 mg /dL
Level of Depression	Score 8	Score 6

Effect of Zn Supplementation

Table 2

Pre-treatment Zinc (mg/dL)	50.2 ± 6.7	Serum urea level After	Serum urea level before
Zinc after 3-month (mg/dL)	67.33±11.21	77.23± 9.26	56.09 ± 2.13

Discussion

- Low zinc concentrations are common in patients with cirrhosis of the liver. Patients with fulminant hepatic failure and subacute hepatic failure have also been shown to have low serum zinc levels. Zinc supplementation has been tried in HE. It may have a role in mild chronic HE, though further trials are necessary.
- Unequivocal evidence of benefit of oral zinc therapy for treatment of acute HE is lacking. Zinc sulfate and zinc acetate have been used at a dose of 600 mg orally every day in clinical trials observed improved in this study; there was also improvement in mental function in this study. Zinc supplementation also significantly decreased HE grade and corrects blood ammonia levels and improved serum urea score and neuropsychological tests compared with standard therapy. Interestingly this study showed as well administration of zinc in combination in improve zinc plasma levels.
- Zinc administration has the potential to improve hyperammonemia by increasing the activity of ornithine transcarbamylase, an enzyme in the urea cycle. The subsequent increase in ureagenesis results in the loss of ammonia ions. More ammonia is converted in Urea in the presence of enough Zn in the serum, thus the cirrhosis complications related with the higher levels of ammonia were significantly corrected.
- The effect of Zn supplementation was also reflected on corrected platelet count, reduction in serum bilirubin levels, corrected enzymatic profile of hepatic enzymes, normal platelet count, thus we can conclude that Zn supplementation with restoring the normal serum level of Zn is beneficial for the cirrhosis patients.

Conclusion

A limitation of the present study is the small sample size. However, to date, there have been few reports on the long-term clinical efficacy of zinc other than short-term data. Therefore, we believe that the data in the present study are important, and hopefully will lead to further large-scale studies. In conclusion, our results showed that administration of zinc does improve

nitrogen retention in blood. However, a serum zinc level greater than 65 µg (1.22 µmol)/dL after zinc supplementation might be a good predictor of complication free survival of cirrhosis patients.

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