Evaluation of Kidney Function Parameters in Children with Vitamin B12 Deficiency

Vitamin B12 Eksikliği Olan Çocuk Hastalarda Böbrek Fonksiyon Parametrelerinin Değerlendirilmesi

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ABSTRACT

Objective: Vitamin B12 is crucial for cell metabolism, deoxyribonucleic acid synthesis and cell proliferation. Hematological and neurological systems are known to be affected in vitamin B12 deficiency. The aim of this study was to research the effects of vitamin B12 deficiency on kidney function parameters.

Material and Methods: Thirty-four children with vitamin B12 deficiency and 36 sex and age-matched healthy controls were included in the study. Complete blood count, serum urea, creatinin, vitamin B12, holotranscobalamin, methyl malonic acid, homocysteine, ferritin, folate and estimated glomerular filtration rate were recorded. Additionally, spot urine protein, microalbumin and neutrophil gelatinase-associated lipocalin were measured.

Results: Kidney function parameters were normal for childrens that participated the study. Serum kidney function parameters adjusted for age also showed no significant difference between the two groups. No correlation was found between serum vitamin B12 and neutrophil gelatinase-associated lipocalin; however, a negative correlation was detected between neutrophil gelatinase-associated lipocalin and holotranscobalamin ($r = -0.24$, $p = 0.045$). Holotranscobalamin was substantially lower in the group with vitamin B12 deficiency ($p<0.001$).

Conclusion: No negative influence of B12 deficiency on kidney function was found in non-anemic children.

Key Words: Child, Holotranscobalamin, Kidney function, Vitamin B12 deficiency

ÖZ

Amaç: Vitamin B12, hücre metabolizması, deoksiribo nükleik asit sentezi, hücre proliferasyonu için yaşamsal öneme sahiptir. Vitamin B12 eksikliğinde nörolojik ve hematolojik sistemin etkilendiği bilinmektedir. Bu çalışmada da, vitamin B12 eksikliğinin böbrek fonksiyonları üzerine etkisinin incelenmesi amaçlandı.

Gereç ve Yöntemler: Vitamin B12 eksikliği olan 34, yaş ve cinsiyet özellikleri açısından benzer özellikler gösteren 36 sağlıklı çocuk çalışmaya dahil edildi. Hastalara ait tam kan sayımı, serum üre, kreatinin, vitamin B12, holotranskobalamin, metil malonik asit, homosistein, ferritin, folat düzeyleri ve tahmini glomerüler filtrasyon hızı kaydedildi. Ayrıca, idrarda protein, mikroalbumin ve nötrofil jelatinaz ilişkili lipokalin değerleri ölçüldü.

Bulgular: Çalışmaya katılan çocukların böbrek fonksiyonlarını gösteren parametreler normal değerlerdeydi. Böbrek fonksiyonları ile ilgili parametreler açısından vitamin B12 eksikliği olan ve olmayan hastalar arasında anlamlı fark yoktu. Her iki grup arasında vitamin B12 ve nötrofil jelatinaz ilişkili lipokalin arasında korelasyon bulunmadı, ama holotranskobalamin ve nötrofil jelatinaz ilişkili lipokalin arasında negatif korelasyon saptandı ($r = -0.24$, $p = 0.045$). Holotranskobalamin vitamin B12 eksikliği olan grupta anlamlı derecede düşüktü ($p<0.001$).

Sonuç: Anemisi olmayan çocuklarda vitamin B12 eksikliğinin böbrek fonksiyonları üzerine belirgin olumsuz etkisi bulunamamıştır.

Anahtar Sözcükler: Çocuk, Holotranskobalamin, Böbrek fonksiyonu, Vitamin B12 eksikliği

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INTRODUCTION

Vitamin B12 (cobalamin) is one of the most important water-soluble complex vitamins. Vitamin B12 is vital for nucleic acid synthesis in cell metabolism and protein biosynthesis. Deficiency of vitamin B12 is common in childhood period, especially in infancy and adolescence along with multiple nutritional deficiency. The frequency of vitamin B12 deficiency was found to be 22% to 65% in studies conducted in regions with low socioeconomic level (1, 2).

Strict vegetarian nutrition of the mothers of breast-fed infants and dietary vitamin B12 deficiency often cause vitamin B12 deficiency in the children. Absorption disorders also cause vitamin B12 deficiency in childhood. Congenital pernicious anemia, juvenile pernicious anemia and transcobalamin 2 transport deficiency are among the most frequent B12 absorption disorders. Because of vitamin B12 role in cell proliferation, hematological and neurological problems including megaloblastic anemia, peripheral neuropathy, posterior spinal cord degeneration and developmental delay can be observed in children with B12 deficiency (3).

It is important to start the treatment early in children with B12 deficiency to have a better clinical course. Because measurement of the serum total vitamin B12 level quantitates both the inactive forms(transcobalamin I and transcobalamin III bound) and active form (transcobalamin II-bound) of B12, it may not exactly represent real vitamin B12 status (4). Elevated plasma levels of methyl malonic acid (MMA) and homocysteine in combination with low vitamin B12 level are more reliable markers of vitamin B12 deficiency.

Urinary neutrophil gelatinaseassociated lipocalin (uNGAL) is a biomarker reflecting renal tubular damage, and it has been shown to be increased urinary excretion in renal damage (5). Although there are many studies in the literature on the neurological and hematological effects of vitamin B12 deficiency, there are scarce data investigating the effect on renal functions (6-8).

The aim of this study was to research the effects of vitamin B12 deficiency on renal function parameters.

MATERIALS And METHODS

Patients admitted to our Pediatric Hematology and Oncology Department between February 2018 and 2019 with vitamin B12 deficiency were evaluated prospectively in this study. Children aged 2-18 years with a body weight of 3-97 percentile and a vitamin B12 level of <200 pg/ml without any chronic illness or infection were included in the study. Children with chronic illness including hypothyroidism, diabetes, obesity or malnutrition, iron or folate deficiency and signs of dehydration (serum urea/creatinine > 20, urine density> 1020) were excluded from the study. The study group was consisted of a total of 34 patients. Thirty-six sex and age-matched healthy children who accepted outpatient clinics for routine control formed the control group. This study was approved by the local ethics committee by the number of 24.01.2018/2012-KAEK-15/1583. The study complied with the Decleration of Helsinki, and the informed consent was obtained from the all patients and/or their parents. Patient demographics, blood pressure, height and weight percentiles, physical examination were recorded. Complete blood count including hemoglobin (Hb), white blood cell count (WBC), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT) were registered. Anemia was described as a Hb concentration below cut off levels established by the World Health Organization (WHO) (9). Serum urea, creatinine(Cre), urinalysis and microalbumin in spot urine, serum vitamin B12, ferritin, folate levels of all subjects; additional homocysteine and MMA levels of the patients with B12 deficiency were recorded. Diagnosis of vitamin B12 deficiency was declared as a serum level of vitamin B12 below 200 pg/ml and eleveated plasma homocysteine and/or MMA with the outside of other causes of anemia (10). Urine samples of 2 ml was taken directly into the Eppendorf tubes and stored at -80ºC until measurement of uNGAL by enzyme linked immunosorbent assay (ELISA) method. The cut off values of the uNGAL were determined on the values reported in the manufacturer's book and 131.70 ng/ml was accepted as upper limit. Two ml of venous blood sample was drawn and centrifuged at 1500 rpm for 10 minutes, the serum was separated and stored at -80 ºC until measurement of holotrascobalamin by Abbot Architecht i2000 (Abbott Park, IL, USA). The values of the holotranscobalamin below 35 pmol/l was accepted as B12 deficiency according to manufacturer's book. The estimated glomerular filtration rate (eGFR) was calculated with serum creatinine according to Schwartz formula for children (11).

Statistical Analysis

The distributions of continuous variables were analysed with Kolmogorov-Smirnov or Shapiro-Wilk tests based on normality of distribution. Descriptive statistics were defined as mean ± standard deviation, and median (minimum-maximum). Categorical data was compared using chi-square and Fisher's exact tests. To compare independent groups, students-t test and Mann-Whitney U test was used based on normality of distribution. Pearson or Spearman correlation analysis was used to test relationships between variables. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc.s, Chicago, IL, USA). When $p \leq 0.05$ was considered as statistically significant.

RESULTS

A total of 34 children (9 girls, 25 boys) with vitamin B12 deficiency and 36 healthy controls (17 girls, 19 boys) were analysed in the study. There was no difference between the groups in terms of median age [13 (range, 4-17) vs. 12.5 (range, 5-17)]. Physical examination was unremarkable in all subjects participated in

Table I: Comparison of complete blood count parameters of the subjects (mean ± SD).

Parameters (mean ± SD)	Vitamin B12 deficient group (n = 34)	Control group (n = 36)	p
Hb (g/dl)	14.40 ± 1.32	13.80 ± 1.18	0.064
MCV (fL)	85.30 ± 4.72	81.50 ± 3.36	< 0.001
RDW(%)	13.80 ± 1.51	13.50 ± 1.12	0.939
MCH (pg)	30.50 ± 8.90	28.00 ± 1.27	0.002
MCHC (g/dL)	34.00 ± 1.33	34.20 ± 0.88	0.638
RBC ($x10^6$/µl)	5.00 ± 0.45	5.00 ± 0.43	0.780
WBC ($x10^3$/µl)	7.00 ± 14.30	8.00 ± 2.26	0.032
PLT ($x10^3$/µl)	284.50 ± 52.34	308.00 ± 59.87	0.084

Hb: *hemoglobin,* ***MCH:*** *main corpuscular hemoglobin,* ***MCHC:*** *mean corpuscular hemoglobin concentration,* ***MCV:*** *mean corpuscular volume,* ***PLT:*** *platelet,* ***RBC:*** *red blood cell,* ***RDW:*** *red cell distribution width,* ***WBC:*** *white blood cell*

Table II: Homocystein end MMA levels in patients with vitamin B12 deficiency.

Patients with vitamin B12 deficiency (n=34)	Increased	Normal
Homocystein	27 (79%)	7 (21%)
MMA	8 (24%)	26 (76%)

MMA: *Metil malonic acid*

the study. Hypertension was detected in none of the patients. No significant difference was found in body mass index (BMI) between the two groups. Mean vitamin B12 levels were 147 ±30 pg/ml in the vitamin B12 deficient group and 411±168 pg/ml in control group. There was a negative correlation between age and vitamin B12 levels of the patients (r=-0.31, p=0.008).

Comparison of complete blood count parameters between children with vitamin B12 deficiency and control group is shown on Table I. Hemoglobin levels, RBC and PLT counts were not different between the two groups, but MCV was higher in the vitamin B12 deficient group (p<0.001). A weak-moderate negative correlation was found between MCV and vitamin B12 levels (r=-0.31, p=0.008), but no correlation was found between MCV and holotranscobalamin. Platelet counts and vitamin B12 levels of the subjects were positively correlated (r= 0.36, p=0.002). White blood cell counts was also statistically significantly lower and MCH was higher in the vitamin B12 deficient group (p=0.032, and p=0.002 respectively). White blood cell count showed a positive correlation with B12 levels of the subjects (r=0.35, p=0.003).

Homocystein levels were found to be increased in the 79% of the vitamin B12 deficient patients (Table II). No correlation was found between homocystein and holotranscobalamin levels. Additionally, there was no correlation between homocystein and uNGAL. Methyl malonic acid were increased in 24% of the vitamin B12 deficient patients, yet, no correlation was found between MMA and uNGAL levels.

Comparison of biochemical and nutritional parameters showed no meaningful difference between the two groups (p> 0.05) (Table III). Serum creatinine adjusted for age also showed no remarkable difference between the two groups.

Median spot urine microalbumin level of the vitamin B12 deficient group was 7.20 mg/l (0.18-29 mg/l) while median spot urine microalbumin level was 6.10 mg/l (0.20-29.0 mg/l) in the control group. Proteinuria was not found in urinalysis obtained as the first urine sample in the morning in all subjects. No significant difference was found in terms of microalbuminuria between the two groups (p=0.630).

Median uNGAL was 8.90 ng/ml (1.10-121.00 ng/ml) in the patient group with vitamin B12 defiency. In the control group,

Table III: Comparison of Biochemical and Nutritional Parameters of the subjects (mean ± SD).

Parameters (mean ± SD)	Vitamin B12 deficient group (n = 34)	Control group (n = 36)	p
Urea (mg/dL)	21.00 ± 5.94	22.60 ± 4.22	0.855
Creatinine (mg/dL)	0.68 ± 0.09	0.64 ± 0.12	0.960
Albumin (g/dl)	4.50 ± 0.33	4.20 ± 0.32	0.112
ALT (IU/L)	12.50 ± 4.94	12.20 ± 4.69	0.568
AST (IU/L)	19.50 ± 4.49	21.50 ± 5.79	0.078
Ferritin (ng/ml)	39.20 ± 21.20	34.80 ± 12.10	0.658
Folate (ng/ml)	6.80 ± 1.85	7.60 ± 2.06	0.811
eGFR (mL/min / 1.73m^2)	102.60 ± 13.59	93.90 ± 8.70	0.309

ALT: *alanine amino transferase,* ***AST:*** *aspartate amino transferase,* ***eGFR:*** *estimated glomerular filtration rate*

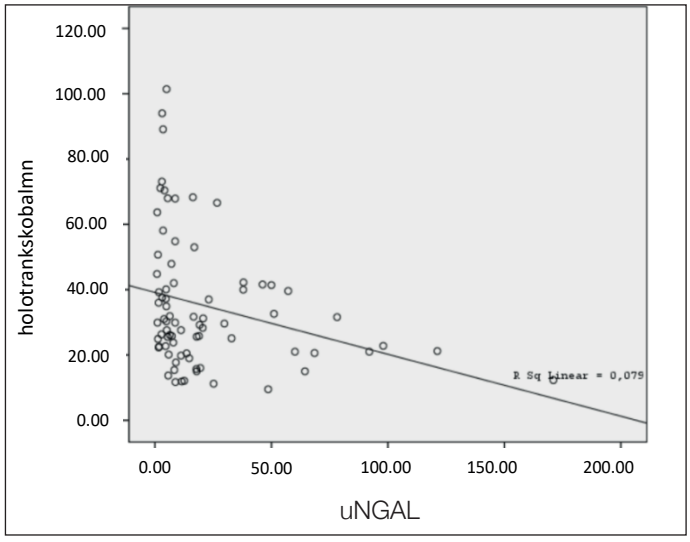

Figure 1: Correlation between holotranscobalamin and uNGAL levels of the subjects.

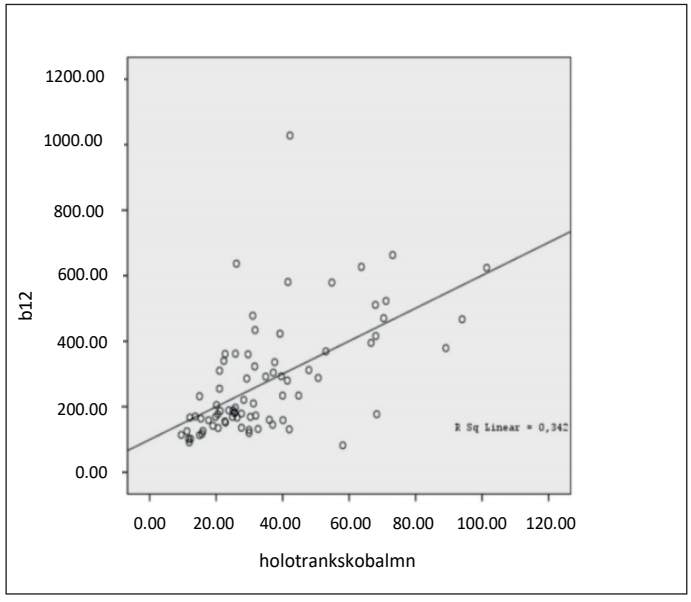

Figure 2: Correlation between vitamin B12 and holotranscobalamin of the subjects.

median uNGAL was 6.1 ng/ml (0.90-92.00 ng/ml). None of the patients included in the study showed no pathological level of NGAL excretion in the urine, but the NGAL level was close to the upper limit in some patients. There was no significant difference between the two groups in terms of uNGAL levels (p=0.290). No significant correlation was found between vitamin B12 and uNGAL; however, there was a weak negative correlation between uNGAL and holotranscobalamin levels (r= -0.24, p =0.045) (Figure 1).

Median holotranscobalamin level was 24.30 pmol/ l(9.50-68.30 pmol/l) in the patient group and 39.80 pmol/l (20.00-101.40 pmol/l) in the control group. Holotranscobalamin levels were found to be low (<35 pmol/l) in 28 patients in the vitamin B12 deficient group and 14 patients in the control group. Holotrascobalamin levels were significantly lower in the vitamin B12 deficient group than the controls (p <0.001). A positive correlation was also found between vitamin B12 and holotranscobalamin levels (r=0.57, p <0.001) (Figure 2).

DISCUSSION

Vitamin B12 is for cell metabolism, nucleic acid synthesis and cell proliferation. This study revealed that no negative effects of vitamin B12 deficiency on renal functions in non-anemic children. Vitamin B12 is needed more during periods that cell regeneration is rapid growth and development like infancy and adolescence. For this reason, its deficiency is most frequently seen in the periods when the need is not adequately met with nutrients. Frequency of vitamin B12 deficiency in adolescents was reported to be %10.5 in our country (12). In our study, the median age of vitamin B12 deficient group was 13 years, and 27 of 34 patients (80%) were between the ages of 12 and 18 years that, they were in adolescence period likewise that study. We found also a negative correlation between the age of patients and vitamin B12 levels.

Deficiency of vitamin B12 results in hyperhomocysteinemia and increased MMA levels in the plasma. Kumagai et al.(13) analysed the renal effects of hyperhomocysteinemia, and showed increased arterial and arteriolar wall thickness and focal tubulointerstitial fibrosis in the kidneys of rats. They reported a negative correlation between creatinine clearance and homocysteine levels. In the present study, comparison of kidney functions between the groups with and without vitamin B12 deficiency revealed no significant difference in urea, creatinine, GFR and albumin levels, although hyperhomocysteinemia was present in 79% of those with vitamin B12 deficiency patients.

Miliku et al.(8) also investigated the effects of maternal and fetal B12 and homocysteine levels on the renal function of children, they observed high GFR values in those with high fetal B12 values. It has been concluded that those with higher levels of homocysteine may also have smaller kidney sizes and decreased GFR values.

Serum creatinine is a late indicator of kidney damage, however uNGAL can provide the earliest detection of renal damage. Zappitelli et al.(14) detected the development of acute kidney injury in critically ill children earlier by uNGAL evaluation. Gunes et al. Analysed human kidney injury molecule-1 (KIM-1), liver-type fatty-acid binding protein (L-FABP) and N-acetyl-bD-Glocosaminidase A (NAG) in addition to uNGAL to detect early renal damage in children with B12 deficiency. Evaluation of these markers and urine electrolytes were found to be similar in the vitamin B12 deficient and control groups. However, the rate of NGAL, KIM-1, L-FABP and NAG proteins in urine to creatinine was found to be higher in patients with vitamin B12 deficiency compared to controls. This difference suggests the presence

of a subclinical renal injury in patients with B12 deficiency. They concluded that hypoxia may develop secondary to anemia and renal damage may developed accordingly (6). In our study, uNGAL values were found within normal limits in all patients. In 34 children in this study group, vitamin B12 deficiency did not cause a pronounced anemia. No significant difference was found in terms of uNGAL values between the vitamin B12 deficient and the control groups. However, there was a negative correlation between holotranscobalamin and uNGAL. This negative correlation isn't enough evidence to said that supports the entity of a chronic process that kidney injury increases parallel to severity of vitamin B12 deficiency. In our study, urinary creatinine couldn't analyses, so the rate of uNGAL to creatinin in urine couldn't be compared. This is a important deficiency of the study.

The transcobalamin, which enables the transport of vitamin B12 in the plasma, is bound to vitamin B12, then holotranscobalamin is formed. Holotranscobalamin is the component of vitamin that reaches the cells, therefore it shows the adequacy of the metabolic function of vitamin B12 and called active B12. Studies showed that holotranscobalamin is a more sensitive marker than serum vitamin B12 in defining vitamin B12 deficiency (15,16). In the study conducted by Bamonti et al.(17), a strong correlation was found between holotranscobalamin and vitamin B12 values. In our study, in accordance with the literature, holotranscobalamin was found to be positively correlated with vitamin B12 levels.

The most important limitation of the study is that urinary creatinine can not be studied. Urine creatinine to uNGAL ratio could provide a more sensitive result to compare renal damage. Another limitation is small sample size. Additionally, vitamin B12 deficient patients were not anemic, which may have prevented hypoxia-induced renal damage.

CONCLUSION

In conclusion, vitamin b12 deficiency seems to no effect on kidney functions in non-anemic children. Further large scale studies are warranted to evaluate the indicators of renal damage in vitamin B12 deficiency.

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