Does High Vitamin B12 Levels Cause Proteinuria?

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ABSTRACT

Aim: Cubilin receptors, which are responsible for the absorption of vitamin B12 from the intestine, are also found in the kidney and regulate protein reuptake from urine. We claimed that vitamin B12 deficiency may lead to less proteinuria as a result of increased Cubilin receptors, or higher vitamin B12 levels may cause proteinuria by down regulating the receptors. To support this hypothesis, the relationship between vitamin B12 and urine protein levels was investigated.

Methods: The last two-year hospital records of patients between the ages of 18-65 were retrospectively scanned. Patients who were tested for the vitamin B12, plasma creatinine, and urine protein analyses and whose eGFR value was calculated as >60mL/min were included. Patients diagnosed with diseases that may cause proteinuria such as diabetes, malignancies, or kidney diseases were excluded. Vitamin B12 level <200 pg/ml was accepted as a deficiency, 200-1000 pg/ml as normal, and >1000 pg/ml as hypervitaminosis.

Results: Of the 31,776 patients who met the criteria, vitamin B12 deficiency was found to be 38.6% in men; 33.1% in women, and 35% in total. Rates of hypervitaminosis B12 were 1.4% in both men and women, and overall. Urinary protein was observed in 13% of all patients. A statistical significance was found between vitamin B12 levels and proteinuria. In post-hoc analysis, this statistical difference was detected between the hypervitaminosis group and the other levels.

Conclusion: Vitamin B12 hypervitaminosis was found to be statistically associated with proteinuria, indicating that caution should be exercised in vitamin B12 treatment.

Keywords: vitamin B12, hypervitaminosis, proteinuria
**Introduction**

Vitamin B12 is a water-soluble vitamin and its deficiency can cause megaloblastic anemia, anorexia, bilateral peripheral neuropathy and demyelination, paresthesia, memory loss, dementia, depression, psychotic symptoms, and cognitive disorders (1). Ingested vitamin B12 is absorbed in the terminal ileum by a peripheral membrane protein called Cubilin (2). The Cubilin receptors are also found in the renal proximal tubules and take part in the reuptake of protein and albumin from urine (3). Through the hereditary mutation of the Cubilin-coding gene, megaloblastic anemia (due to the vitamin B12 absorption disorder, which occurs in the neonatal period) and proteinuria (not accompanied by renal impairment) are detected and called Imerslund-Gräsbeck Syndrome (1,4,5).

Since, the Cubilin receptor has a key role in vitamin B12 absorption in the intestines, in case of the vitamin B12 deficiency in individuals without congenital disorders, it is claimed that the expected change is the increase of numbers of Cubilin receptors. Also, Cubilin receptor amounts may be increased correspondingly in renal tubules as a reflex response to the vitamin B12 deficiency, and therefore, protein and albumin loss in urine may be decreased. As another hypothesis, it can also be claimed that higher vitamin B12 levels will lead to a decrease in the number of Cubilin receptors and therefore, may increase proteinuria.

In this study, to support the hypothesis that consisting “low vitamin B12 levels decrease proteinuria” and “higher vitamin B12 levels may lead to proteinuria and thus worsen the renal course” were examined by investigating the relationship between protein excretion in the urine and the vitamin B12 levels.

**Methods**

A retrospective, cross-sectional study was conducted in a tertiary-level hospital. Before starting the study, the local ethics committee approval was obtained (ethics committee approval number: 27.02.20/02-26). The Helsinki Declaration on Good Clinical Practice rules were taken into consideration in the study. Since our study was planned as a retrospective screening of hospital records, no informed consent was provided from the patients.

In the last two years (from March 2018 to March 2020) blood and urine test records were scanned, and patients aged 18-65 who underwent serum vitamin B12, creatinine, and dipstick urine protein tests were included in the study. Patients missing any of these tests were excluded from the study. All tests of the patients had to be performed on the same day with the same application protocol. Estimated Glomerular Filtration Rate (eGFR) was calculated and patients with eGFR > mL/min/1.73 m² were included in the study. All patients were scanned for their diagnoses, and it was explored whether they were diagnosed with possible conditions affecting urine protein levels. Patients diagnosed with any of the diseases that may cause proteinuria, such as diabetes mellitus, oncologic diseases (including all solid and hematological malignancies), acute or chronic kidney diseases, nephrotic or nephritic syndromes, and hypertensive patients who are likely to use antihypertensive pills that may cause proteinuria were excluded from the study.

Vitamin B12 was measured by the chemiluminescence method (Beckman Coulter Access® 33000). The deficiency was considered if the level of the vitamin B12 was lower than 200 pg/ml, the vitamin B12 level was accepted as normal if it was between 200-1000 pg/ml, and hypervitaminosis if it was >1000 pg/ml. Anemia was diagnosed if the hemoglobin value was lower than 13 g/dL in men and 12 g/dL in women.

For the evaluation of kidney functions of patients, creatinine was measured by modified Jaffé’s Kinetic method (Beckman Coulter® AU5400), and the estimated Glomerular Filtration Rate (eGFR) was calculated by using the Modification of Diet in Renal Disease (MDRD) formula (6). The eGFR calculation formula that we used in our study is as follows:

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\text{eGFR (mL/min/1.73m²)} = 186.3 \times \text{Plasma Creatinine}^{-0.154} \times \text{Age}^{-0.203} \times (0.742 \text{ if woman})
\]

Patients whose eGFR values were measured as
≤60 mL/min/1.73m² were considered as mildly to moderately decreased kidney functions adopted by using KDIGO categorization and were excluded from the study (7).

The results were evaluated with the SPSS 23.0 (IBM, USA) package program. Categorical data were presented with numbers and percentages and the Chi-squared test was used for their statistical analyses. Kolmogorov-Smirnov test was used for the determination of the normal distribution. If the compared groups are three or more and they do not distribute normally, the Kruskal Wallis test was performed. Statistical significance was taken as p<0.05 in the whole study.

Results

The number of patients whose vitamin B12, serum creatinine, and urine protein levels were examined was 46,460 from the patients aged 18-65 years in the last two years. While 12,509 patients diagnosed with some diseases possibly affecting protein levels were excluded from the study, 480 patients were excluded due to missing data. By calculating their eGFR estimations, 1,701 patients with eGFR values lower than 60 mL/min/1.73 m² were excluded from the study and as a result, a total of 31,770 patients were included in the study. The flow chart of the study was given in Figure 1.

When the patients’ data was evaluated, the mean age of the patients was 42.3±13.4 years; 65.7 percent were women, and the mean vitamin B12 level was 280.3±188.3 pg/ml. Vitamin B12 deficiency (<200 pg/ml) was found to be 38.6% in men, 33.1% in women, and 35% in total. The incidences of high vitamin B12 levels (>1000 pg/ml) were 1.4% in both men and women, and overall. Proteinuria was found in 13% of all patients (Table 1).

Table 1. Frequencies and percentages of vitamin B12 categories according to age, gender and urinary protein.

| Vitamin B12 Category | Age (Mean, St. Deviation) | Gender (% , n) | Urinary Protein (% , n) | P *
|----------------------|--------------------------|---------------|------------------------|---
| Deficient            | 40.7 ± 13.4              | Men: 38.6% (4207) | Negative: 86.9% (9668) | <0.001 * |
|                      |                          | Women: 33.1% (6914) | Positive +1: 2.0% (220) | <0.001 ✡ |
|                      |                          |               | Positive +2: 0.4% (50)  | <0.001 ✡ |
|                      |                          |               | Positive +3: 0.3% (35)  |         |
|                      |                          |               | Total: 100% (11121)    |         |
| Normal Vitamin B12   | 43.1 ± 13.2              |               |                       |         |
|                      |                          |               |                       |         |
| Hypervitaminosis     | 45.1 ± 13.4              |               |                       |         |

*Kruskal Wallis test; ‡Chi-squared test

Vitamin B12 levels were found to be statistically significant with age, gender, and urine protein (p<0.001, p=0.015, and p<0.001, respectively).

Vitamin B12 values were classified into three categories (those less than 200 pg/mL as the vitamin B12 deficiency, normal: between 200-1,000 pg/ml, and hypervitaminosis: if over 1,000 pg/ml). The relationship of vitamin B12 categories with age,
According to the results, there was a statistically significant difference between vitamin B12 categories with age, gender, and proteinuria (p<0.001).

In the post-hoc analysis of the urinary protein and the vitamin B12 categories, the adjusted residual values were analyzed, and it was seen that this statistical significance was between the group with hypervitaminosis and the other two groups (p<0.05) (Figure 2).

**Figure 2.** Post-hoc adjusted residual analysis between vitamin B12 categories and urinary protein levels (A different letter at the top of each column indicates that the column ratios differ significantly from each other by the 0.05 level. Vitamin B12 deficiency<200 pg/ml; normal: 200-1000 pg/ml; hypervitaminosis>1000 pg/ml.)

**Discussion**

Vitamin B12 deficiency is a common vitamin deficiency in all age groups (8-10). In our study, vitamin B12 deficiency was found to be more frequent (33.3%) than in the literature and we obtained a different result from the literature and found that vitamin B12 deficiency was more common in men and at younger ages (8,11). These unlikely results in our study can be attributed to the fact that older age patients were not included in our study due to the upper age limit of 65 years. However, the high ratio of vitamin B12 deficiency in adults detected in our study is quite remarkable.

On the other hand, vitamin B12 hypervitaminosis has been less researched in the literature as a less noticeable condition and has been associated with malignancies in some studies (12,13).

In our study, the relationship between the vitamin B12 levels and urine protein levels was investigated in a large group of patients (about 42,000 people) with normal eGFR levels, and a statistically significant difference was found (p<0.001). It is noteworthy that this statistical difference was particularly between the hypervitaminosis group and the groups with normal or low vitamin levels. This reveals that vitamin B12 height is significantly associated with protein loss in urine compared to groups with normal or low vitamin levels.

The fact that vitamin B12 elevation is associated with proteinuria can be interpreted as causing protein loss in kidneys by proposing that the Cubilin receptors are down-regulated in response to the hypervitaminosis state. The importance of increased proteinuria due to the vitamin B12 levels is that high-dose vitamin B12 supplementation can deepen hypoproteinemia in individuals with protein-losing kidney or liver diseases. Therefore, it is necessary to be more careful in the use of vitamin B12 in order not to exceed high doses.

There are many studies in the literature investigating the relationship between vitamin B12 and proteinuria through Imerslund-Gräsbeck Syndrome. However, there are few studies in the literature investigating this relationship in patients without Imerslund-Gräsbeck syndrome. A cohort study conducted on the Framingham Heart Study Group investigated albumin excretion in urine in patients with vitamin B12 deficiency and found that there was no relationship between the vitamin B12 deficiency and albuminuria (14). In this study, although there was no correlation between vitamin B12 deficiency and proteinuria, the presence of proteinuria in the group with a high vitamin B12 level was interpreted as a condition associated with higher homocysteine levels in renal failure patients. In our study, patients with low eGFR levels were excluded from the study. Therefore, the relationship between hypervitaminosis and proteinuria that we obtained is an important result, in which the decrease in kidney function is omitted from the evaluations.
Conclusion

In our study, a statistically significant relationship was found between hypervitaminosis B12 and proteinuria in a large group of patients (approximately 32 thousand). This reveals the importance of hypervitaminosis values to be considered in terms of the risk of proteinuria in the treatment of vitamin B12 and aimed to disseminate information and raise awareness on this issue. Future studies are needed to explain the pathogenesis by investigating the change in the number of renal Cubilin receptors.

References