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Serum Vitamin B_6 Levels in Hemodialyzed Patients

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Abstract

Vitamin B_6 is a water-soluble molecule that serves as a coenzyme in many biochemical reactions, but mainly in the metabolism of proteins. It is found in many dietary sources and its stored quantities in the body are exhausted in 3 - 4 months. Among the people who can develop B_6 deficiency are those on hemodialysis (it is lost during cooking and during the dialysis session). Due to the lack of drug with the B_6 complex in our country, our patients did not take B_6 in any form. The serum B_6 levels of 46 (26M/20F) of our hemodialyzed patients (after 5 months without receiving corresponding treatment) were studied, to determine whether they were normal (it is noted that 31/46 had symptoms that could be attributed to B_6 deficiency). The levels were determined both before any therapeutic intervention and after the administration of B_6 tablets (initially 1 tablet of 200 mg/week for 8 months and then 1 tablet/24 hours for 4 months, because there was no injectable drug on the market). Then, the administration continued with an intravenous drug (because it was now available) at a dose of 1 ampule of 100 mg B_6 every 15 days (during the dialysis session) for 12 months. With this treatment, serum levels of B_6 improved (ANOVA, $p = 0.001$). The number of patients with neurological symptoms decreased over time and symptoms improved, both in terms of the number of patients in whom they were detected (from 31/46 at the beginning of the study, only 6/46 had symptoms at the end), and in terms of serum B_6 levels (20/46 had lower than normal serum B_6 levels at the beginning and 9/46 at the end). No one achieved B_6 levels above the upper normal with the treatment. It is concluded that serum B_6 levels in hemodialyzed patients are significantly reduced when they do not receive B_6 supplements. The symptoms of B_6 deficiency are basically neurological and the administration of tablets of 200 mg B_6/day or 1 ampule of 100 mg B_6/15 days is satisfactory for most patients, without the risk of hypervitaminosis.

Keywords

Vitamin B_6 Deficiency, Conventional Hemodialysis, Online Hemodiafiltration, Pyridoxal 5'-Phosphate

1. Introduction

Vitamin B_6, usually administered as pyridoxine hydrochloride, is a water-soluble molecule that is important for the normal function of many organ systems. It is metabolized as an active molecule, pyridoxal 5'-phosphate (PLP), which serves as a coenzyme in many biochemical reactions, especially in protein metabolism [1]. It is found in avocado, spinach, carrots, peas, potatoes, cereals and legumes, fruits, dairy products, meat, fish and eggs [2]. Due to its wide occurrence in food sources, it is difficult to reduce its dietary intake. Its stored quantities in the body (reserves) are exhausted in 3 - 4 months [3], which explains why B_6 deficiency is more common than B_{12} deficiency.

While isolated vitamin B_6 deficiency is rare, it usually occurs in combination with a deficiency of other B vitamins. Reduced vitamin B_6 levels have been reported in contraceptive users, smokers, alcoholics, and in patients with celiac disease, malabsorption and malnutrition syndromes, or diabetes mellitus [4] and in people who consume an incorrectly balanced vegan diet [5].

Pyridoxal 5'-phosphate has a molecular weight of 247.14 g/mol (clearly lower than folate and B_{12}) and is lost during cooking and during hemodialysis session. Deficiency is well known in hemodialyzed patients. Its serum levels are further reduced with high-flux dialyzers and with the initiation of erythropoiesis-stimulating agents (due to increased erythropoiesis) [6] or phosphate binders. The possibility of vitamin B_6 deficiency in hemodialyzed patients is of clinical interest and presents with peripheral neuropathy as weakness of the extremities and numbness in the form of socks on the feet or gloves on the hands, but also with irritability, muscle cramps, and more severe manifestations in the feet compared to the hands. It also presents with insomnia, depression, confusion, stomatitis and cracks at the corners of the mouth (angular cheilitis), glossitis (with a smooth tongue), conjunctivitis, irritability and exhaustion [2] [7]. It also causes hypochromic microcytic anemia, convulsions, skin rashes and immunodeficiency. Because many of the signs and symptoms of this condition (immunological disorders, metabolic disorders) [8] [9] are like those of uremia, it may be incorrectly attributed to the latter.

B_6 vitamin deficiency can lead to a variety of sickly conditions. For this reason, clinical practice guidelines recommend their systematic supplementation in hemodialyzed patients [10]. However, these recommendations are based on studies with conventional hemodialysis and may not be appropriate for online hemodiafiltration (HDF), which is now widely available, especially in European dialysis centers [11].

In this prospective, single-center, uncontrolled repeated-measures study, the serum B_6 levels of patients in our unit were examined and a method of restoring them was investigated, at a time when the only drug with $B_1 + B_6 + B_{12}$ complex in intravenous form was not available in our country.

2. Patients-Methods

2.1. Patients

Serum B_6 levels were studied in hemodialyzed patients, 5 months after the discontinuation of any intravenous preparation containing $B_1 + B_6 + B_{12}$ in our country (early 2024). The inclusion criteria were adult patients > 18 years of age, on dialysis with for at least 12 months, undergoing at least 4-hour sessions of conventional hemodialysis or online HDF (with pre- or post-dilution) 3 times per week, with vascular access that allowed a blood supply to the dialyzer of 350 - 400 ml/min. Exclusion criteria included a history of active infectious, inflammatory and malignant disease or intestinal malabsorption and malnutrition. Patients with cognitive impairment, disorientation, and inability to use medications, and the use of any drug and water-soluble vitamin supplement in the last 4 weeks. Patients with hypersensitivity to polyethersulfone filters were also excluded.

2.2. Methods

After five months without any treatment with a drug containing B_6, its serum levels were determined (May 2024), to see if there was any problem. Because serum levels were found to be lower than normal in 20 of 46 patients and in 31 of 46 had symptoms of its deficiency (numbness, restless legs, burning pain), it was decided to administer one tablet/week with the vitamin B complex ($B_1 + B_6 + B_{12}$) containing 200 mg of pyridoxine-B_6 (a dose that was much higher compared to that recommended in the literature for such a period) [12]. The dose was administered in the unit by nurses to ensure patient compliance with the instruction. After 8 months (Jan 2025) with this dose and because their serum B_6 levels were not restored, the same drug was administered at a dose of 200 mg/24 hours, a dose 7 fold higher than the first (this time the patients took the drug themselves at home). At that time (*i.e.* after 8 months of treatment with 200 mg B_6/24 hours) 16 patients continued to have serum B_6 levels below the lower normal level, while clinically 12 of the patients continued to have symptoms such as numbness (5), restless legs (4), itching (1), burning (1) and cramps (1), of which 4 did not follow the treatment according to the instructions.

The symptoms of numbness, restless legs, itching and cramps are manifestations of other conditions except hypovitaminosis B_6. However, we asked patients to specify when they had such manifestations (before the beginning of dialysis program or after its initiation) to distinguish whether they were due to the loss of B_6 during the sessions, the period when they did not receive it exogenously. In addition, at the re-examination, we asked patients to answer us whether the symptoms they reported to us the first time (before any of our interventions) continued

to exist or whether they improved.

At the end of January 2026, treatment with an injectable complex $B_1 + B_6 + B_{12}$ began, because the medicine was now on the market in this form (1 ampoule containing 100 mg B_6) at a dose of 1 ampule/15 days at the end of the dialysis session, to ensure that the drug was administered. With this dose, serum B_6 levels were checked after 12 months, to see its effectiveness.

All patients underwent hemodialysis for 4 - 4.5 hours/session. For those on with predilution online HDF, the exchange volume was ≥48 L/session and with post-dilution ≥ 24 L/session. The dialysate used was individualized for each patient (potassium 2 - 3 mmol/L, bicarbonate 30 - 33 mmol/L, sodium 138 - 140 mmol/L, chloride 110 mmol/L, calcium 1.5 mmol/L, magnesium 0.50 mmol/L, and glucose 5.5 mmol/L).

The dialyzers used were polyethersulfone (Polynephron low-flux, Elisio, surface area 2.1 m^2 and Polynephron high-flux, Elisio, surface area 2.1 m^2). The blood pump supply was 350 - 400 ml/min, while the dialysate was constant at 500 ml/min for everyone. Low molecular weight heparin (vemiparin) was used at a dose of 2500 and 3500 IU depending on their body weight. The machines used were Nikkiso DBB EXA.

Blood samples for PLP determination were taken after a 12-hour fast, before a midweek dialysis session (Wednesday or Thursday). PLP was determined by HPLC (high-performance liquid chromatography), which has also been used by others to estimate B_6 levels (normal range 12.6 - 45.2 μg/L) [13].

2.3. Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (mean ± SD) or median (range), according to normality of the distribution of each variable. Categorical variables were expressed as absolute frequencies and percentages. Comparisons between the groups were performed using the t-test, paired t-test and ANOVA. The analysis was conducted with the Statistical software MedCalc (version. 20.218). Probability values of $p < 0.05$ (two-tailed) were considered statistically significant for all comparisons.

2.4. Ethical Committee

The study was completed at the "Dimokrition" Renal Unit of Komotini, which was approved by the Scientific Council of Komotini General Hospital (No. 8/2024) and was conducted in accordance with the Declaration of Helsinki and the Guidelines for Ethics in Medical and Health Research Involving Humans. Written consent for participation was obtained from each patient, after being thoroughly informed about the protocol.

3. Results

Forty-six patients (20F/26M), aged 40 to 93 years (median 77 years), who had been on a dialysis program for 18 to 450 months, were included [another 5 patients

who were initially included in the study were finally excluded because they did not complete it (two were transferred to another dialysis unit, two died and one was transplanted)]. Of these (46 patients), 21 patients were on online HDF (8 with predilution and 13 with postdilution) and 25 were on conventional hemodialysis (Table 1). The primary diseases of the patients were: unknown 3, diabetic nephropathy 16, glomerulonephritis 6, unilateral nephron with nephrosclerosis 5, cardiorenal syndrome 5, hypertensive nephrosclerosis 5, adult-onset polycystic kidney disease 3, obstructive uropathy 1, lithium nephropathy 1, and nephrolithiasis 1.

Table 1. Contains gender, age, months on dialysis, type of dialysis, and presence or absence of diabetes (HD = hemodialysis, HDF = hemodiafiltration).

Parameters	Results
Patients (n)	46
Sex (male/female)	(26/20)
Age (mean ± SD) (range) (median)	(73.1 ± 13.2) (40 - 93) (77)
Months on dialysis (mean ± SD) (range)	(78.3 ± 79.4) (18 - 450)
Type of dialysis (conventional HD/HDF)	(25/21)
Diabetics (Yes/No)	(16/30)

During the first determination of serum B_6 levels in our patients (before any pharmaceutical intervention), they were found to be on average equal to 14.5 ± 5.2 μg/L (1st blood sample). In about 50% of our patients (20/46) B_6 serum levels were found to be lower than the lower normal limit (<12.6 μg/L), at which time they were clinically tested to determine whether they had B_6 deficiency symptoms. Numbness in the fingers was found in 12 patients, burning in the fingers in 10 patients, cramps in 1 patient and restless legs in 8 patients (n = 31). Instructions were given to take one tablet/week, containing 200 mg of B_6 (administered in the dialysis unit by nurses). In the repeat exam of serum B_6 levels, *i.e.* after 8 months (2nd blood sample), approximately the same results were found (mean ± SD = 13.5 ± 3.3, p = NS, paired t-test) (Table 2), a difference that was not statistically significant (p = NS), where again several patients (16/46) had serum B_6 levels < 12.6 μg/L (lower normal limit). The medical instruction to take one tablet of the B complex/24 hours was given. After 4 months (3rd blood sample), serum B_6 levels were checked again and were found to be significantly increased (mean ± SD = 17.0 ± 6.9 μg/L), however, 13 of the 46 patients had B_6 levels < 12.6 μg/L (while the highest value found was 33.2 μg/L). It is noted that four patients did not take the drug (at home) according to the instructions, so the levels of B_6 (mean ± SD) in their serum decreased from 11.62 ± 2.40 μg/L to 8.17 ± 1.18 μg/L. When comparing the results between the end of taking 1 tablet/week and after 4 months at a dose of 1 tablet of 200 mg/24 hours, a statistically significant increase in B_6 levels was found (mean ± SD = 13.5 ± 3.3 Vs 17.0 ± 6.9 μg/L, paired t-test, p < 0.002)

(Table 2). At the end of this period, because the injectable drug with $B_1 + B_6 + B_{12}$ (with 100 mg B_6/ampule) began to be on the market, the administration of 1 amp/15 days started. For the next 12 months, the same dose of this preparation continued to be administered and at the end of this period, serum B_6 levels were again determined (4^{th} blood sample), which was mean ± SD = 17.5 ± 5.7 μg/L, (p = NS, paired t-test), where again 9 patients had serum B_6 levels below 12.6 μg/L (while the maximum value found was 31 μg/L in the same patient who had increased relative serum levels even with the administration of 1 tablet/24 hours) (Table 2).

Table 2. Contains the mean ± SD of serum levels of B_6 before any treatment (A), after 8 months of treatment with 1 tablet B_6 of 200 mg per week (B) and after 4 months of treatment with 200 × 7 mg B_6 per week (C), after 12 months on IV treatment with complex $B_1 + B_6 + B_{12}$ twice/month and the statistical differences between them (ANOVA).

Control periods	Mean ± SD (μg/L)	p
Before any treatment (A, 29 May 2024)	14.5 ± 5.2	
After 8 months of treatment with 200 mg/w on B_6 (B, 29 Jan 2025)	13.5 ± 3.3	
After 4 months of treatment with 200 mg × 7/w on B_6 (C 22 May 2025)	17.0 ± 6.9	0.001
After 12 months on treatment with IV ampule with $B_1 + B_6 + B_{12}$ (with 100 mg B_6 in each amp) twice per month (D, 8 June 2026)	17.5 ± 5.7	

Serum B_6 levels in diabetic patients (n = 16) before the start of the study were 16.3 ± 4.9 μg/L and in non-diabetics (n = 30) 13.6 ± 5.2 μg/L, p = 0.051), nearly statistically significant. As for patients on conventional hemodialysis (n = 25) at the start of the study, serum B_6 levels were 15.1 ± 5.5 μg/L, while those on online HDF (n = 21) had 13.7 ± 4.7 μg/L (p = NS). At the end of the study after our pharmaceutical interventions, the following results were noted: diabetics had B_6 levels of 17.8 ± 5.9 μg/L and non-diabetics 17.1 ± 5.2 μg/L (t-test, p = NS), while those on conventional hemodialysis had levels of 17.3 ± 6.0 and those on online HDF had levels of 17.8 ± 5.8, (t-test, p = NS).

Of the clinical findings of the 31 symptomatic patients who had B_6 deficiency before the start of B_6 substitution, only 6 had certain symptoms at the end of the study (restless legs, numbness, burning and pain in the extremities).

4. Discussion

Vitamin B_6 is a general term that refers to 6 interconvertible molecules, pyridoxine (PN), pyridoxamine (PM), pyridoxal (PL) and their phosphorylated derivatives such as pyridoxine 5'-phosphate (PNP), pyridoxamine 5'-phosphate (PMP) and pyridoxal 5'-phosphate (PLP). Its 3 natural forms are pyridoxine, pyridoxal and pyridoxamine. The pharmaceutical form of vitamin B_6 or pyridoxine hydrochloride (marketed as a medical preparation and which is converted in the liver to the

active pyridoxal 5'-phosphate), is an important coenzyme, associated with over 100 enzymes.

Vitamin B_6 is of great nutritional importance. It is involved in the metabolism of carbohydrates, proteins and lipids, as well as in the production of neurotransmitters. It participates in biochemical reactions of homocysteine, cell proliferation, DNA/RNA synthesis and gene expression modulation. It is essential for gluconeogenesis and participates in the production of antibodies [14]. In addition, it can cause immune dysfunction, increased oxalate production and polyneuropathy.

Serum water-soluble vitamin levels (except folate and vitamin B_{12}) are not often measured, which can lead to deficiencies in hemodialysis patients that often remain undiagnosed. Without B_6 supplementation, at least one-third of patients in various studies have low serum vitamin B_6 levels before the beginning of hemodialysis, as we have found, with levels significantly low in 50% of cases [15] [16]. Proposed mechanisms for impaired vitamin B_6 metabolism include inadequate or reduced dietary intake [17] [18]. The use of erythropoiesis-stimulating agents (erythropoietins) and intestinal phosphate-binding resins (resins can absorb a variety of trace elements and vitamins) [19] has been shown to negatively affect vitamin B_6 levels in the body [16] [20]. Vitamin B_6 intake is often limited in dietary studies of hemodialyzed patients [21]. Lower vitamin B_6 intake was found when hemodialyzed patients consumed processed food meals instead of home-cooked meals [22]. Intake below the required amounts of vitamin B_6 (due to lower energy intake) and diseases (especially kidney diseases) are risk factors for B_6 deficiency. Other causes include malabsorption [23], loss across the dialyzer [24] or peritoneal membrane (in those on peritoneal dialysis), inhibition of PLP activity or metabolism by uremic toxins [23], decreased phosphorylation of pyridoxal kinase, or increased pyridoxal degradation activity [25]-[27]. The Second National Report on Biochemical Nutritional Indicators in the US Population, published by the Centers for Disease Control in 2012, found that B_6 deficiency was the most common nutrient deficiency.

It seems that hemodialyzed patients are at risk of developing water-soluble vitamin deficiency. A review of 6 of the included studies found a loss of B_6 during hemodialysis of 24% - 56% [16]. Excessive renal loss of B_6 is expected in diabetics and hemodialyzed patients [2] [28]. However, while it is considered in the literature that diabetics have lower levels of B_6 in their serum, others did not find this difference [13], while we, on the contrary, found nearly statistically higher levels of B_6 in them, before any administration of a drug containing it (16.6 ± 4.9 Vs 13.6 ± 5.2, $p = 0.051$), levels that were equalized between the two groups at the end of our study, after the pharmaceutical intervention.

Conventional hemodialysis is associated with losses of water-soluble vitamins and trace elements, and recommendations have been made regarding their possible supplementation. The removal of vitamin B_6 by dialyzer has been discussed to explain the low levels of PLP in their plasma [29]. However, the amount of vitamin

B_6 removed by hemodialysis has not been clearly demonstrated. Lacour *et al.* did not find PLP in the ultrafiltrate obtained from conventional hemodialysis [30]. This is not surprising, as it is highly bound to albumin. However, it should be noted that longer hemodialysis sessions may increase the loss of water-soluble vitamins. In fact, poor PLP was found in a group of patients undergoing home dialysis [31].

Although water-soluble vitamins are relatively small molecules, losses are likely to be greater with online HDF than with conventional hemodialysis [32]. It should be noted, however, that water-soluble vitamins are partially bound to albumin and albumin losses occur more frequently with online HDF, so this may contribute to the greater loss of B_6 with HDF [33].

Bévier *et al.*, in 39 patients, determined the losses of water-soluble vitamins and B_6, as well as trace elements during a postdilution online HDF session. Blood and ultrafiltrate samples were collected before and after a 4-hour session. Serum levels were significantly reduced for B_6 (25.4%). The losses of B_6 in the ultrafiltrate per session were 0.33 ± 0.09 mg, (noting that only pyridoxal was found in the ultrafiltrate), corresponding to 22% of the recommended dietary intake/24h in healthy adults [34]. However, others found a lower loss of vitamin B_6 in the ultrafiltrate in patients undergoing online HDF [35], corresponding to about half of the loss found by Bévier *et al.* in their study, although the latter used different membranes and the substitution volumes were slightly smaller. These findings certainly highlight the need for regular monitoring of serum B_6 levels and systematic supplementation [34].

Without systematic supplementation, vitamin B_6 deficiency is common in hemodialyzed patients [16] [30]. The NKF/KDOQI recommends a daily supplement of 10 mg pyridoxine for adult hemodialyzed patients [10] [36], others recommend a dose of 100 - 150 mg/week [3], although we administered 200 mg/week, which did not achieve improvement in serum levels and had to increase the dose 7 fold to restore its levels.

The need for vitamin B_6 supplementation has been demonstrated in the past in some studies of hemodialyzed patients and patients undergoing continuous ambulatory peritoneal dialysis [8] [37] [38]. Based on these reports, the recommended oral dose of B_6 varies between 10 mg and 300 mg per 24 hours [28] [34]. The wide range of recommendations for vitamin B_6 supplementation may be due, at least in part, to differences in the methods used to determine vitamin B_6 in foods.

Schwotzer *et al.* found that the serum levels of most vitamins are above the normal range in hemodialyzed patients and receiving a classic dose of vitamin supplements, except for vitamin C. Specifically, they administered a capsule containing 40 mg of vitamin B_6 to 24 patients undergoing online HDF after each session. With this dose, serum B_6 levels were above the normal range in the patients and for this reason it was suggested that the classic dose of vitamin B_6 supplements after hemodialysis session be reduced [35]. Therefore, as suggested [36], system-

atic vitamin B_6 supplementation should also be considered for online HDF patients (34). The NKF/KDOQI guidelines recommend a daily supplement of 10 mg of pyridoxine in adult hemodialyzed patients [10]. However, our experience with the administration of 200 mg/week showed that this dose was low and serum B_6 levels were restored with a 7 fold increase in this dose. This fact certainly raises concerns because the administration of B_6 is also associated with overdose and toxicity (after prolonged intake of its supplements in high doses, but not with food intake) [35]. For this reason, taking large doses of B_6 supplements should only be done under medical supervision. Taking more than 1000 mg of B_6/24 hours can cause nerve damage and pain or numbness in the hands or feet. Some of these side effects have been recorded even after just 100 - 300 mg of B_6 per day (the maximum tolerated dose of vitamin B_6 administered is 100 mg per day for adults), however, with great difficulty, we managed to improve serum B_6 levels in most patients with a dose of 200 mg/24 hours and maintained them with intravenous administration of a dose of 100 mg/15 days.

It is concluded that B_6 deficiency is common in hemodialyzed patients. The administration of tablet B_6 of 200 mg/24 hours can and does maintain the serum levels almost normal in most patients (without an increase in serum B_6 levels above the upper normal range and without manifestations of hypervitaminosis), while intravenous dose of an ampule of 100 mg/15 days has the same results, without the risks of overdose.

Author Contributions

Conceptualization: K.S.M.; formal analysis: P.S.P. and P.A.K.; investigation: K.S.M. and I.M.K.; data curation: I.M.K. and E.S.I.; writing-original draft: K.S.M.; writing-review and editing: I.M.K.; supervision: K.S.M.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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