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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 probableassociationamongvitamin B12 and folic acid with nephrotic patients in various stages comparing to healthy ones among Egyptian children.
Patients and methods: The current researchwasdone 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 majorvariation was found among 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 relapse. Understanding the correlation of vitamin B12 and folate supplementation in childrenwith NS mightputphysicians 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**(1)**with aprevalence of 2-16.9 per 100.00 childrenuniversally **(2).**Idiopathic NS is the suprememutual form of NS in babyhood is representing approximately 90% of all cases.Itconsidered a primary glomerular disease without an identifiable causative disease or infection**(3).**About 85%-90% of children with idiopathic NS are steroid sensitive, and the majority of cases follow a relapsing and remitting course**(4).** About half of relapsed patients, show frequently relapsing or steroid dependent course**(5).**

As reported in 2012, NS is a major health trouble in Egyptian children**(6).**Associated with increasingoccurrence of recently diagnosed cases through years and increasing inincidence of steroid-resistant cases that are relatedtouse of multiple immunosuppressive drugs, complications, recurrent hospital admission, and renal functionretro-gradation**(7).**Thrombo-embolic complications in patients with NS is a serious problem its frequency about 3%. Venous thrombosis is threefold more commonin comparison to arterial thrombosis(8). Abnormality inHomocysteine (Hcys) metabolism by increasing in its level has been independent risk factor for both arterial and venous thrombosis (9).

Vitamin B12 and folate have an essential role in homocysteine metabolism. Not only as cofactors howevermight their homeostasis disturbance be directly related to cardiovascular risk and CKD progression**(10).**

In the bowel,folate is resulting from polyglutamateswhich are changed into monoglutamates, and folic acid carried by a specific carriertransport it across mucosal epithelia and produced another component called 5-methyltetrahydrofolate (5-MTHF)**(11).**

In the duodenum, vitamin B12is taken with nutrients as cobalamin, complexes with salivary haptocorrin. Pancreatic proteases liberates this complex abruptly from cobalamin. After thatcobalaminfastens 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**(12).** Urinary excretion of vitamin B12 is minimumbecause of reabsorption in the proximal tubule**(13).**

Several metabolic alterations have been occurred in CKD patients, comprising hormonal dysregulation, acidosisand 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 asgastroparesis, anorexia, diarrhea,or slow intestinal transit, augmentedgut microbiota impairment and gut mucosal permeability might represent deterioratethe condition**(14,15).**

Our aim was to investigate theprobableassociation between vitamin B12 and folic acid withidiopathic NS patients in different stages comparing to healthy onesamong Egyptian children.

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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 [(16)]. Participants were between the ages of 4 and 14.

Regarding to laboratory investigations and clinical status we choose 30 children in the relapse phase (**Group1**). On follow up, theyhaveimpairment 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[(17)].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 inurine was more than 4 mg/m2/h; for 3 successive days [(17)].

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 (>90ml/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 onplain 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 serumaccording 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 partialquantity of obtainable binding sites on its specific binder, sodecreasing the quantity of labeled folate or vitamin B12 bound. Consequently, the radioactivity bound level is contrariwiseassociated to the amount in the sample. Amount of folate and vitamin B12 weremeasuredsynchronously in a single tube. The two tracers, [125I] for folate and [57Co] for vitamin B12 yield energies at levels thatcould be separated easily by numerous two-channel counters.

Assessment of albumin and cholesterol:

Serum cholesterol and albumin were evaluatedutilizing colorimetric enzymatic methods by means ofAuto-analyzer 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 Walk test. Qualitative data were represented as frequencies and relative percentages. Chi-square test ($\chi 2$) 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

Ourpatient groups consisted of 30 nephrotic patients in relapse stage;14 (46.7%) females and16 (53.3%) males.Their age ranged from6 to 13years with a mean of 9.60 (SD 1.92) years. The other 30 nephroticpatients were in remission stage [16 (53.3%) female and 14 (46.7%) males; age rangedfrom6 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 13years with a mean of 9.2 (SD 1.9) years.There was no statistical significant differences between the 3 groupsregarding age or gender (**Table 1**).

Table 1: Demographic characteristics of the studiedgroups.

Variable	Group	Mean	SD	F test	P value
Age (years)	**Relapse**	9.60	1.92	0.389	0.666
	Remission	9.27	1.82		
	Control	9.20	1.90		
Sex (M/F)	**Relapse**	16/14	X^2		0.585
	Remission	14/16	1.071		
	Control	12/18			

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

Table 2 showed comparison of serum albumin, cholesterol, vitamin B12 and folic acid betweennephritic 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.

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

*P ≤0.05 is significant.

On comparingthe 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.

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

*P ≤0.05 is significant.

serum albumin was substantially greater in the control group compared to remission patients.Serum cholesterol was significantly higherin the remission group than in thecontrol 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**).

Table 4:Association of albumin, cholesterol, vitamin B12, and folic acid of remissionand control groups.

Variable	Group	Mean	SD	T test	P value
Albumin(g/dl)	**Remission**	2.37	0.31	-10.112	0.000*
	control	3.55	0.56		
Cholesterol(mg/dl)	**Remission**	289.60	35.44	18.763	0.000*
	control	128.8	30.78		
Vitamin B 12 (pg/mL)	**Remission**	240.63	31.97	-8.111	0.000*
	control	289.39	7.89		
Folic acid(ng/mL)	**Remission**	12.99	3.08	0.337	0.737
	control	12.73	3.02		

*P ≤0.05 is significant.

Table 5 showed multivariate logistic regression predictors for relapse in nephrotic patient compared with the controls. NS relapse was substantially associated with low serum B12 levels.

Table 5: Association between nephrotic syndrome and serum B12 and folic acid (relapse and control).

Variable	B	S.E.	Sig.	Exp(B)	95% confidence interval for EXP(B)	
					Lower	Upper
Age	0.318	0.172	0.065	1.374	0.980	1.926
Sex	-0.260	0.583	0.655	0.771	0.246	2.414
Vitamin B_{12}	-0.036	0.013	0.005*	0.965	0.941	0.989
Folic acid	0.102	0.105	0.330	1.108	0.902	1.360
Constant	6.135	3.088	0.047	461.698		

*P ≤0.05 is significant.

DISCUSSION

Our study discussedthe relation between idiopathic NS (relapse andremission) 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 asin the study of **Kundal*et al.*** [18], whostated 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.**,**Kundal*et al.*** and**Orimadegun*et al.*** [18,19,20]revealed that children with first episode nephrotic syndrome(FENS) had significant statistical difference in mean serum level of folic acid with control group.Low serumfolate and vitamin B12 may be attributed toaugmentedvitamin loss in urine as a result of proteinuria[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

1. **McCaffrey J, Lennon R, Webb N (2016):** The non-immunosuppressive management of childhood nephritic syndrome. Pediatr Nephrol., 31:1383-1402.
2. **Chanchlani R, Parekh R (2016):** Ethnic Differences in Childhood Nephrotic Syndrome. Frontiers in Pediatrics, 4:39.
3. **Pais P, Avner E (2016):** Nephroticsyndrome. In: Kliegman RM, StantonBF, Geme JW, Schor NF editors:Nelson Textbook of pediatrics, 20th ed, Philadelphia, Elsevier Inc. https://www.elsevier.com/en-xm.
4. **Larkins N, Kim S, Craig J, Hodson E (2016):** Steroid-sensitive nephritic syndrome: an evidence-based update of immuno-suppressive treatment in children. Archives of Disease in Childhood, 101(4):404-40.
5. **Sureshkumar P, Hodson E, Willis N, Barzi F, Craig J (2014):** Predictors of remission and relapse in idiopathic nephrotic syndrome a prospective cohort study. Pediatr Nephrol., 29:1039-46.
6. **Bakr A, Eid R, Sarhan A, Hammad A, *et al.* (2014):** Pathological profile of biopsied Egyptianchildren with

primary nephrotic syndrome: 15-year single centerexperience. Journal of Nephrology, 27(4):419-23.

7. **Eid R, Fathy A, Hamdy N (2020):** Health-related quality of life in Egyptian children with nephritic syndrome. Quality of Life Research, 29:2185-96.
8. **Lilova M, Velkovski I, Topalov I (2000):** Thromboembolic complications in children with nephrotic syndrome in Bulgaria. PediatrNephrol., 15(1–2):74-8.
9. Cattaneo M**(1999).**Hyperhomocysteinemia, atherosclerosis and thrombosis**.**ThrombHaemost.,81:165-76.
10. **Soohoo M, Ahmadi S, Qader H, Streja E, *et al.*(2017):** Association of serum vitamin B12 and folate with mortality in incident hemodialysis patients. NephrolDialTranspl., 32:1024-32.
11. **Randaccio L, Geremia S, Demitri N, Wuerges J (2010):** Vitamin B12: Unique metalorganic compounds and themost complex vitamins. Molecules, 15:3228-59.
12. **Kang S, Wong, P, Malinow M (1992):**Hyperhomocysteinemia as a risk factor for occlusive vascular disease. Annu RevNutr., 12:279:98.
13. **Capelli I, Cianciolo G, Gasperoni L, Zappulo F, *et al.* (2018):** Folic Acid and Vitamin B12 Administration in CKD, Why Not? Nutrients, 11:383(1-20).
14. **Rowland I, Gibson G, Heinken A, Scott K. *et al.*(2018):** Gut microbiota functions: Metabolism of nutrients and other food components. Eur JNutr., 57:1-24.
15. **Zha Y, Qian Q (2017):** Protein Nutrition and Malnutrition in CKD and ESRD. Nutrients, 27:208.
16. **International Society of Kidney Disease in Children (1981):** The primary nephrotic syndrome in children: Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. J Pediatr., 98:561-4.
17. **Bagga A, Srivastava R (2005):** Nephrotic syndrome. In: Srivastava RN, Bagga A, ed. Pediatric Nephrology, 4thed. New Delhi. Jaypee. 159-200.
18. **Kundal M, Saha A, Dubey N, Kapoor K*et al.*(2014):** Homocysteine Metabolism in Children with Idiopathic Nephrotic Syndrome. Clin Transl Sci., 7(2):132-6. doi: 10.1111/cts.12145., 7(2): 132-6.
19. **Podda G, Lussana F, Moroni G, Faioni M. *et al.* (2007):** Abnormalities of homocysteine and B vitamins in the nephrotic syndrome. Thromb Res., 120:647-52.
20. **Orimadegun B, Orimadegun A, Ademola A, Agbedana E (2014):** Plasma homocysteine and B vitamins levels in Nigerian children with nephrotic syndrome. Pan African Medical Journal, 18:107.