INTRODUCTION

Steroid therapy results in remission of proteinuria in steroid sensitive nephrotic syndrome (SSNS) but develop relapses in 40-50% of cases usually following infections. Infections are the most important complications responsible for mortality and morbidity in the form of poor response to corticosteroid therapy or exacerbation of relapse in patients with stable remission, particularly in developing countries like Pakistan. Minimal change disease is T-cell disorder and is associated with disturbance in switch mechanism of B-cell function from IgM to IgG leading to high IgM levels. Mechanism of steroid sensitivity and the basis for infection triggered relapses are unclear. However, evidence of abnormal cell-mediated immunity and its association with atopy, high immunoglobulin E and up-regulated gene-expression for interleukin (IL)-4 and IL-13 suggest a type-2 cytokine disturbance. Type-1 cytokine predominates in cell mediated immunity and works through gamma interferon.

Management of children with frequent relapsing nephrotic syndrome (FRNS) is challenging one and many strategies have been used to treat and prevent these relapses. Low Zinc levels during active nephrotic state might lead to a down regulation of type-1 cytokine, a relative type-2 cytokine bias and an increased risk of infections. Zinc supplement may lead to decreased episodes of infections presumably due to augmentation of gene expression for IL-2 and interferon, thereby restoring the cytokine-1 immune response.

Zinc deficiency is common and associated with a high mortality in developing countries. Low Zinc levels have been described in children with severe malnutrition, malabsorption and nephrotic states due to either lack of intake, decreased absorption or loss of Zinc in diarrheal stool and in urine. There is strong evidence from developing countries that Zinc supplementation can reduce morbidity and mortality, especially among children due to gastro-

ABSTRACT

Objective: To determine whether Zinc supplementation could reduce relapse rate in children with nephrotic syndrome. Study Design: Randomized-controlled trial. Place and Duration of Study: National Institute of Child-Health and The Kidney Centre, Karachi, from January 2008 to June 2009. Methodology: Sixty nephrotic children aged 2 - 15 years were selected. Baseline data including age, number of infections and relapses during pre and post study one year were recorded. Randomization was done to divide into Zinc group (Zg) to receive Zinc versus placebo (Pg) for 6 months. Relapses and infections were treated with standard therapy. T-test and chi-square tests were used to compare the mean values and proportions respectively with significance at p < 0.05. Results: Among 60 children, 54 completed trial (Zg = 25, Pg = 29). Forty (74%) were males and 14 (26%) females. Mean age, pre study relapses and Zinc level in the two groups were similar. Overall, infections and relapses were observed in 43 (79.62%) and 17 cases (31.48%) respectively. There was no significant difference in frequency of infections and mean infection rate in Zg (20, 80% and 1.92 ± 1.47) compared to Pg (23, 79.3% and 2 ± 1.53, p = 0.950). Relapses occurred in 7 (28%) in Zg compared to 10 (34%) in Pg which was not significant (p = 0.609). Mean infection and relapse rate per patient per year (PPPY) in Zg was 1.92 ± 1.47 and 1.14 ± 0.37 compared to 2 ± 1.53 and 1.3 ± 0.48 in Pg respectively (p=0.846, 0.464). Pre study relapses in two groups were similar (Zg vs. Pg = 96 vs. 96.6%) whereas post study relapses in Zg were lower (7, 28%) compared to Pg (10, 34.5%). Post study mean relapse rate in Zg was 1.14 ± 0.37 PPPY compared to 2.71 ± 1.11 in pre study (p = 0.005). In Pg, post study mean relapse rate PPPY was 1.30 ± 0.48 compared to 1.70 ± 0.48 in pre study period (p = 0.037). Relapse rate reduction was 43% after Zinc supplementation compared to 27% reduction in placebo. Metallic taste was observed in 10% of cases.


Zinc Supplement in Reduction of Relapses in Children with Steroid Sensitive Nephrotic Syndrome

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intestinal and respiratory diseases. But Zinc supplementation as preventive strategy has not been found in a Cochrane database system review on interventions for prevention of infections in nephrotic syndrome.

Keeping in mind the low Zinc levels in precipitating relapses into consideration, we hypothesized that Zinc supplementation may reduce the frequency of infections and thereby may decrease relapse rate in SSNS so that recommendation may be made for its use in prevention of infection associated relapses. In Pakistan, no study has been carried out so far on role of Zinc in reduction of infections and infection triggered relapses in children with SSNS.

The objective of the study was to determine whether Zinc supplementation could reduce the relapse rate in children with frequent relapsing nephrotic syndrome.

**METHODOLOGY**

This double blind randomized placebo controlled trial was conducted from January, 2008 to June, 2009 in the Department of Paediatric Nephrology, National Institute of Child Health (NICH) and The Kidney Centre, Karachi. Approval from the Hospital's Ethical Committee was taken prior to conducting the study.

Sixty children of 2-15 years with FRNS followed-up at both the institutes with standard care for minimum of 12 months duration were included after informed consent from parents. Baseline data including gender, present age, age of onset of NS, number of relapses and infections associated with relapses during the preceding one year were recorded.

Serum Zinc was measured by calorimetric method at enrolment in all cases and in 25 cases that received Zinc supplement at the end of trial. Patients were randomized into two groups (Zinc and placebo) to receive either oral Zinc sulphate (10 mg/day) or placebo blindly (researcher and parents were unknown about bottles containing either Zinc or B-Complex but were coded by third party) and were followed fortnightly to ensure compliance and supply of Zinc for 6 months and then monthly during post supplementation. All patients enrolled in the trial were monitored for side effects and were documented. Infections and relapses associated with relapses during the preceding one year were recorded.

Data including current age, age of onset of nephrotic syndrome, gender, number of relapses and infectious episodes, infections associated with relapse before and after Zinc supplement, side effects and serum Zinc levels, was collected and analyzed using Statistical Package for Social Sciences (SPSS) version 16.

Qualitative variables like gender, relapses and infections were represented by frequencies and percentages. Mean ± SD was calculated for quantitative variables like age, number of relapses, number of infections and serum Zinc level. Student t-test was applied to determine the significance between two groups and paired t-test was applied to determine the significance between pre and post comparison within a groups (Zinc/placebo). Chi-square test was used for comparison of qualitative variables between groups. P < 0.05 was taken as significant and 95% confidence interval was used to see effect size.

**RESULTS**

Sixty children with FRNS were enrolled (30 in each group) but 54 completed the trial. Six lost to follow-up, 5 from Zinc group (Zg) and one from placebo (Pg). Among 54 children who completed trial, 25 (46.29%) were in Zg and 29 (53.70%) in Pg.

Overall, mean age at enrolment and at initial diagnosis of nephrotic syndrome in two groups was 7.65 ± 3.20 and 3.3 ± 3.14 years respectively.

Table I shows baseline characteristics in Zg vs. Pg. Pre study mean age at enrolment and age at initial diagnosis, relapse rate and serum Zinc level in the two groups were not different. Respective p-values for above variables (age at enrolment 0.370, age at initial diagnosis 0.450, relapse rate 0.377 and serum Zinc level 0.916) in two groups were not significant.

Table II shows the comparative frequency of infections and relapses after Zinc supplementation in Zg vs. Pg group. Overall, infections and relapses were observed in 43 (79.62%) and 17 (31.48%) cases respectively. There was no significant difference in the frequency of infections (p = 0.950) and relapses in two groups (p = 0.609). There was no significant difference in infection and relapse rate per patient per year (PPPY) in two groups (p = 0.840 and 0.1813 respectively).

Table III shows pre and post study comparative relapse rates in two groups before and after Zinc supplement.
Mean infection associated relapse rate PPPY after Zinc supplementation was 1.14 ± 0.37 (43% lower) compared to 2.71 ± 1.11 (27% lower) in the pre Zinc supplementation. This is statistically highly significant (p < 0.005). Post-study relapse rate PPPY in Pg was 1.30 ± 0.48 compared to 1.70 ± 0.48 pre-study period. This was significant (p = 0.037).

Most common types of infections were acute respiratory infection and diarrhea observed in 29 and 9 cases respectively.

**Table I: Comparison of infection and relapses in children with Zinc versus placebo group after Zinc supplementation.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Zinc group (n = 25)</th>
<th>Placebo (n = 29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without infection</td>
<td>5 (20.0)</td>
<td>6 (20.7)</td>
<td>0.950</td>
</tr>
<tr>
<td>With infection</td>
<td>20 (80.0)</td>
<td>23 (79.3)</td>
<td></td>
</tr>
<tr>
<td>Without relapse (remission)</td>
<td>18 (72.0)</td>
<td>19 (65.5)</td>
<td>0.609</td>
</tr>
<tr>
<td>With relapses</td>
<td>7 (28.0)</td>
<td>10 (34.5)</td>
<td></td>
</tr>
<tr>
<td>Mean infection rate / patient / year (mean ± S.D)</td>
<td>1.92 ± 1.47</td>
<td>2.0 ± 1.53</td>
<td>0.846</td>
</tr>
<tr>
<td>Mean relapse rate / patient / year (mean ± S.D)</td>
<td>1.14 ± 0.37</td>
<td>1.3 ± 0.48</td>
<td>0.464</td>
</tr>
</tbody>
</table>

**Table II: Pre and post study comparison of relapse in two groups before and after study.**

<table>
<thead>
<tr>
<th>Mean relapse rate / patient / year</th>
<th>Zinc group (n=25)</th>
<th>Placebo (n=29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre study</td>
<td>7 2.71 ± 1.11</td>
<td>10 1.70 ± 0.48</td>
<td>0.005</td>
</tr>
<tr>
<td>Post study</td>
<td>7 1.14 ± 0.37</td>
<td>10 1.30 ± 0.48</td>
<td>0.037</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Relapses in nephrotic syndrome are often triggered by various infections particularly acute respiratory tract infections (ARI). Many different interventions for prevention of infectious and infection associated relapses like use of prophylactic antibiotics, intravenous immunoglobulin, pneumococcal vaccine and Chinese medicinal herbs (*Tiaojining*) in addition to non-pharmacological strategies have been studied. In addition to treatment of infection in SSNS, increasing the dose of corticosteroids on daily basis for 7-10 days during infection has also been suggested to prevent full blown relapse in cases of ARIs.

Studies have shown a high prevalence of Zinc deficiency in developing countries. The beneficial effects of Zinc supplementation in reduction of morbidity and mortality in children with ARIs and diarrheal diseases has lead researchers to a concept of use of Zinc in infections associated with nephrotic syndrome.

This double blind RCT has shown that though frequency of infection in Zinc supplement group (80%) was not significantly different (p = 0.950) from placebo (79.3%) but relapses were 43% lower in Zinc group compared to 27% observed in placebo group. This study also showed a significant reduction in relapse rate after Zinc supplementation (1.14 ± 0.37) compared to pre Zinc supplementation (2.71 ± 1.11) with p-value of 0.005. These findings suggest that Zinc supplementation in children with SSNS may be beneficial in prevention of infectious and infection associated relapses. This observation has also been shown by Arun et al. in a recently published randomized controlled trial that Zinc supplementation was associated with fewer relapses and higher likelihood of maintaining remission in frequent and infrequently relapsing NS. In their study, the observed response was better in frequent relapers in whom 28% reduction in relapse rate with Zinc supplementation. They found that 44.7% of patients in Zinc supplementation maintained sustained remission compared to 27.5% in placebo group. These results are lower than our findings that 72% in Zinc group maintained remission compared to 65.5% in placebo.

Zinc deficiency (42%) in this study among 55 patients at enrolment and 24% at the end of study in randomly selected 25 patients is much higher than 6.17% in above quoted study from India. This could be explained on the basis of different assay methods used in two studies as we used calorimetric method whereas spectrophotometry was used in a study by Arun et al.

Though in this study, post Zinc supplementation level was not done in all cases but there was no significant improvement compared to baseline as found by Arun et al. which suggest that either higher dose is required or Zinc deficiency may not be contributing factor for infection and relapses in nephrotic children. There may be another mechanism along with Zinc deficiency which may be associated with decreased production of Th1 cytokines leading to Th2 cytokines bias and its supplementation may result in restoration of Th1 immune response due to presumed augmented gene expression for IL-2 and interferon-alfa. It is also interesting to note that same mechanism is working in the effectiveness of levamisole in prevention of frequent relapses in steroid dependent NS, based on the evidence that it up-regulate Th1 cytokines similar to Zinc. Since the Th1 and Th2 cytokine imbalance is believed to result in relapses in SSNS and we think that restoration of this balance with use of Zinc may be the mechanism preventing relapses rather than reduction in infections and thereby relapses indirectly.

**CONCLUSION**

Findings of this RCT suggest that oral Zinc supplementation seems to be helpful in reducing the relapse rate (43%) in children with frequent relapsing steroid sensitive nephrotic syndrome. However, further studies on the efficacy of Zinc supplementation in
frequent relapsing nephrotic syndrome on long-term basis in a larger cohort are required to validate our findings before recommending Zinc supplementation in clinical practice.

REFERENCES