Original Article
Trace Elements and Minerals Status in Pediatric Onset Nephrotic Syndrome and Their Relation to Proteinuria: a Multicenter Cross-Sectional Study.

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ABSTRACT
Introduction: Studies on the status of trace elements and minerals in pediatric patients with idiopathic nephrotic syndrome (INS) are lacking.
Aim of the Study: To study trace element status in a cohort of children with INS and the relationship with INS activity to advise the need for monitoring & supplementation during the disease course.
Methods: Material and methods: We studied 191 children with INS and 105 healthy children as a control group from two pediatric nephrology centers. We divided them into two groups: 86 patients in the relapse group and 105 patients in the remission group. We measured the serum levels of Zinc (Zn), Copper (Cu), Selenium (Se), Manganese (Mn), Iron (Fe), Calcium (Ca), Phosphorus (P), and Magnesium (Mg) in all of them.
Results: Significantly lower serum levels of Zn, Cu, and Fe levels were observed in patients with active disease than in the other groups, without remarkable differences between the remission and control groups. Significantly lower serum Ca, Mg, and Se serum levels were in the active disease group than those in remission and lower in the two disease groups than in the controls. Serum Mn and P were significantly elevated in patients with activity compared to those in remission and higher in the two groups than in the controls. Significant correlations were found between proteinuria & the studied elements except for Mn.
Conclusion: Children with INS have low levels of Zn, Cu, Fe, Se, Ca & Mg, while high levels of P & Mn during proteinuria. Follow-up of mineral & trace elements in children with Nephrotic Syndrome may be recommended, especially in patients with prolonged proteinuria.
Keywords: Trace elements; Calcium; Phosphorus; Magnesium, Manganese; Nephrotic Syndrome.

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INTRODUCTION

Idiopathic nephrotic syndrome (INS) is a pediatric kidney disorder where hypoalbuminemia, hyperlipidemia, proteinuria, and edema are the primary manifestations of this disorder [1]. Trace elements are essential for metabolism and are commonly found in the circulation coupled to proteins. Their absence might have an inverse effect on children's growth and development. Zinc, copper, and selenium are crucial cofactors for numerous enzymes that have a key role in maintaining DNA integrity [2]. Manganese is an essential trace mineral which requires multiple enzymes as a cofactor [3]. Manganese is involved in various metabolic processes through these enzymes, including detoxifying reactive oxygen species, bone formation, reproduction, and immune response [4].

The mineral metabolism can be disrupted in children with nephrotic syndrome (NS), regardless of the range of glomerular filtration rate (GFR). Hypocalcemia and hyperphosphatemia are two disorders that lead to osteomalacia and excessive bone resorption [5]. Magnesium is required for nearly every bodily function, and its deficiency is relatively frequent. Magnesium is essential in producing energy and synthesis of protein, as well as in helping regulate blood sugar levels and maintain appropriate blood pressure [6].

We aimed to evaluate if monitoring and supplementation of minerals and trace elements that are included in the current study are necessary during the disease course by assessing their relationship with disease activity.

METHODS

This cross-sectional study was carried out from June 2023 to December 2023, where we included one hundred ninety-one (191) children with INS recruited from the pediatric nephrology unit at our university hospitals and 105 healthy children as the control group participated in this research. The controls were chosen from siblings and closely related relatives who shared the same socioeconomic status and eating habits as the patients, aged within a range of ± 2 years as the patients' ages. We classified patients into two groups based on disease activity: eighty-six patients in Group 1 were in relapse. One hundred five patients in Group 2 were in remission for at least three months. We included patients who were more than one year of age at the onset of illness, who were on either regular or interrupted therapy for at least six months before enrolment, with GFR of ≥ 60 mL/min/1.73m², and no active infection, one month preceding the study. Patients with congenital or secondary nephrotic syndrome, patients or controls with suspected nutritional deficiency due to extremely low economic standards or poor dietary patterns, severe malnutrition, patients consuming multivitamins, iron, or calcium supplements, and acute infections in the month before the start of the study were all excluded.

A complete medical and pharmacological history was obtained for all patients. The clinical data collected included the patient's age at diagnosis, duration of illness, response to steroid therapy, and use of immunosuppressive drugs. A detailed clinical examination was
also conducted, which involved measuring weight, height, and body mass index (BMI), as well as taking arterial blood pressure measurements using an automated Dinamap device (GE Dinamap Procare 300, GE Healthcare, US) with standard techniques. The kidney function test was done, including serum levels of creatinine and blood urea. Additionally, trace elements and minerals such as serum albumin, calcium (Ca), phosphorous (P), magnesium (Mg), copper (Cu), selenium (Se), iron (Fe), zinc (Zn), and manganese (Mn) were all evaluated. The urinary protein/creatinine ratio (uPtn/Creat) was also measured. In patients with hypoalbuminemia, the corrected Ca was calculated.

Blood samples: Four millilitres of fresh venous blood from peripheral veins were collected through sterile venipunctures. Samples were divided into three parts. Two parts were sent for haemoglobin and serum creatinine using the Jaffe method. The last part for blood chemistry, including urea, albumin, Ca, P, Mg, Fe, Se, Mn, Zn, and Cu, using the atomic absorption spectrophotometry technique using AU5800 spectrophotometer (Beckman Coulter, Inc. 250 S. Kraemer Blvd. Brea, U.S.A.). Fresh urine samples were collected from the patient in the early morning to measure uPtn/creat. The normal ranges for the studied elements were as follows: Calcium 8.8-10.8 mg/dl, Phosphorus 4-7 mg/dl, Magnesium 1.7 to 2.2 mg/dl, Manganese 5-12 µg/dL, Zinc 60-120 µg/dL, Copper 70-140 µg/dL, Selenium 110-165 µg/L, Iron 50-120 µg/dL.

**The following definitions were adopted:**
Nephrotic syndrome: occurrence of edema, hypoalbuminemia (<2.5gm/dl), proteinuria (> 2mg/mg creatinine in spot urine sample), and hypercholesterolemia (>250mg/dl).
Relapse (active disease): A random spot of urine with a ratio of protein/creatinine > 2.
Remission: A urinary protein/creatinine ratio <0.2.

**Statistical analysis**
We used the SPSS v.22.0 (IBM Corporation, Armonk, NY) for analysis. Chi-square was used to compare qualitative data. Continuous data were expressed as mean, standard deviation, or median and interquartile range. As appropriate, the T-test or the Mann-Whitney test was used to compare these variables." All data were normally distributed except for the uProtein/Creatinine ratio in the total patient group. The ANOVA test was used to compare continuous variables between three groups. Multivariate regression was used to determine the effect of INS and proteinuria as independent factors for mineral derangements. The Pearson correlation coefficient test was used to assess the correlations. There was no missing data; all data were available for all included children.

**RESULTS**

Data of the two groups are shown in **Table 1**, where the mean age of diagnosis and disease duration was 4.8± 2.8 (range 1.3-14) years and 38.7 ± 29.1 (range 8-180) months, respectively. Regarding steroid response, IFNS was reported in 59 (31%), SDNS in 97 (51%), and SRNS in
35 (18.%) of patients. Regarding immunosuppressive therapy other than steroids, 60 patients (31%) received calcineurin inhibitors, and 20 (11%) received mycophenolate mofetil. Serum albumin levels in patients were remarkably lower than in controls, while the uPtn/creat ratio was significantly higher in patients than in controls. Serum Zn, Cu, Fe, Se, Ca, and Mg levels were considerably lower in patients than in controls. However, significantly higher serum levels of Mn and P levels were observed in patients. The most often deficient nutrient in the patient group was Se (deficient in 100% of patients), followed by Zn and Ca (in 63% of patients for each). Iron (Fe) deficiency was reported at 47%, and Cu deficiency was recorded at 34%. In addition, hyperphosphatemia was found in 36% of the patients, while hypermagnesemia was found in 29.0%.

We observed significantly lower serum levels of Zn, Cu, Fe, and Se in patients with activity than others. However, there were no considerable variations between the remission and controls regarding Zn, cu, and Fe. We also observed significantly lower serum Ca, Mg, and Se serum levels in the active disease group compared to the remission one and significantly lower in the two disease groups than in the controls. Serum Mn and P levels significantly increased in patients with activity compared to those in remission and substantially higher in the two groups than in the controls Table 2.

When comparing patients with SSNS and SRNS, we couldn't report significant changes in trace elements and mineral levels between the groups during activity and remission. NS was independently correlated to low Ca levels (B; -0.75, p; 0.034, CI 0.24 - 0.95) high P levels (B; 1.87.5, p; <0.001, CI; 3.18 – 13.21), and low Mg (B; -2.17, p;0.006, CI; 0.024 – 0.543), while proteinuria was independently associated with all studied elements' abnormalities except for ca and Mn Table 3.

There were significant negative correlations between uPtn/creat ratio and serum levels of zinc (r; -0.61, p; 0.01), Cu (r; -0.55, p;0.03), Fe (r; -0.38, p;0.01), Se (r; -0.51, p;0.04), Ca (r; -0.39, p;0.01), and Mg (r; -0.43, p;0.03). Serum P levels were positively correlated significantly to uPtn/creat ratio (r; 0.53, p;0.001), and we did not report any significant correlation between Mn and uPtn/creat ratio Figures 1 and 2. We did not report significant correlations between trace element levels and age of onset or disease duration.

Table 1: Clinical and laboratory characteristics of the patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients (no= 191)</th>
<th>Controls (no= 105)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>130 (68%)</td>
<td>63 (60%)</td>
<td>0.167</td>
</tr>
<tr>
<td>Age (years)</td>
<td>8.4± 3.7</td>
<td>8.5± 2.8</td>
<td>0.933</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.1± 0.2</td>
<td>1.2± 0.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Height percentiles</td>
<td>30±13.6</td>
<td>46±14.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>29.1± 13.4</td>
<td>28.1± 8.9</td>
<td>0.489</td>
</tr>
<tr>
<td>Weight percentiles</td>
<td>56±16.7</td>
<td>52±9.3</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI</td>
<td>18.4± 4.</td>
<td>18.9± 2.7</td>
<td>0.351</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>101.4± 10.3</td>
<td>94.3± 12.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure percentiles</td>
<td>53 ±11.7</td>
<td>49.5±3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>64.6± 8.2</td>
<td>60.8± 10</td>
<td>0.008</td>
</tr>
<tr>
<td>Diastolic blood pressure percentiles</td>
<td>52± 4.9</td>
<td>50.1±2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>11.7± 1.6</td>
<td>12.2± 1.2</td>
<td>0.006</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>29.1± 13.3</td>
<td>25.7± 11.3</td>
<td>0.119</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.5± 0.3</td>
<td>0.5± 0.2</td>
<td>0.099</td>
</tr>
</tbody>
</table>

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Table 1: Clinical and laboratory characteristics of the patients and controls. (Continued)

<table>
<thead>
<tr>
<th></th>
<th>Patients (no=191)</th>
<th>Controls (no=105)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dl)</td>
<td>2.9 ± 0.7</td>
<td>4.2 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary protein/creatinine (mg/mg creatinine)</td>
<td>3.2 (2.1-8.6)</td>
<td>0.01 (0.01-0.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zinc (µg/dL)</td>
<td>66.6 ± 21.9</td>
<td>88.1 ± 14.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Copper (µg/dL)</td>
<td>79.6 ± 23.2</td>
<td>101.8 ± 17.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Selenium (µg/dL)</td>
<td>41.6 ± 18.6</td>
<td>124.2 ± 10.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Manganese (µg/L)</td>
<td>13.8 ± 1.2</td>
<td>8.3 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
<td>63.2 ± 14.3</td>
<td>92.8 ± 39.7</td>
<td>0.002</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.5 ± 0.9</td>
<td>9.6 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>6.6 ± 1.1</td>
<td>4.1 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>1.4 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values expressed as median (IQR)

Table 2: Comparison between active disease, disease in remission, and controls groups regarding laboratory data.

<table>
<thead>
<tr>
<th></th>
<th>Active (no=86)</th>
<th>Remission (no=105)</th>
<th>Control (no=105)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>10.1 ± 1.5</td>
<td>12.2 ± 1.5</td>
<td>12.2 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>32.9 ± 15.9</td>
<td>26.1 ± 9.7</td>
<td>25.7 ± 11.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.6 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.1 ± 0.7</td>
<td>3.9 ± 0.4</td>
<td>4.2 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary protein/creatinine (mg/mg)</td>
<td>3.4 ± 1.4</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zinc (µg/dL)</td>
<td>39.6 ± 15.8</td>
<td>83.9 ± 16.4</td>
<td>88.1 ± 14.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Copper (µg/dL)</td>
<td>57.9 ± 15.7</td>
<td>96.5 ± 21.3</td>
<td>101.7 ± 17.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Selenium (µg/dL)</td>
<td>20.5 ± 10.7</td>
<td>57.0 ± 9.8</td>
<td>124.2 ± 10.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Manganese (µg/L)</td>
<td>13.8 ± 1.2</td>
<td>10.5 ± 1.0</td>
<td>8.3 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
<td>36.9 ± 26.4</td>
<td>83.5 ± 45.9</td>
<td>92.8 ± 39.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>6.8 ± 0.9</td>
<td>9.2 ± 0.9</td>
<td>9.6 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>7.2 ± 1.2</td>
<td>5.2 ± 0.9</td>
<td>4.1 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>1.5 ± 0.2</td>
<td>1.8 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Significant differences between the active and remission groups without significant differences between the remission and control groups

Table 3: Multivariate analysis for proteinuria as an independent factor for elements derangements

<table>
<thead>
<tr>
<th></th>
<th>Parameter Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>activity</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>-0.123-</td>
</tr>
<tr>
<td>Copper</td>
<td>.043-</td>
</tr>
<tr>
<td>Iron</td>
<td>.037-</td>
</tr>
<tr>
<td>Selenium</td>
<td>-0.059-</td>
</tr>
<tr>
<td>Manganese</td>
<td>.175</td>
</tr>
<tr>
<td>Calcium</td>
<td>.085-</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>.558</td>
</tr>
<tr>
<td>Magnesium</td>
<td>-2.452-</td>
</tr>
</tbody>
</table>

a. Significant differences between the three groups.

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DISCUSSION

Earlier studies on minerals and trace elements status in pediatric patients with INS are seldom, with small samples focused mainly on studying the levels of Zn, Cu, and Se. This study is the first to assess eight trace elements and minerals in multicenters of children with INS and the first to evaluate Mn in children with INS. Our study reported significant differences between cases and controls regarding body weight and height percentiles. Differences in weight can be explained by weight gain due to edema during activity or steroid therapy [7], while the differences in height percentiles can be attributed to the effect of steroids on linear growth via the inhibition of osteoblastogenesis [8]. We also reported higher blood pressure (both systolic and diastolic) in cases than controls. This finding can be explained by salt retention due to increased aldosterone as a response to hypovolemia during disease activity or due to medication side effects [9].

Our study reported low serum Zn, Cu, Se, Fe, Ca, and Mg levels and elevated P and Mn levels during disease activity. During remission, we also revealed the same abnormalities in Ca, P, Mg, Se, and Mn. Patients had lower serum levels of Zinc (Zn) and Copper (Cu) than the control
group in active disease and remission states. No significant variation was seen between remission and the control group. These findings align with earlier studies [10,11]. Mumtaz and his colleagues found that Zn and Cu levels were remarkably lower during the active phase than during remission. Additionally, they found that serum Zn levels negatively correlated with 24-hour urinary protein, which was statistically significant, and serum Cu was negatively correlated to 24-hour urinary protein/creatinine ratio [10]. Similarly, Tulpar et al. [12] found lower serum Zn in activity than remission. Moreover, Hamik et al. [13] found that Zn deficiency notes in frequently relapsing nephrotic syndrome, followed by steroid-resistant NS.

A study of 60 pediatric patients with SSNS were randomly allocated to receive Zn or a placebo in addition to standard treatment. Compared to those who received a placebo, patients treated with Zn experienced a 43% reduction in relapses [14]. Zinc deficiency can increase the sustainability of infection due to decreased Th1 cytokines production. Also, the imbalance in Th1–Th2 cytokine imbalance can result in relapses of SSNS [15,16]. Zinc supplementation may boost the expression of IL-2 and IFN-γ genes, thus restoring the immune response of Th1 [17]. Cogan et al. [18] reported an excess urinary loss of Zn, copper, and iron transport proteins, in addition to the protective high-density lipoproteins in NS, the increased urinary excretion of trace elements and the loss of transport proteins. Proteinuria and zincuria were linearly related in patients with NS, according to Freeman et al. [19].

Serum albumin is a transporter for several metabolites, where albumin's metal binding potential has long been recognized. Albumin's metal binding sites bind to several important metal ions, including Cu, Zn, and Ca, with distinct specifications for each metal ion [10]. Hence, hypoalbuminemia can produce Zn deficiency. Ceruloplasmin binds 90% of the Cu, while albumin binds 10%. According to Ito et al., Cu deficiency was thought to result from urinary ceruloplasmin losses in nephrotic syndrome [20]. The explanations for Zn and Cu deficiency in NS are increased 24-hour urine protein losses, reduced food intake, and urinary losses of trace elements [10].

Copper deficiency plays a vital role in the development of multiple renal disorders. Earlier animal studies have shown that rats fed a copper-deficient diet showed atrophy of renal tubules and fragmentation in one study [21] and effacement of foot processes in another study [22]. On the other hand, some adult case reports showed that some cases of NS with copper deficiency experienced an improvement in proteinuria following the administration of copper [23,24]. Cu has a vital role in decreasing oxidative stress, where previous studies reported that Cu deficiency induces oxidative damage, glucose and cholesterol metabolism alteration, and alterations of circulating blood and immune cells [25].

In our study, all NS patients were deficient for Se, and serum Se levels were significantly lower in active NS than in remission and controls, while levels remained low during remission. Parallel to our findings, Tulpar et al. [11] reported that plasma Se levels were lower in active NS than in controls (p=0.001), and their levels did not return to normal during remission as per our findings. They also reported that urinary Se levels were higher during activity than in remission (20.9±5 vs. 8.6±2.6 µg/L, p=0.0001). Also, Mishra [26] found that plasma Se was
significantly diminished in active NS, and Se levels returned to normal after prolonged remission.

Previous studies have reported that Se in the serum is largely protein bound and have reported a significant association between urinary protein loss and urinary excretion of Se [27]. Selenium is a glutathione peroxidase (GPx) enzyme cofactor with an antioxidant function [26]. The production of free radicals during the pathogenesis of NS causes oxidative stress, which promotes glomerular permeability and proteinuria [28]. Selenium and GPx are important in maintaining cell membrane integrity and reducing oxidative stress damage [29].

In our results, selenium levels increased during remission but were still lower than controls and were independently associated with NS. These findings may suggest that selenium deficiency may play a role in the pathogenesis of NS. The results of a previous study on animal models with experimental NS could support this suggestion. Their study showed that rats fed on a selenium-deficient diet experienced more severe proteinuria than those fed a selenium-rich diet [30].

This research noted significantly higher blood Mn levels in INS patients than in controls and active disease compared to remission. However, we did not detect an association between Mn and proteinuria. Our study is the first to evaluate serum Mn in children with NS. Our interpretations of these results might be related to numerous aspects; first, although Mn is absorbed through active transport, the gut is deficient for a specific Mn transporter [31]. Prior research showed that various Fe transporters are important for Mn absorption [32]; therefore, the lack of Fe and Cu leads to enhanced absorption of Mn. Third, Mn is largely removed via the gastrointestinal tract (GIT) after conjugation with bile, while small quantities are excreted through other routes, including the kidneys [33].

Previous studies on the Mn levels in chronic kidney disease (CKD) patients reported high Mn levels in these patients and explained that by their low Ca and Fe levels, they linked Mn levels to decreased GFR [34]. Meanwhile, some other studies showed low Mn levels in CKD. They explained their findings by increased Mn consumption due to increased manganese superoxide dismutase activity and oxidative stress [35]. A study on animal models had shown that oral supplementation with Mn was associated with more severe proteinuria and tubulointerstitial injury [36]. In our study, serum levels of Mn did not return to normal levels during remission, which may indicate a role of Mn in the development of NS that necessitates more investigations in future studies.

In our study, serum Fe levels were significantly reduced in active NS, while serum Fe levels were negatively correlated to the degree of proteinuria and Hb levels. Iron deficiency anemia is reported in both adults and children with NS. Several studies [37-38] have linked iron deficiency anemia to increased urine iron and transferrin losses in nephrotic syndrome. Iron enters bound to transferrin and stays associated with it in alkaline urine. In this study, serum levels of Fe and hemoglobin were lower in active disease and correlated to proteinuria. This finding supports what was reported in a previous study that linked the drop in hemoglobin levels during the active NS to the urinary losses of iron and transferrin, where it is recommended to give iron
supplementation during disease activity [39].

In this study, significantly higher serum P levels were seen in patients than in controls and active disease groups compared to the remission group. In addition, serum P levels were positively correlated to proteinuria. These findings coincide with those reported by other researchers [40,41]. In the study by Feinstein et al. [40], they found that the mean plasma phosphate levels were significantly elevated in SSNS patients in relapse compared to patients in remission and control groups (p<0.001). Contrarily, some other studies revealed no significant difference regarding phosphorus levels between active NS and NS in remission [41,42].

The principal regulatory mechanism of plasma phosphate is its reabsorption by the proximal tubules, which is sodium-dependent [43,44]. Severe NS is frequently associated with depleted intravascular volume, enhanced tubular salt and phosphate reabsorption, and hyperphosphatemia. On the other hand, massive proteinuria results in enhanced salt and phosphate reabsorption [45]. That explains why the P levels returned to normal in remission and normalization of intravascular volume.

Regarding calcium, serum calcium levels were lower in the active group than in remission. Previous research has found that low serum calcium levels in NS occur during disease activity and then return to normal during remission [41,46,47]. In nephrotic syndrome, an imbalance in calcium homeostasis is frequent, owing to the loss of vitamin D and calcium-binding proteins, causing low levels of serum calcium and vitamin D. Glucocorticoids may also reduce intestinal calcium absorption [48]. Several studies have been conducted to investigate calcium metabolism in nephrotic syndrome.

Sato et al. [5] found that patients with the nephrotic syndrome had higher urinary 25(OH)D excretion, reduced urinary 25(OH)D and urine protein, as well as a connection between serum vitamin D binding protein (DBP) and urine. Even with a normal glomerular filtration rate, children with NS are frequently exposed to multiple calcium homeostasis derangements such as hypocalcemia, hyperphosphatemia, low vitamin D metabolites, poor intestinal calcium absorption, and increased serum level of parathyroid hormone (PTH), a process of metabolic bone disease (MBD) [49]. A previous longitudinal follow-up study that included 88 children with NS reported improvement in bone marrow density in those children following vitamin D3 and calcium supplementation [50].

Our study noted significantly lower serum Mg levels in patients with active NS than in remission and controls, which was negatively correlated to proteinuria. Previous studies have reported comparable results [6,51]. It is well-determined that aldosterone significantly regulates magnesium metabolism by enhancing its renal excretion [52]. In nephrotic syndrome, the development of hypovolemia and hyponatremia with subsequent increment in aldosterone levels can lead to increased renal Mg loss and hypomagnesemia.

Our findings of lower Mg in the remission group compared to the controls can be explained by previous research that suggested a relationship between serum and urinary Mg and tubulointerstitial function, where the structural or functional alteration in the renal epithelium impedes the reabsorption and tubular loss of magnesium since both the reabsorption of
filtered magnesium and the retention of serum magnesium take place in renal tubules, resulting in increased Mg urinary excretion. About 12% of our patients in the remission group are diagnosed with FSGS, which explains the still lower Mg concretions in patients in remission than controls due to the associated tubulointerstitial alterations in these patients. Also, it is reported that some medications used for the treatment of NS, such as furosemide, corticosteroids, and cyclosporine, can cause hypomagnesemia [53]. Hypercholesterolemia is a characteristic feature of NS, and magnesium plays a vital role in regulating lipid metabolism and reducing serum cholesterol [54].

In performing the regression test to study the effect of NS and proteinuria on the levels of trace elements and minerals, we found that NS can significantly affect the levels of Ca, P, and Mg, regardless of the status of disease activity, suggesting the possible associations between NS as a disease by itself or the effect of treatment, especially steroids. Glucocorticoids induce hypocalemia through decreasing intestinal Ca absorption and increased renal Ca excretion [48]. This can explain why Ca levels remained low even during remission. Steroids can induce hyperphosphatemia through their inhibitory effect on fibroblast growth factor 23 (FGF23), which enhances phosphorus renal excretion [55] and can explain why P levels did not return to normal during remission despite the correction of volume depletion. Also, steroids can affect Mg levels by increasing renal magnesium excretion [56]. Similarly, cyclosporine can affect mineral status, including Ca, P, and Mg. Cyclosporine induces hypercalciuria, hypophosphatemia, and hypomagnesemia by decreasing their tubular reabsorption [57].

LIMITATIONS OF THE STUDY
First, we did not perform functional essays confirming the actual deficiency of the studied elements (e.g., measuring oxidative stress status assessment of bone marrow density); anyhow, some of the studied elements showed abnormalities even during remission. Second, as it is a cross-sectional study, we cannot elucidate the causality between INS and mineral abnormalities in the settings of proteinuria as these changes can be attributed to proteinuria, disease pathogenesis, disease complications, and medications, especially steroids. Further prospective studies that follow-up patients to assess the mineral status during activity and remission in the same patients and studying the mineral status at the presentation of the first attack before and after steroid treatment may help clarify any associations.

RECOMMENDATIONS
Regular follow-up of trace elements and minerals status in children with INS and to give supplementations when needed.

CONCLUSIONS
Children with INS have low levels of trace elements (Zn, Cu, Fe, Se) and minerals (Ca, Mg) and high levels of P and Mn during proteinuria. Furthermore, even during remission, children with NS may have hypocalemia, hypomagnesemia, low Se, and elevated P and Mn levels. The findings of our study may draw attention to trace elements and mineral derangements in children with INS and the possible need for treatment.
ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>BP</td>
<td>Blood pressure</td>
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<td>Ca</td>
<td>Calcium</td>
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<td>Copper</td>
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<td>Fe</td>
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<td>GFR</td>
<td>Glomerular filtration rate</td>
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<td>INS</td>
<td>Idiopathic nephrotic syndrome</td>
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<td>Mg</td>
<td>Magnesium</td>
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<td>P</td>
<td>Phosphorous</td>
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<td>S</td>
<td>Selenium</td>
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<tr>
<td>uPtn / Creat</td>
<td>Urinary protein/creatinine</td>
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REFERENCES


Authors’ contributions
The submitted manuscript is the work of the author & co-author.
All authors have contributed to authorship and read and approved the manuscript.
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STATEMENTS
Ethics approval and consent to participate
This study was approved by the Ethical Committee of Beni-Suef University, Faculty of Medicine and the ethics code was FWA00015574 FMBSUREC /606023 and by the ethical committee of Ain Shams University (FMASU R222/2023). Also, written informed consent was obtained from the parents of the participating children.

Consent for publication
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