Serum Level of Vitamin B12 And Folic Acid in Egyptian Children with
Idiopathic Nephrotic Syndrome

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ABSTRACT
Background: Nephrotic syndrome (NS) is one of the most widespread chronic renal illnesses in childhood. Homocysteine (Hcys) metabolism uses vitamin B12 and folic acid as a cofactor.

Objective: The current study investigated the probable association among vitamin B12 and folic acid with nephrotic patients in various stages comparing to healthy ones among Egyptian children.

Patients and methods: The current research was done on 60 patients in relapse and remission compared to 30 healthy children. Folic acid, vitamin B12, albumin and cholesterol in serum were measured in all patients and controls.

Results: Level of vitamin B12 was significantly lower in relapse than controls. Mean vitamin B12 was significantly higher in relapse group than in the remission one. Vitamin B12 was significantly lower in remission group than in the control group. No major variation was found amongst patients in relapse and controls as regard serum folic acid level. No significant alteration was found amongst patients in remission and relapse as regard serum folic acid level. Same results were found between patients in remission and control as regard to folic acid. Conclusions: Decreased levels of B12 were associated significantly with NS in remission and relapse. Understanding the correlation of vitamin B12 and folate supplementation in children with NS might put physiologists and scientists in preferable situation to create informed remediation policy and decisions.

Keywords: Nephrotic syndrome, B12, Folate, Children, National Research Centre, October 6th University.

INTRODUCTION
Nephrotic syndrome (NS) is the prevalent chronic kidney diseases (CKD) in babyhood with a prevalence of 2-16.9 per 100.00 children universally. Idiopathic NS is the supremaetal form of NS in babyhood representing approximately 90% of all cases. It is considered a primary glomerular disease without an identifiable causative disease or infection. About 85%-90% of children with idiopathic NS are steroid sensitive, and the majority of cases follow a relapsing and remitting course. About half of relapsed patients show frequently relapsing or steroid dependent course.

As reported in 2012, NS is a major health trouble in Egyptian children. Associated with increasing occurrence of recently diagnosed cases through years and increased incidence of steroid-resistant cases that are related to the use of multiple immunosuppressive drugs, complications, recurrent hospital admission, and renal function regression. Thrombo-embolic complications in patients with NS is a serious problem its frequency about 3%. Venous thrombosis is threefold more common in comparison to arterial thrombosis. Abnormality in homocysteine (Hcys) metabolism by increasing in its level has been independent risk factor for both arterial and venous thrombosis.

Vitamin B12 and folate have an essential role in homocysteine metabolism. Not only as cofactors however might their homeostasis disturbance be directly related to cardiovascular risk and CKD progression.

In the bowel, folate is resulting from polyglutamates which are changed into monoglutamates, and folic acid carried by a specific carrier transport it across mucosal epithelia and produced another component called 5-methyltetrahydrofolate (5-MTHF). In the duodenum, vitamin B12 is taken with nutrients as cobalamin, complexes with salivary haptocorrin. Pancreatic proteases liberates this complex abruptly from cobalamin. After that cobalamin fastens to an intrinsic factor released from the stomach parietal cells. In the distal ileum this compound is endocytosed from the enterocytes through cubilin. In plasma, plasma transport protein named transcobalamin carry cobalamin. Urinary excretion of vitamin B12 is minimum because of reabsorption in the proximal tubule.

Several metabolic alterations have been occurred in CKD patients, comprising hormonal dysregulation, acidosis and systemic inflammation, together with co-morbidities and multi-drug remedies, could lead to malnutrition with subsequent deficiency of vitamin B12 and folate. Also there are other factors such as gastroparesis, anorexia, diarrhea, or slow intestinal transit, augmented gut microbiota impairment and gut mucosal permeability might represent deterioration condition.

Our aim was to investigate the probable association between vitamin B12 and folic acid with idiopathic NS patients in different stages comparing to healthy ones among Egyptian children.
PATIENTS AND METHODS
A cross-sectional study was conducted on 30 children from Cairo University's Children's Hospital's Nephrology Department. These children have idiopathic NS, according to the diagnosis. Idiopathic NS diagnosis was based on the International Society of Kidney Disease in Children's (1981) definition. Participants were between the ages of 4 and 14.

Regarding the laboratory investigations and clinical status we choose 30 children in the relapse phase (Group1). On follow up, they have impairment in the level of albumin to creatinine ratio, albumin and cholesterol or the excretion of proteins in urine was more than 40 mg/m2/h; for 3 successive days. In Group2, 30 children were in the remission phase. On follow up, they showed an amelioration in albumin to creatinine ratio, serum level of cholesterol and albumin or protein excreted in urine was more than 4 mg/m2/h; for 3 successive days.

Control group (Group 3): It included 30 healthy, sex and age -matched children.

Inclusion criteria: The patients group included:
1. Patient's age <15 years.
2. Normal GFR (>90 ml/min/1.73 m2).
3. Systemic glucocorticoids therapy for ≥1 month.

Exclusion criteria:
1. Congenital and secondary NS.
2. History of any other chronic medical disease.
3. Vitamin supplementation

All participants were subjected to:
1. Careful history taking with particular emphasis on onset and duration of the disease, duration, dose and course of steroid treatment, and any other pharmacological treatment (including immunosuppressive drugs).
2. Medical examination: Full clinical examination which included general, chest, cardiac, abdominal examination.
3. Samples of blood (1ml) were obtained on plain tubes and centrifuged. Sera were stored at -80°C until analyzed. Albumin, cholesterol, vitamin B12 and folic acid in serum were measured in all controls and patients.

Quantification of folic acid and vitamin B12:
Vitamin B12 and folate levels were measured in serum according to the producer's directions using SimulTRAC-SNB Radioassay kit, ICN Pharmaceuticals Inc. (USA). The unlabeled folate or vitamin B12 competes with its labeled species for the partial quantity of obtainable binding sites on its specific binder, sodecreasing the quantity of labeled folate or vitamin B12 bound. Consequently, the radioactivity bound level is contrariwise associated to the amount in the sample. Amount of folate and vitamin B12 were measured synchronously in a single tube. The two tracers, [125I] for folate and [57Co] for vitamin B12 yield energies at levels that could be separated easily by numerous two-channel counters.

Assessment of albumin and cholesterol:
Serum cholesterol and albumin were evaluated utilizing colorimetric enzymatic methods by means of Autoanalyizer Hitachi 704 (Roche Diagnostics, Switzerland).

Ethical considerations:
This study design was approved by the Scientific Ethical Committee of the National Research Centre (No: 1412032022). Guardians of all patients provided written informed consent. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis
The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 21 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro-Wilk test. Qualitative data were represented as frequencies and relative percentages. Chi-square test (χ2) and Fisher's exact test to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean and standard deviation (SD). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). Analysis of Variance ANOVA (F test) used to compare between more than 2 independent groups of normally distributed variables. For the purpose of predicting relapse in NS, multivariate logistic regression analysis was performed. Calculated odds ratios included a 95% confidence interval. P value ≤0.05 was considered significant.

RESULTS
Our patient groups consisted of 30 nephrotic patients in relapse stage:14 (46.7%) females and 16 (53.3%) males. Their age ranged from 6 to 13 years with a mean of 9.60 (SD 1.92) years. The other 30 nephrotic patients were in remission stage [16 (53.3%) female and 14 (46.7%) males; age ranged from 6 to 13 years with a mean of 9.27 (SD 1.82) years]. The other group was a control group consisted of 30 healthy child age and sex matched with patients group;18 (60%) females and 12 (40%) males. The range of their age was between 6 and 13 years with a mean of 9.2 (SD 1.9) years. There was no statistical significant differences between the 3 groups regarding age or gender (Table 1).
Table 1: Demographic characteristics of the studied groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>F test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Relapse</td>
<td>9.60</td>
<td>1.92</td>
<td>0.389</td>
<td>0.666</td>
</tr>
<tr>
<td></td>
<td>Remission</td>
<td>9.27</td>
<td>1.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>9.20</td>
<td>1.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>Relapse</td>
<td>16/14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Remission</td>
<td>14/16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>12/18</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P ≤0.05 is significant  
X^2 = Chi square.

Table 2 showed comparison of serum albumin, cholesterol, vitamin B12 and folic acid between nephritic patients in relapse and control groups. Mean serum albumin was significantly lower in relapse group than control group. Mean serum cholesterol was significantly higher in relapse patients than controls. Comparing relapse to controls, the mean serum vitamin B12 level was considerably lower. There was no statistical significant difference between relapse patients and controls as regard serum folic acid level.

Table 2: Evaluation of the control and nephrotic syndrome relapse groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>T test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dl)</td>
<td>Relapse</td>
<td>2.16</td>
<td>0.52</td>
<td>-9.971</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.55</td>
<td>0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol(mg/dl)</td>
<td>Relapse</td>
<td>309.47</td>
<td>8.97</td>
<td>13.102</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>128.80</td>
<td>30.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit. B 12(pg/mL)</td>
<td>Relapse</td>
<td>268.34</td>
<td>42.69</td>
<td>-2.655</td>
<td>0.010*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>289.39</td>
<td>7.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid (ng/mL)</td>
<td>Relapse</td>
<td>12.72</td>
<td>2.91</td>
<td>-0.024</td>
<td>0.981</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>12.73</td>
<td>3.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P ≤0.05 is significant.

On comparing the remission group and the relapse group, there was no discernible difference between the two groups regarding mean serum albumin, cholesterol, and folic acid. In comparison to the remission group, the mean serum vitamin B12 level was considerably greater in the recurrence group (Table3).

Table 3: Comparisons between albumin, cholesterol, vitamin B12, and folic acid of relapse and remission groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>T test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dl)</td>
<td>Relapse</td>
<td>2.16</td>
<td>0.52</td>
<td>-1.953</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>Remission</td>
<td>2.37</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol(mg/dl)</td>
<td>Relapse</td>
<td>309.47</td>
<td>8.97</td>
<td>0.166</td>
<td>0.462</td>
</tr>
<tr>
<td></td>
<td>Remission</td>
<td>289.60</td>
<td>35.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B 12 (pg/mL)</td>
<td>Relapse</td>
<td>268.34</td>
<td>42.69</td>
<td>2.846</td>
<td>0.006*</td>
</tr>
<tr>
<td></td>
<td>Remission</td>
<td>240.63</td>
<td>31.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid (ng/mL)</td>
<td>Relapse</td>
<td>12.72</td>
<td>2.92</td>
<td>-0.367</td>
<td>0.715</td>
</tr>
<tr>
<td></td>
<td>Remission</td>
<td>12.99</td>
<td>3.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P ≤0.05 is significant.

serum albumin was substantially greater in the control group compared to remission patients. Serum cholesterol was significantly higher in the remission group than in the control group. The remission group’s mean serum vitamin B12 level was considerably lower than the control group. Between the two groups, there was no statistical significant difference in serum folic acid (Table 4).
DISCUSSION

Our study discussed the relation between idiopathic NS (relapse and remission) and vitamin B12, folic acid levels in the Egyptian children.

Our data has revealed that Egyptian children with idiopathic NS had high serum level of cholesterol, low serum levels of albumin as in the study of Kundalet et al.\(^\text{[18]}\), who stated that children with NS had low serum total proteins and albumin. Also, they had elevated serum cholesterol. Mean vitamin B12 level in serum was significantly lower in our patients than controls.

Our patients showed no significant statistical difference in mean serum level of folic acid with control group but still lower than control, however Poddaet al., Kundalet al.\(^\text{[18,19,20]}\) and Orimadegun et al.\(^\text{[18,19]}\). revealed that children with first episode nephrotic syndrome(FENS) had significant statistical difference in mean serum level of folic acid with control group. Low serum folate and vitamin B12 may be attributed to augmented vitamin loss in urine as a result of proteinuria\(^\text{[18]}\). Also prolonged hospital admission and drug intake may lead to loss of appetite and decrease micronutrients intake.

In conclusion, understanding the supplementation of folic acid and vitamin B12 in children with NS could place physicians and scientists in preferable situation to createwell-versedremedy policy and decisions.

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REFERENCES


