Research Article

Role of Zinc in Patients with Nephrotic Syndrome

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Introduction: Nephrotic syndrome (NS) is one of the most common causes of chronic morbidity in developing countries. This study was conducted to assess the effect of zinc supplementation in patients with NS and to evaluate its association with the serum albumin level, relapse rate, and infection frequency.
Materials and Methods: In this randomized, double blind, placebo-controlled trial study, 60 patients with NS, both with the first episode and first relapse, aged 2-10 years were included. Among the 60 patients, 30 patients with NS receiving zinc were in the zinc group and 30 patients with nephrotic syndrome receiving placebo were in the placebo group. The zinc status was assessed before and after receiving zinc or placebo.
Results: The serum zinc level was significantly lower during relapse (0.54±0.18 and 0.56±0.22), and increased during remission, which is (0.85±0.42) normal in zinc group and remained low (0.69±0.14) in the placebo group. The mean serum albumin level was low during relapse in both groups; it increased 14 days later but was still low.
The difference in the mean percentage of increase of height after 6 months was not statistically significant (3.3±1.2 % vs. 3.3±1.9 %) between the two groups. Nineteen patients (63.3%) in the zinc group developed relapse compared to 15 patients (50%) in the placebo group, but the difference was not statistically significant. Infection occurred in 73.3% after zinc supplementation as compared with 63.3% in the placebo group.
Conclusions: When zinc was given in RDA for short duration doesn't reduce relapse in NS and doesn't change zinc level compared to placebo.

Keywords: Nephrotic Syndrome; Recurrence; Outcome; Zinc, Child.

Running Title: Role of Zinc in Patients with Nephrotic Syndrome

Introduction

Zinc is an important trace element second to iron in the human body. Zinc is present in nearly all body tissues, especially the thyroid, pancreas, and reproductive organs. This mineral is involved in the body's enzymatic reactions, synthesis of proteins, and carbohydrate metabolism. Many studies have shown zinc deficiency by demonstrating clinical response with zinc supplementation without biochemical zinc assessment in Bangladesh [1,2] and by serum zinc assessment around the world [3,4].
Reduced immunological competence, particularly in malnourished children, is attributable to zinc deficiency, an effect that can be reversed by zinc supplementation [1,5,6].

Critical functional consequences of zinc deficiency include childhood growth retardation, impaired immune function, increased rate of infections such as diarrhea and pneumonia, possibly increased rates of mortality as a result of infections, adverse outcomes of pregnancy, and abnormal neurobehavioral development [1-8].
  Children with tall stature have higher hair and plasma zinc levels and children with chronic zinc deficiency have a lower zinc status and short stature [9]. Zinc supplementation increases linear growth and weight gain by a small, but highly significant amount. One study by P. N. Singla et al showed a significant positive correlation between serum zinc and height-for-age.

This study also showed hypoalbuminemia (serum albumin <2.5 g/dl), and anemia (hemoglobin ≤8.0 g/dl) in malnourished children were associated with a significant decline in serum zinc and copper levels [10].

Several studies have demonstrated increased urinary loss of zinc, suggesting alteration in renal tubular secretion or reabsorption of zinc [11-13]. The normal serum zinc level is 66-194 µgm/dl [11-22 µmol/L (0.7-1.4 mg/L)]. A positive correlation has been found between urinary zinc and protein excretion. In spite of high dietary intake and normal intestinal absorption, children with idiopathic NS have zinc deficiency caused by increased urinary zinc loss [12].

Zinc is a highly protein bound mineral, 50% of plasma zinc is bound to albumin and the remainder is bound to other plasma proteins [14]. Other mechanisms for the low zinc level in NS include concomitant nutritional deficiency with reduced oral zinc intake, decreased intestinal absorption, and increased intestinal zinc secretion [15,16].

Most of the patients in our country are poor and malnourished. The immunity level is reduced in NS and patients suffer from recurrent infections, particularly respiratory and gastrointestinal tract infections due to overcrowding and malnutrition. Different studies have showed zinc deficiency is associated with respiratory tract and gastrointestinal tract infection. During NS attacks with a low protein level, a reduction in the serum zinc level is possible. This study was designed to assess the effect of zinc supplementation on infection, relapse rate, and growth. No such study has been conducted in Bangladesh.

Materials and Methods

In this randomized, double blind, placebo-controlled trial study, 60 patients with nephrotic syndrome aged 2-10 years were included. These patients had massive proteinuria ($40mg/m^2/h$), hypo albuminemia (serum albumin <2.5 g/dl), hyper cholesterolemia (serum cholesterol >200mg/dl), generalized edema for the 1st time or the 2nd time (had previous one attack). This study was conducted in the Pediatric Nephrology Department of Dhaka Shishu (Children) Hospital and Chemistry Division of Atomic Energy Centre, Dhaka between July, 2011 and June, 2012. Among the 60 patients, 34 cases were 1st attack NS & 26 cases were 1st relapse NS. No patient was lost in this study.

The patients with NS who had hematuria, hypertension, reduced C3 level, and patients with impaired renal function were excluded from the study. Also, patients with secondary NS (e.g. nephrotic syndrome due to hepatitis, malaria, HIV, syphillis, lymphoma, leukemia, drug induced NS (e.g. gold, penicillamine, NSAIDs, etc.) and congenital NS were excluded. None of these patients were suffering from any sort of infection like hepatitis, tuberculosis, etc.

Randomization was done using the lottery method-- 60 small pieces of paper were equally marked with hidden numbers (1001/1002). The patients were allowed to take a piece of paper randomly. Double blinding was done by having identical syrup bottles labeled with numbers (1001 & 1002), which were allocated to the children in the 2 groups chronologically according to their serial number within each group.

NS patients with the 1st attack & the 1st relapse were received prednisolone at a dose of $60mg/m^2$/day. When their urine became protein free for 3 consecutive days, along with prednisolone treatment ($60mg/m^2$/day in patients with the 1st attack of NS & $60mg/m^2$ every alternate day in patients with the 1st relapse of NS), one group received zinc (elemental) at a dose of 2 mg/kg/day (10mg/5ml) 2 hours after meal and the other group received placebo for 14 days. Both drugs were coded by the pharmaceutical company. Serum zinc level was assayed by chemistry division of atomic energy center of Bangladesh. Serum zinc levels were measured during relapse and 14 days after zinc/placebo supplementation. Two milliliter venous blood was collected for measurement of the serum zinc level. The Flame Atomic Absorption Spectrometer (Varian AA Duo 240FS/280Z) was used for analysis.

The concentration of zinc in serum samples was calculated using the following formula:

$$\frac{\text{Absorption of sample}}{\text{Slope of the calibration curve}} \times \text{Dilution factor}$$

A statistical analysis was carried out using the SPSS, version 16.0 (SPSS Inc., Chicago, Illinois, USA). Mean values were calculated for continuous variables. The Chi-Square test was used to analyze categorical variables, shown with cross tabulation. The Student t-test and paired t-test were used for continuous variables. Pearson's correlation coefficient was used to evaluate the correlation between continuous variables. P values <0.05 were considered significant.

Written approval of the Ethics Review Committee of Dhaka Shishu Hospital was obtained. Written consent was obtained from parents (after explanation) before doing the procedure.

Results

Results of the study are classified in tables 1-10. Table 1 shows the age distribution of the study patients. The majority of the patients, 13 (43.3%) in group I and 11 (36.7%) in group II, were 2-4 years in both groups. Other results are presented in the table.

Table 1. Age distribution of the study patients (n=60).

Age (years)	Group I (n=30)		Group II (n=30)	
	n	%	n	%
2-4	13	43.3	11	36.7
>4-6	6	20.0	9	30.0
>6-8	5	16.7	6	20.0
>8-10	6	20.0	4	13.3

ns=not significant
P value by chi square test

Table 2 shows the sex distribution of the study patients. There were 22 (73.3%) males in group I and 15 (50.0%) males in group II. There were 8 (26.7%) and 15 (50.0%) females in group I and group II, respectively. The male to female ratio was 1.6:1. The difference was not statistically significant (P>0.05) between the two groups using the chi square test.

Table 2. Sex distribution of the study patients (n=60).

Sex	Group I (n=30)		Group II (n=30)		P value
	n	%	n	%	
Male	22	73.3	15	50.0	0.063[ns]
Female	8	26.7	15	50.0	

ns=not significant
P value by chi square test

Table 3 shows the BSA status of the study patients. On day 1, ++++ was found in both groups in all children. On day 14, + was found in 14 (46.7%) patients in group I and 11 (36.7%) patients in group II. The difference on day 14 was not statistically significant (P>0.05) between the two groups using the chi square test.

Table 3. Distribution of study patients according to BSA status (n=60).

BSA status	Group I (n=30)		Group II (n=30)		P value
	n	%	n	%	
Day 1					
++++	30	100.0	30	100.0	
Day 14					
Nil	16	53.3	19	63.3	0.432[ns]
(+)	14	46.7	11	36.7	

s= significant, ns= not significant
P value by chi square test

Table 4 shows the mean zinc status of the study patients. In the first sample, the mean zinc level was 0.54±0.18 in group I and 0.56±0.22 in group II. The mean difference was not statistically significant (P>0.05) between the two groups using unpaired t-test.

In the second sample, the mean zinc level was 0.85±0.42 in group I and 0.69±0.14 in group II. The mean difference was not statistically significant (P>0.05) between the two groups using unpaired t-test. There was a statistically significant (P<0.05) difference in the zinc level between the first sample and the second sample in both groups using paired t-test. The mean (±SEM) percentage of increase was 72.16±16.26% and 45.49±11.79% in group I and group II, respectively. The difference was not statistically significant (P>0.05) between the two groups using unpaired t-test.

Table 4. Mean distribution of the study patients according to zinc level (n=60).

Zinc level	Group I (n=30)		Group II (n=30)		P value
	Mean	± SD	Mean	± SD	
First sample	0.54	±0.18	0.56	±0.22	[a]0.837[ns]
Range (min-max)	0.22	-0.88	0.26	-0.94	
Second sample	0.85	±0.42	0.69	±0.14	[a]0.058[ns]
Range (min-max)	0.49	-2.50	0.38	-0.99	
P value	[b]0.001[s]		[b]0.006[s]		
% of Increase (Mean±SEM)	72.16	±16.26	45.49	±11.79	[a]0.189[ns]
Range (min-max)	-22.40	-303.70	-43.4	-193.0	

s= significant, ns= not significant
[a]P value by unpaired t-test
[b]P value by paired t-test.

Table 5 shows the mean haemoglobin level of the study patients. The mean haemoglobin level was 11.89±2.69 in group I and 11.37±2.13 in group II.

The mean difference was not statistically significant (P>0.05) between the two groups using unpaired t-test.

Table 5. Mean distribution of study patients according to hemoglobin level (n=60).

Hemoglobin level	Group I (n=30)		Group II (n=30)		P value
	Mean	**± SD**	**Mean**	**± SD**	
Hemoglobin level	11.89	±2.69	11.37	±2.13	0.418[ns]
Range (min-max)	(7.1	-19.4)	(6.90	-16.8)	

ns= not significant
P value by unpaired t-test

Table 6 shows the mean growth pattern of the study patients. The initial height was 99.9±13.7 cm in group I and 102.67±15.4 cm in group II. The mean difference was not statistically significant (P>0.05) between the two groups using unpaired t-test. After 6 months, height was 103.1±13.5 cm in group I and 106.0±15.1 cm in group II. The mean difference was not statistically significant (P>0.05) between the two groups using unpaired t-test.
A statistically significant (P<0.05) difference was found between the initial height and height after 6 months in both groups using paired t-test.
The mean percentage of increase was 3.3±1.2% and 3.3±1.9% in group I and group II, respectively. The difference was not statistically significant (P>0.05) between the two groups using unpaired t-test.
Table 7 shows the mean alkaline phosphates of the study patients. The mean alkaline phosphates was 300.7±98.3 U/L in group I and 289.0±75.6 U/L in group II. The difference was not statistically significant (P>0.05) between the two groups using unpaired t-test.
Table 8. shows relapse in study patients. No relapse was found in 11 (36.7%) patients in group I and 15 (50.0%) patients in group II. The difference was not statistically significant (P>0.05) between the two groups using Chi square test. The mean relapse was 0.97±0.89 in group I and 0.7±0.79 in group II. The difference was not statistically significant (P>0.05) between the two groups using unpaired t-test.
Table 9 shows the mean serum albumin of the study patients. On day 1, the mean serum albumin was 13.4±1.97 gm/L in group I and 12.8±2.2 gm/L in group II. The mean difference was not statistically significant (P>0.05) between the two groups using unpaired t-test.

Similarly, on day 14, the mean serum albumin was 20.5±1.93 gm/L in group I and 19.6±2.53 gm/L in group II. The mean difference was not statistically significant (P>0.05) between the two groups using unpaired t-test. Paired t-test showed a statistically significant (P<0.05) difference in serum albumin between day 1 and day 14 in both group.
The mean percentage of increase was 54.7±12.2% gm/L and 54.4±13.0% in group I and group II, respectively. Unpaired t-test showed no significant difference (P>0.05) between the two groups.

Table 6. Distribution of study patients according to growth pattern (n=60)

Growth pattern (z score)	Group I (n=30)		Group II (n=30)		P value
	Mean	**± SD**	**Mean**	**± SD**	
Initial height (z score)	99.9	±13.7	102.67	±15.4	[a]0.740[ns]
Range (min-max)	(79.5	-135)	(82	-137)	
height after 6 months (z score)	103.1	±13.5	106.0	±15.1	[a]0.778[ns]
Range (min-max)	(83	-138)	(85	-139)	
P value	[b]0.001[s]		[b]0.001[s]		
Percentage of Increase	3.3	±1.2	3.3	±1.9	[a]0.947[ns]

s= significant, ns= not significant
[a]P value by unpaired t-test
[b]P value by paired t-test.

Table 7. Mean distribution of the study patients according to alkaline phosphates (n=60).

	Group I (n=30)		Group II (n=30)		P value
	Mean	**± SD**	**Mean**	**± SD**	
Alkaline phosphates (U/L)	300.7	±98.3	289.0	±75.6	0.608[ns]
Range (min-max)	(126	-500)	(172	-444)	

ns=not significant
P value by unpaired t-test.

Table 10 shows the infection rate of the study patients. First-time infection was found in 6 (20.0%) patients in group I and 3 (10.0%) patients in group II. Second-time infection was seen in 6 (20.0%) patients in group I and 13 (43.0%)

Table 8. Distribution of the study patients according to relapse (n=60).

Number of relapse	Group I (n=30)		Group II (n=30)		P value
	n	%	n	%	
No relapse	11	36.7	15	50.0	
Had relapse	19	63.3	15	50.0	[a]0.297[ns]
1 time	10	33.3	9	30.0	
2 times	8	26.7	6	20.0	
3 times	1	3.3	0	0.0	
Mean ± SD	0.97	±0.89	0.7	±0.79	[b]0.219[ns]
Range (min-max)	(0	-3)	(0	-2)	

ns=not significant
[a]P value by Chi square test
[b]P value by unpaired t-test.

Table 9. Mean distribution of study patients according to serum albumin (n=60).

Serum Albumin (gm/L)	Group I (n=30)		Group II (n=30)		P value
	Mean	± SD	Mean	± SD	
Day 1	13.4	±1.97	12.8	±2.2	[a]0.322[ns]
Range (min-max)	(8	-17)	(8	-17.4)	
Day 14	20.5	±1.93	19.6	±2.53	[a]0.126[ns]
Range (min-max)	(15	-24)	(13	-25)	
P value	[b]0.001[s]		[b]0.001[s]		
Percentage of Increase	54.7	±12.2	54.4	±13.0	[a]0.912[ns]

s= significant, ns= not significant
[a]P value by unpaired t-test
[b]P value by paired t-test

Table 10. Infection rate in study patients (n=60).

Infection rate	Group I (n=30)		Group II (n=30)	
	n	%	n	%
1st time	6	20.0	3	10.0
2nd time	6	20.0	13	43.3
3rd time	5	16.7	0	0.0
4th time	4	13.3	2	6.7
5th time	1	3.3	1	3.3
6th time	0	0.0	0	0.0
7th time	1	3.3	0	0.0

ns=not significant
[a]P value by Chi square test
[b]P value by unpaired t-test.

patients in group II. Third-time infection was seen in 5 (16.7%) patients in group I and none of the patients in group II. Fourth-time infection was found in 4 (13.3%) patients in group I and 2 (6.7%)

patients in group II. Fifth-time infection was detected in 1 (3.3%) patient in group I and 1 (3.3%) patient in group II. Seventh-time infection was seen in 3 (3.3%) patients in group I and none of the patients in group II.

Discussion

This study showed that abnormalities in the zinc level occur in patients with NS during relapse. The serum zinc level was significantly low in children with NS during relapse in both groups. Reimond EW [14], Tumer N et al [15] and Mahajan et al [17] also found significantly low serum zinc levels in children with NS during relapse. In this study, we found an increase in the serum zinc level during remission in both groups. The mean difference was not statistically significant (P>0.05). The same observation was reported by Reimond EW [14] who compared the serum zinc level with the serum zinc level of the same patient during remission. In our study, the mean percentage of the increase in the zinc level between the two groups was not statistically significant (P> 0.05). In the control group, we did not use zinc supplementation but the zinc level increased, probably due to the increased serum protein level during remission which explains the relationship between zinc with serum protein.

Reimond EW [14] found an increase in the serum zinc-albumin ratio in the presence of proteinuria, indicating a proportionately larger albumin loss. They also found that all patients with NS had decreased serum albumin; they maintained a low serum albumin level as long as proteinuria was present, even during corticosteroid therapy. In our study, the mean serum albumin level was 13.4±1.97 gm/L in the zinc group and 12.8±2.2 gm/L in the placebo group during relapse. After 14 days, it increased but was still low in both groups. The protein status and food values of patients are different in the USA and a developing country like Bangladesh, which might be a probable explanation for different results. We did not measure urinary zinc excretion due to budget limitations.

Singla et al showed a significant positive correlation between serum zinc and height for Age [12]. Zinc deficiency impairs growth by interfering with nucleic acid metabolism & protein synthesis [18-20]. Rivera et al showed daily administration of 10 mg zinc for 7 months positively affected length increments of infants who were initially stunted [21,22]. In our study, the mean percentage of increased height after zinc

supplementation with RDA for 14 days was 3.3±1.2 % and 3.3±1.9 % in two groups respectively, with no significant difference (P>0.05), probably due to the short course of zinc supplementation.

Arun et al showed that patients with SSNS those who receiving RDA level of zinc supplementation for 12-months period had fewer relapses and higher likelihood of remission. Frequent relapsers showed 28% fewer relapses [20]. We did not observe this finding. No change of relapse rate was found 11(36.7%) in zinc group and 15(50.0%) in placebo group. Nineteen (19) patients (63.3%) in the zinc group developed relapse compared to 15 patients (50%) in the placebo group. Among these 19 patients in the zinc group, 9 had frequent relapses and 10 had infrequent relapses; in the placebo group, 6 had frequent relapses and 9 had infrequent relapses. The difference was not statistically significant (P>0.05) between two groups. Arun et al showed supplementation with zinc led to fewer infections, although the difference was not significant [20]. Also, 73.7% of the relapses in the zinc group were preceded by infections compared to 50% in the placebo group. In this study, we found that 76.6% of the infections occurred after zinc supplementation as compared with 63.3% in the placebo group. The most common infection was RTI, followed by UTI, septicemia, and scabies. According to their study, reduce relapse rate in the supplemented patients was not due to either prior zinc deficiency or fewer infection related relapses [20].

The prevalence of hypozincemia in NS is not yet fully understood [19]. We did not find any significant differences in the serum zinc level in the intervention group after supplementation as compared to the placebo group. Since we used zinc at RDA doses for 14 days, if the duration of supplementation was longer, we might have seen different results.

Conclusions

There is no significant increase in the zinc level compared to placebo when zinc is given at RDA doses for a short duration. Zinc supplementation for a short duration does not reduce the relapse rate in NS.

Conflict of Interest

None declared

Financial Support

None declared

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