Original Article (Galley Proof Copy)

Zinc as a Supplement in Reduction of Relapses in Children with Nephrotic Syndrome.

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ABSTRACT

Introduction: 80% of plasma zinc (Zn) is bound to albumin which is lost in urine in nephrotic syndrome (NS) during relapse period. Zinc supplementation reduces mortality and morbidity, especially among children due to infectious diseases, as it also has immune-modulator effects.

Aim of the study: To study the efficacy of zinc supplementation in reducing the relapse and infection rates in children with nephrotic syndrome.

Methods: This observational prospective study was conducted at the pediatric nephrology clinic of our hospital. It comprised 40 patients with frequent relapsing and/or steroid dependent NS. The patients were divided into two groups, the first group received zinc supplements (zinc group) and the second group were maintained on their usual treatment without zinc supplementation (and served as a control group). Both received routine medical care of nephrotic syndrome, but zinc group received also oral zinc supplementation 10 mg/day for 6 months with doubling the dose during infection period and relapse time. Serum zinc was measured at the start and at the end of the study. Relapse rate, hospitalization rate, number of days of hospitalization, time of relapse, and investigations were compared before and after zinc supplementation.

Results: There was low serum zinc level in most cases of frequently relapsing / or steroid dependent nephrotic syndrome. Oral zinc supplementation in FRNS/ or steroid dependent NS, improved serum zinc level by 22.72%, reduced relapse rate by 28% and reduced hospitalization days by 33.7%. Although there was no significant improvement as regards hospitalization rate and time of relapse, sustained remission occurred in 80% of patients of zinc group.

Conclusion: Oral zinc supplementation in frequently relapsing and in steroid dependent nephrotic syndrome children is useful for reduction of relapse rate, hospital stay and for achieving sustained remission.

Key words: Nephrotic syndrome, relapses, serum albumin, serum zinc level

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INTRODUCTION

After initial successful treatment of nephrotic syndrome (NS), about 80% of children suffering from steroid sensitive nephrotic syndrome experience disease relapses which require additional courses of high dose prednisolone. Nearly half develop repeatedly relapsing nephrotic syndrome (at least two relapses during a time span of six months of presentation or 4 relapses within 12 months) or steroid dependent nephrotic syndrome (relapse while receiving prednisolone or within 2 weeks since stopping the drug [1].

Zinc (Zn) is an essential trace element which acts as a cofactor for certain enzymes which is required for metabolism and cell growth and is present in approximately 300 specific enzymes [2]. Nearly 75–80% of plasma zinc is bound to albumin, making up to 98% of the exchangeable fraction of Zn in blood plasma. Serum albumin functions as an extracellular "zinc buffer" which aids in the control of the concentrations of "free" Zn2+ ions that are available to other plasma proteins or for cellular uptake through membrane-bound zinc transporters [3].

There was an observed positive correlation between urinary zinc and protein excretion. Children with NS suffer from zinc deficiency which is caused by an increase in the urinary zinc losses despite high dietary intake and normal intestinal absorption [4]. A decrease in serum zinc level occurs throughout NS attacks with low protein level. Concomitant nutritional deficiency with reduced oral zinc intake, decreased intestinal absorption, and increased intestinal zinc secretion are mechanisms that resulted in decreased zinc level in NS [5].The mean serum zinc level in frequently relapsing cases of nephrotic syndrome is significantly lower than that of infrequently relapsing cases [6].

There is strong evidence from developing countries that zinc supplements are able to decrease morbidity, especially among children due to infectious diseases [7]. There is a suggestion that zinc deficiency might result in down-regulation of T-helper 1 (Th1) cytokines, leading to a relative T-helper 2 (Th2) cell over activity hence a greater risk of clinical infections [8]. Supplementation of zinc could lead to re-establishment of Th1 immune response through a possible augmentation of gene expression for interleukin (IL)-2 and interferon-α [9]. The Th1/Th2 cytokine imbalance is thought to be involved in relapses of steroid sensitive nephrotic syndrome (SSNS) [9].

Our objective was to study the efficacy of zinc supplementation in reducing the relapse and infection rates in children with frequently relapsing or with steroid dependent nephrotic syndrome.

METHODS

Patients

This interventional study was conducted at the pediatric nephrology clinic at our hospital. It was conducted on 40 patients with frequently relapsing and/or steroid dependent nephrotic syndrome, divided into two groups, 20 patients in the first group with zinc supplementation, the other group, served as control group, and consisted of 20 patients, without zinc supplementation. The work was approved by the Ethical Committee of Pediatric Department, Faculty of Medicine at Ain Shams University, and an informed written

consent was obtained in every case from their legal guardians.

Population

The study included children above 2 years old with frequent relapsing and/or steroid dependent nephrotic syndrome with at least one year from the initial diagnosis. Children with congenital nephrotic syndrome, malnutrition, malabsorption, hepatic problems, and those with other chronic systemic illness were excluded from the study.

Patients were divided into 2 groups:

- 1st group: frequent relapsing and steroid dependent nephrotic syndrome continued on the standard therapy of nephrotic syndrome in addition to zinc supplementation on 10 mg daily for 6 months. Dose has been doubled to 20 mg/day for two weeks in case of relapse, infection, and recorded deficient serum zinc level [14].
- 2nd group: frequent relapsing and/or steroid dependent nephrotic syndrome continued the standard (steroids and other immunosuppressive medications as Mycophenolate Mofetil or calcineurin inhibitors) therapy and served as a control group.

Methods

All patients were subjected to the following:

a) Thorough history taking & medical records revision laying stress on:

- Demographic data (age and sex)
- Age of onset of NS, number of relapses and infections associated with relapses during the preceding one year before the start of the study.
- Detailed dietetic history stressing on zinc intake.

Patients were monitored for episodes of infections, number of relapses, infections associated with relapse before and after zinc supplements, and side effects have been reported and analyzed.

b) Clinical examination:

Routine general clinical examination and stress on complications of nephrotic syndrome, and infections.

c) Laboratory & Radiological evaluation:

Serum total zinc level was done for all patients of the two groups at start of the study and after 6 months, beside to routine laboratory investigations (CBC, urine analysis, 24 hours urinary proteins, serum albumin, serum cholesterol).

Statistical Analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (IBM Corp. Released 2017). (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

a) Descriptive statistics:

1. Shapiro wilk's test was used to evaluate normal distribution of continuous data.

2. Frequency and percentage of non - numerical data.

b) Analytical statistics:

1. Student T Test.
2. Mann Whitney Test (U test)
3. Chi-Square test
4. Fisher's exact test
5. Paired t-test
6. Wilcoxon signed rank test
7. McNemar test

P- value: level of significance: P>0.05: Non significant (NS), P< 0.05: Significant (S), P<0.01: Highly significant (HS).

RESULTS

No significant differences were observed between zinc and control groups concerning age, sex, age of onset of nephrotic syndrome, number of relapses 12 months prior to the study, previous episodes of infections at the start of the study, serum albumin, 24 hours urinary proteins, urinary albumin, serum zinc level at the start of the study. There was a high significant change of serum zinc level after 6 months supplementation of zinc, with no abnormally high serum zinc level (toxic level) was recorded after 6 months of zinc supplementation.

At the end of the study the number of relapses, urinary albumin, and the duration of hospitalization were significantly decreased in the zinc group compared to the control group, also the serum albumin was significantly higher in the zinc group than the control group. However, no significant differences were found between both groups regarding hospitalization rate and time of occurrence of relapse after the start study.

Table 1: Comparison between the two study groups at start of the study as regards demographic data, medical history and lab investigations

		Zinc group		Control group		P	Sig
		Mean	±SD	Mean	±SD		
Age (years)		8.85	3.47	10.15	3.41	0.239*	NS
Sex	**Male**	15	75.0%	14	70.0%	0.723**	NS
	Female	5	25.0%	6	30.0%		
Age of onset of NS (years)		4.65	2.23	4.15	1.42	0.403*	NS
Number of relapses one year prior to the study		5.55	1.43	5.05	0.83	0.184*	NS
Infections one year prior to the study	**No**	11	55.0%	9	45.0%	0.527*	NS
	Yes	9	45.0%	11	55.0%	*	
Serum albumin (g/dl)		2.61	0.29	2.70	0.28	0.147*	NS
24 hours urinary proteins (g/day)		2.40	0.90	2.04	.76	0.179*	NS
Urine cast	**Negative**	8	40.0%	8	40.0%	1.0*	NS
	Positive	12	60.0%	12	60.0%		
Urinary Albumin	**0**	7	35.0%	5	25.0%	0.596*	NS
	1+	1	5.0%	3	15.0%	*	
	2+	3	15.0%	4	20.0%		
	3+	9	45.0%	8	40.0%		

*Student t Test **Chi-Square Tests

Table 2: Zinc compliance and side effects among zinc group cases

Zinc compliance	**Good**	16	80.0%
	Poor	4	20.0%
Side effects during 6 months	**Negative**	17	85.0%
	Positive(GIT upset)	3	15.0%

Table 3: Comparison between the two study groups as regards serum zinc level at the start and at the end of the study and degree of change before and after supplementation.

	Zinc group		Control group		P	Sig
	Mean	±SD	Mean	±SD		
Serum zinc at start of study (µg/dl)	69.70	10.28	73.40	10.53	0.268*	NS
Serum zinc at end of study (µg/dl)	84.80	11.14	72.30	6.71	**0.001***	**HS**

*Student t Test *Mann-Whitney Test

Table 4: Medical history and lab investigations among the two study groups at the end of the study

		Zinc group		Control group		P	Sig
		Mean	±SD	Mean	±SD		
Duration of hospitalization (in days)		3.38	0.92	5.10	1.20	**0.004***	**HS**
Number of relapses		1.90	0.79	2.55	0.69	**0.008***	**HS**
Time of relapses from study start (in weeks)		7.70	3.53	8.65	3.79	0.417*	NS
Hospitalization	**Negative**	15	75.0%	14	70.0%	0.525**	NS
	Positive	5	25.0%	6	30.0%		
Serum albumin (g/dl)		2.86	0.25	2.71	0.20	**0.042***	**S**
Urinary Albumin	**0**	16	80.0%	6	30.0%	**0.009‡**	**HS**
	1+	4	20.0%	2	10.0%		
	2+	0	0.0%	3	15.0%		
	3+	0	0.0%	9	45.0%		

*Student t Test **Chi-Square Tests ‡ Fisher's Exact Test

Table 5 : % of change (improvement) of duration of hospitalization and rate of relapses between the two study groups at end of the study

% Of change between the two study groups at end of the study	Duration of hospitalization	Number of relapses
	33.7%	25%

DISCUSSION

A high prevalence of zinc (Zn) deficiency was observed in patients with nephrotic syndrome. This could be attributed to hypoalbuminemia and increased twenty four hour urinary protein losses **[10].**

At the start of the study, the two studied groups showed no significant differences as regards age, sex, age of onset of nephrotic syndrome, number of relapses 12 months prior to the study and last year episodes of infections. Also, there were no significant differences in 24 hours urinary proteins or urinary albumin.

Serum zinc level done for patients of the two groups at the start of the study which was abnormal low level in 90% of patients of zinc group (mean 69.70µg/dl) and 80% of patients of control group (mean 73.40µg/dl). These results are consistent with Ghafoorimehr et al., 2019 **[11]** who reported that, Zinc deficiency is common in children suffering from nephrotic syndrome and is related to recurrence of relapses. Also Hashim & Jabur, 2020 **[6]** reported that in children with frequently relapsing NS, the mean serum zinc level (58.45 µg/dl) was significantly less than that of infrequent relapsers (61.58 µg/dl) and the control normal group (89.64 µg/dl) respectively.

In the present study, after 6 months zinc supplementation, serum zinc level showed a high significant change (mean 84.80µg/dl) by the end of the study and improvement of serum zinc level by 22.72%. Diet modification before and

during study time presented no significant differences between the patients of the two groups. This indicated that there was no influence of diet on the results of the study. No abnormal high level (toxic level) was recorded during the study time and side effects were mild GIT upset and recorded in 3 cases (15 %). However, poor compliance was reported in 4 cases (20%).

In the present study, serum zinc level at the start of the study was not significantly different (P = 0.268) compared to its level at the end of the study in the control group. At the end of the study, serum zinc level was significantly increased (P = 0.001) in patients supplied with zinc than in patients of the control group.

Oral zinc supplement seems to be effective for treating zinc deficiency of nephrotic syndrome with no fear of zinc toxicity. This is consistent with Haque et al, 2017 [4] who showed that serum zinc level was significantly lower during relapse (0.54±0.18) increasing throughout remission, which is (0.85±0.42) normal in zinc group (receiving zinc supplement) and remained low (0.69±0.14) in placebo group.

In the current study, after 6 months oral zinc supplementation, serum albumin, urine casts and urine albumin showed a high significant improvement in the zinc group, also no nephrotic range proteinuria (3+ Albumin) was reported after 6 months zinc supplementation.
Eighty percent of cases of zinc group were without proteinuria after 6 months zinc supplementation. These results indicated that, sustained remission occurred in 80% of cases after zinc supplementation during the short period of the study (6 months).

After 6 months of zinc supplementation, relapse rate was significantly (P=0.008) reduced in zinc group with improvement by 25%. These results are consistent with Arun et al, 2009 [12] who reported 28% reduction in relapse rates and a significantly higher likelihood of sustained remission was observed in patients with frequent relapses receiving zinc.

In the current study: serum albumin, and urine albumin before and after the study showed no significant differences in the control group. These results emphasize the importance of zinc supplements for reducing relapse rate and obtaining more sustained remission in frequently relapsing nephrotic syndrome. The present study reported no significant difference between the two study groups as regards time of relapse. These results could be attributed to the short period of zinc supplement which were only 6 months.

In the present study, duration of hospitalization was reduced significantly (p=0.004) in patients supplied with zinc compared to the control group patients. Hospital stay in zinc group was 3.38±0.92 days and in control group was 5.10±1.20 days during study period with improvement by 33.7%. Kumar et al., 2017 [13] also concluded that Zinc significantly reduced the duration of hospital stay and remission in children suffering from nephrotic syndrome.

The present study results are consistent with the systematic review of Bhatt et al., 2016 [14] which reported that zinc reduced the frequency of relapses, induced sustained remission (no relapse), reduced the proportion of infection episodes associated with relapse. On the other hand, Bhatt et al, 2016 [14] did not report significant differences in episodes

of infection in zinc group which can be attributed to low number of cases, some cases still have abnormal low level of serum zinc (40%), and poor compliance to zinc supplementations in 20% of cases.

CONCLUSION

Zinc supplementation in frequently relapsing and/or steroid dependent nephrotic syndrome in children, can be safely added to the usual treatment. This supplement may reduce relapse rate, reduce hospital stay, and may be an adjuvant in obtaining sustained remission.

ABBREVIATIONS

Zn	Zinc
NS	Nephrotic syndrome
FRNS	Frequently Relapsing Nephrotic Syndrome
SDNS	Steroid Dependent Nephrotic Syndrome
SSNS	Steroid Sensitive Nephrotic Syndrome
Th1	T-helper 1
Th2	T-helper 2
IL-2	Interleukin 2

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AUTHORS' CONTRIBUTIONS

The submitted manuscript is the work of the author and co-authors.

All authors have contributed to authorship, and have read and approved the manuscript.

Conception and design of the study: all authors

Acquisition of data: 3rd author

Analysis and/or interpretation of data: first and second authors

Drafting the manuscript: all authors

Revising the manuscript critically for important intellectual content: 1st and last author

Approval of the version of the manuscript to be published: all author.

STATEMENTS

Consent for publication

The attached manuscript its contents and materials have not been previously reported at any length or being considered for publishing elsewhere.

Ethics approval and consent to participate

This study protocol and the consents were approved and deemed sufficient by Ethical Committee of Pediatric Department, Faculty of Medicine, Ain Shams University. And informed written consent was obtained in every case from their legal guardians

Availability of data

The authors have indicated that the data and material are factual and genuine.

The corresponding author, [YR], may provide the data that back up the study's conclusions upon request.

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Conflict of interest

No

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