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BRIEF REPORT

Efficacy of zinc supplements in reducing relapses in steroid-sensitive nephrotic syndrome

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Abstract Relapses in steroid-sensitive nephrotic syndrome (SSNS) often follow infections of the respiratory or gastrointestinal tract. Based on data that zinc supplements reduce the risk of infections, we examined the efficacy of such supplements in reducing relapse rates in these patients. Eighty-one patients with SSNS (1–16 years old) were stratified into frequent (n=52) and infrequent (n=29) relapsers and randomized to receive 12-months of therapy with the recommended dietary allowance of zinc (10 mg/day) (n=40) or placebo (n=41). Patients with frequent relapses also received long-term, alternate-day prednisolone. Subjects receiving zinc showed a 20% lower frequency of relapses, with 44.7% of the patients having sustained remission compared to 27.5% in the placebo group (P>0.05). Patients with frequent relapses receiving zinc showed a 28% reduction in relapse rates and a significantly higher likelihood of sustained remission (P=0.02). Findings from this double blind, randomized study suggest that zinc supplementation results in trends towards remission and reduced relapses, especially in patients with frequent relapses. Prospective, adequately powered studies are required for confirmation of these findings.

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Introduction

Patients with steroid-responsive nephrotic syndrome (SSNS) show remission of proteinuria with steroids, but often relapse following infections of the respiratory or gastrointestinal tracts [1]. The pathogenesis of SSNS and the basis for infection-triggered relapses are unclear. Evidence of perturbed cell-mediated immunity and its association with atopy, elevated levels of immunoglobulin E and upregulated gene expression for interleukin (IL)-4 and IL-13 suggest T-helper 2 (Th2) cytokine bias [2, 3].

Studies in patients with SSNS show low blood levels of zinc [4, 5]. While the consequences of zinc deficiency are unclear, there is evidence that this might lead to a downregulation of Th1 cytokines, a relative Th2 bias and an increased risk of infections [6, 7]. Data from meta-analyses suggest that the administration of zinc to children results in a reduced incidence of diarrhea and respiratory infections [8]. Taking the pivotal role of infections in precipitating relapses into consideration, we hypothesized that zinc supplementation should reduce the frequency of infections and thereby relapses. The aim of the study reported here was to examine if the administration of zinc (at the recommended daily allowance, RDA) for 1 year to patients with SSNS would reduce relapse rates.

Subject and methods

This randomized, double blind, placebo-controlled study was conducted from June 2004 to May 2006. Children aged 1–

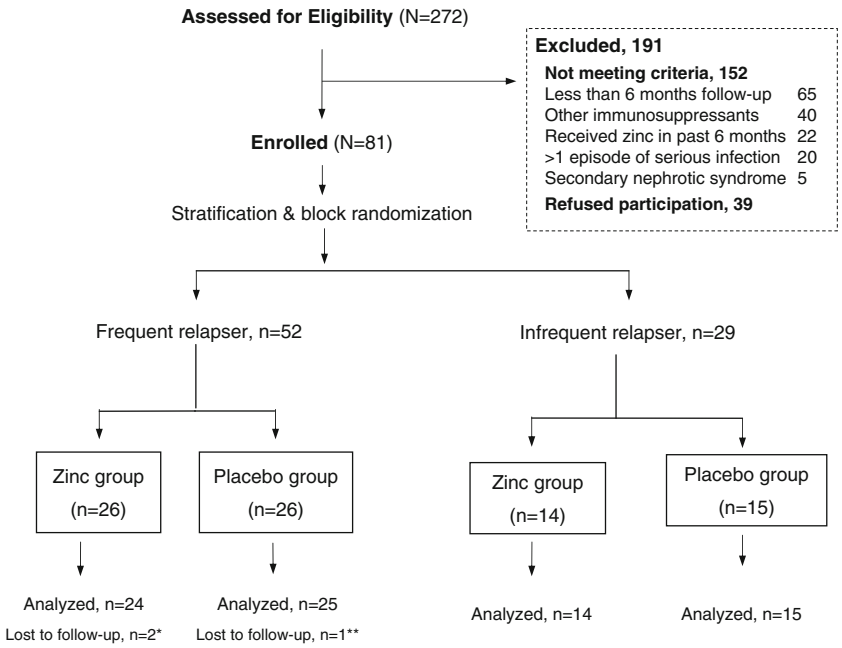

Fig. 1 Trial flow. Of 272 patients assessed for eligibility, 81 (52 frequent relapsers, 29 infrequent relapsers) were enrolled. Three patients with frequent relapses were lost to follow-up: one each at 3 months and 9 months (*single asterisk*), and one at 3 months (*double asterisk*)

16 years who either had SSNS with infrequent relapses or who were frequent relapsers (>2 relapses in 6-months) [1] and had a prednisolone requirement ≤0.75 mg/kg on alternate days were eligible. Patients with severe malnutrition, chronic infections (e.g. tuberculosis, kala azar) and concurrent or recent (within 6 months) immunosuppressive treatment other than prednisolone were excluded.

Following approval from the Ethics Committee and consent from the parents, the patients were enrolled during remission, when free of infections. They were stratified into infrequent and frequent relapsers and block randomized to receive zinc supplements (10 mg as zinc sulfate syrup) or placebo daily, 1 h before or 2 h after meals for 12 months. Follow-up visits were scheduled at 1, 3, 6, 9 and 12 months. The parents monitored urine protein (dipstick) at home. Relapses (dipstick 3–4+ for 3 consecutive days) were treated with prednisolone at a dose of 2 mg/kg per day until remission, followed by 1.5 mg/kg on alternate days for 4 weeks; treatment was then stopped in those with infrequent relapses. For frequent relapsers, the dosage of prednisolone was tapered by 0.25 mg/kg every 4 weeks until 0.75 mg/kg or less, for 12 months.

Trial deviates were subjects having two relapses in 6 months despite prednisolone dose of ≥0.75 mg/kg on alternate days, or those showing steroid toxicity. Zinc levels were estimated by spectrophotometry (GBS Avanta FS 3000 Flamemeter); levels <65 μg/dl were considered to be deficient [9]. Serum levels of interferon (IFN)-γ, soluble IL-2 receptor (sIL-2R) and IL-4 were measured by an enzyme-linked immunosorbent assay (ELISA), at enrollment and at the end of study (Immunotech; Beckman Coulter, Fullerton, CA).

Based on the annual relapse rate of 1.6±0.8 [10], 41 patients were required per group to show a 30% reduction in relapses with treatment, at a power of 80% and alpha error of 5%. Statistical analysis was carried out using Stata ver. 9.0 (StataCorp, College Station, TX). The analysis was based on the intention to treat. Data were expressed as the

Table 1 Baseline characteristics

Variables	Zinc (n=40)	Placebo (n=41)
Age at onset of nephrotic syndrome (months)	46.2±26.1	41.4±26.7
Age at enrollment (months)	95.2±41.8	88.4±38.3
Boys (n)	27 (67.5%)	26 (63.4%)
Adequate treatment of first episode (n)[a]	34 (85%)	38 (92.7%)
Relapses in preceding 6 months	1.2±0.6	1.2±0.7
Cumulative prednisolone dose (mg/kg per 6 months)	58.3±43.9	60.5±52.5
Serum zinc level (μg/dl)	114.5±71.9	108.1±39.7
Serum creatinine (mg/dl)	0.57±0.13	0.58±0.12
Serum albumin (g/dl)	4.0±0.6	4.0±0.6

Unless otherwise indicated, values are given as the mean ± standard deviation

[a] Prednisolone 2 mg/kg for 6-weeks, then 1.5 mg/kg/alternate days for at least 6-weeks

Table 2 Number of relapses (mean) in patients with steroid-sensitive nephrotic syndrome receiving either zinc supplements or placebo

Duration of therapy	Zinc (n=40)	Placebo (n=41)	Effect size (95% CI)
At 6 months	n=39	n=40	
	0.49±0.79	0.68±0.92	-0.19 (-0.57, 0.19)
At 12 months	n=38	n=40	
	1.0±1.16	1.2±1.11	-0.2 (-0.71, 0.31)
Frequent relapser subgroup			
At 6 months	n=25	n=25	
	0.52±0.0	0.68±0.8	-0.16 (-0.6, 0.3)
At 12 months	n=24	n=25	
	1.04±1.2	1.32±1	-0.28 (-0.9, 0.4)

Values are given as the mean ± standard deviation, with the exception of "Effect size" for which the difference in means and the 95% confidence (CI) are given

Number of patients (n) is different at follow-up, since two patients in zinc and one in the placebo groups were lost to follow-up

mean ± standard deviation (SD) and by the difference in means with 95% confidence intervals (CI); the effect size was expressed as the risk ratio (RR). The Cox time proportion method was used to determine the hazard ratio (HR) and person time-based analysis for the number of relapses.

Results

Of the 120 eligible patients, 39 refused consent; ultimately, 52 patients with frequent relapses and 29 with infrequent relapses were included. Baseline characteristics were similar in patients allocated to treatment with zinc and placebo (Table 1). Thirty-eight subjects in the zinc group and 40 in the placebo group completed the study (Fig. 1). Three patients in each group were trial deviates and were treated with cyclophosphamide or levamisole.

There was a 20% reduction in mean relapse rates in zinc-supplemented patients as compared to those receiving the placebo (P>0.05) (Table 2). While a higher proportion of patients receiving supplements had sustained remission at 12 months (Table 3), the differences were not significant. The former group had a 31% lower risk of relapse (RR 0.69, 95% CI 0.45–1.07; P>0.05) and showed a trend for longer time to first relapse (mean 7.9 vs. 6.4 months, respectively; P>0.05). There was no correlation between the time to first relapse or number of relapses and baseline zinc levels (P=0.4). After adjusting the number of relapses for age at onset and enrollment, adequacy of initial steroid therapy and zinc levels, the placebo group showed a 16% higher risk of relapses (HR 1.16, 95% CI 0.55–1.30) compared to those receiving zinc. Three patients receiving zinc complained of metallic taste, which resolved without intervention.

Subgroup analysis among the frequent relapsers showed that the mean number of relapses was 28% less among patients receiving zinc (P>0.05) (Table 2). A significantly higher proportion of these patients showed sustained remission (RR 0.64, 95% CI 0.43–0.96; P=0.02) (Table 3). Although patients receiving zinc showed fewer episodes of diarrhea (0.1±0.4 in zinc vs. 0.2±0.5 in placebo groups) and upper respiratory infections (2.1±1.1 vs. 2.4±1.6, respectively), the differences were not significant. Further, the proportion of relapses that were preceded by infections in the zinc group was 73.7% (28/38), compared to 50% (24/48) in the placebo (RR 1.46, 95% CI 1.05–2.04; P=0.02). Five patients (two in zinc and three in placebo group) had a zinc deficiency at inclusion. At 12 months, the respective zinc levels were 99.3±65 and 78.6±25 μg/dl. Blood levels of sIL-2R, IFN-γ and IL-4 were similar at enrollment and follow-up (data not shown).

Table 3 Patients with sustained remission among those receiving zinc supplements or placebo

Number of relapses	Zinc (n=38)	Placebo (n=40)	P value
No relapses	17 (44.7)	11 (27.5)	
≥1 relapses	21 (55.3)	29 (72.5)	0.1
Frequent relapser subgroup	n=24	n=25	
No relapses	11 (45.8)	4 (16)	0.02
≥1 relapses	13 (54.2)	21 (84)	

Values in parenthesis show proportions

Discussion

Our findings suggest that patients with SSNS receiving supplementation with RDA of zinc show a trend towards fewer relapses and higher likelihood of remission. The response was better in the frequent relapsers who showed 28% fewer relapses, with a significantly increased proportion of patients in this group showing sustained remission. While previous studies have reported low blood levels of zinc in patients with SSNS [4, 5], only five subjects in this study had zinc deficiency. Supplementation with this micronutrient led to fewer infections, although the differences were not significant. Furthermore, 73.7% relapses in the zinc group were preceded by infections compared to 50% receiving the placebo. These results imply that the trend towards a reduction of relapses in the supplemented participants was not due to either prior zinc deficiency or fewer infection-related relapses. While this conclusion is contrary to our initial hypothesis, the results suggest that the administration of zinc benefits patients with SSNS through as yet unexplained mechanisms.

Mild zinc deficiency is believed to result in a reduced production of Th1 cytokines, resulting in Th2 cytokine bias [6, 7]. In contrast, its supplementation is proposed to augment gene expression for IL-2 and IFN-γ, thereby restoring the Th1 immune response [11]. Since the Th1–Th2 cytokine imbalance is also believed to result in relapses of SSNS, we propose that the benefits of supplementation in these patients may be associated with its ability to rectify the immune defect. Although we did not find differences in the blood levels of the representative cytokines, sensitive assays may be required to examine the impact of therapy. It is noteworthy that treatment with levamisole, which reduces relapse rates in patients with SSNS, is understood to upregulate Th1 cytokines in a manner akin to zinc [12].

This study has a number of limitations. Firstly, it was underpowered to show significant differences in the groups. While subgroup analysis did reveal a benefit in the frequent relapsers, further evidence is required to verify these results. Given a difference of 20% in relapse rates, 70 patients with SSNS are necessary in each group to confirm these findings at 80% power and 5% significance. Secondly, the inclusion of infrequent relapsers may have diluted the impact of the intervention. Further studies on the efficacy of zinc administration should be limited to patients with frequent relapses or steroid dependence. Finally, clarification of the amount of supplementation required and the immunological basis of the observed benefits is necessary.

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