DO WE OPTIMIZE VITAMINS AND FOILIC ACID TREATMENT IN DIALYSIS PATIENTS? NEW AND OLD CHALLENGES

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SUMMARY: It has been known for many years that the administration of Folic acid (FA) and Vitamin B12 is a necessary replacement therapy for chronic renal failure patients on periodic hemodialysis due to a number of dietary restrictions in these patients. There is a lot of writing, talking and research about Vitamin B12 and FA deficiency. However, there are only sporadic publications on Vitamin B12 and FA hypervitaminosis and its consequences. ABOPHOLIC META is available in Bulgaria since 2011, and it has been used in the Dialysis Department at Sveta Anna Hospital, Sofia ever since. It is noteworthy that during the years of use of this preparation, using additional amounts of Vitamin B12 is becoming rarer and in fewer patients.

The purpose of this follow-up is to:

Determine whether patients maintain stable (within the reference range) serum levels of Vitamin B12 and FA during a 6-month period of ABOPHOLIC META administration. Confirm the hypothesis of a real difference (association) between the outcomes in patients with good compliance of both sexes.

Material and methods:

57 dialysis patients were followed up in the Dialysis Department at UMHAT "Sveta Anna", Sofia. Hemoglobin, Vitamin B12 and FA results were compared. The methods of prospective follow-up and the following Data analyses were used: t-Test: Two-Sample Assuming Unequal Variances, Descriptive statistics: point estimates of parameters-finding of averages, Deductive statistics, Parametric analysis.

Abbott Diagnostics' CLIA Aliniy ci-series was used as the method of testing of Vitamin B12 and FA serum levels.

Hemoglobin was tested by a colorimetric method.

Conclusions: At a dose of ABOPHOLIC META 1 tab/day, the patients maintained stable Vitamin B12 and FA serum levels and no further Vitamin B12 containing preparation was required. There were no patients with FA level above the reference range. There was a real difference (association) between the results of patients of both sexes with good compliance for both Folic acid (P-value = 0.030) and Vitamin B12 (P-value = 0.032).

No similar data on available sex differences are reported in the scientific literature. This is the first time this has been proven in those patients.

***Key words**: Vitamin B12, Folic acid, hemodialysis*

Introduction: Hyperhomocysteinemia (Hhcy) occurs in approximately 85% of patients with chronic kidney disease and patients with chronic kidney failure (CKF) due to impaired renal metabolism and reduced renal excretion. Folic acid (FA), a synthetic form of Vitamin B9, is critical for the conversion of homocysteine (Hcy) to methionine. If there is not enough FA intake, there is not enough conversion, and Hcy levels are elevated. Hhcy is considered an independent predictor of cardiovascular morbidity and mortality in end-stage kidney disease. Hhcy exerts its pathogenic effect on the major processes associated with the progression of vascular impairment. Studies show: Increased Hcy – an increased risk of inflammation and endothelial damage leading to cardiovascular disease (CVD), stroke and CKF. FA has also been shown to improve endothelial function without lowering Hcy level, which suggests an alternative explanation for FA effect on endothelial function. The role of FA and Hhcy in CVD and in the development of CKF has recently been resumed in some randomized trials. In the general population and in patients with CKF, it is still to be discussed whether to mention any beneficial effects of FA therapy and Hhcy direct action or reduction. While the results of confirmatory trials are pending, it is reasonable to consider FA with or without the addition of methylcobalamin as an appropriate suitable adjunctive therapy in CKF patients.

Patients affected by chronic kidney disease (CKD) or terminal renal failure (ESRD) have also a shorter life expectancy than those with normal renal function, mainly due to the dramatic increase in cardiovascular mortality [1]. Chronic hemodialysis treatment is associated with a 10 to 50-fold higher risk of premature death than in the general population, and cardiovascular disease (CVD) is the leading cause of death in hemodialysis patients [2,3]. However, an increased cardiovascular risk also exists in earlier stages of CKD [4]. There is an increased attention to non-traditional cardiovascular risk factors, in particular oxidative stress, endothelial dysfunction, chronic inflammation, vascular calcification, chronic kidney disease – mineral and bone disorder and hyperhomocysteinemia (Hhcy) [5]. The “homocysteine hypothesis” originated from the observation showing that subjects with very high blood levels of homocysteine due to congenital damage to homocysteine metabolism were more susceptible to the development of severe form of progressive atherosclerosis. Thus, over the years, a possible link between even a moderate elevation of homocysteine levels and the cardiovascular risk and mortality has been found, with conflicting results [6,7].

Although CKF and ESRD patients show elevated levels of homocysteine, the role of Hhcy as a cardiovascular and risk factor for mortality in this population is not yet fully understood and deserves further research [8–12]. The high rate of Hhcy in CKF patients has also increased the interest and discussion about the role of Hhcy as a risk factor for CKF progression [13,14]. The role of folic acid and the role of Vitamin B12 is well known, as they are not only essential cofactors for homocysteine metabolism, but their impaired homeostasis may be directly related to cardiovascular risk and CKD progression [11,15].

Adequate FA intake is required for the formation of tetrahydrofolate (THF). The lack of THF means a single carbon transfer, where the required DNA synthesis reactions are limited.

The minor symptoms of FA deficiency are: fatigue, weakness, irritability, insomnia, forgetfulness.

The main symptoms of FA deficiency are: anemia, muscle cramps, confusion.

The nutritional intake in patients with renal replacement therapy may be suboptimal due to therapeutic, dietary restrictions and cooking procedures.

Disease status and comorbidities, e.g. uremia, cause reduced vitamin absorption and/or activity. Interactions with nutrients as well as the elimination of fluids during dialysis are also causes of folate deficiency.

Laboratory and epidemiological evidence suggest that FA addition has a role in cancer prevention, with antineoplastic effect and cardioprotection by reducing homocysteine levels. However, it is mostly randomized, controlled studies that have shown that FA supplements do not reduce the risk of colorectal adenoma and may in fact increase the risk of advanced lesions and adenoma multiplicity (16,17). With regard to cardiosurgery, the evidence is weak in a recent randomized trial showing that FA additive does not reduce the combined endpoint of cardiovascular events, despite a significant reduction of homocysteine levels (18).

Based on the lack of convincing support evidence and the potential tumor-stimulating effect, it is considered reasonable to avoid excessive addition. All you need to do is provide the recommended dose to prevent deficiency. FA plays a role in cancer prevention by having antineoplastic and cardioprotective effects by reducing Hcy levels.

There are currently ongoing daily recommendations for the FA: NKF (2002) – 1 mg; Renal Dietetic Practice INDI, RIG (2006) – 1 mg; Group of the ADA (2004) – 1 mg; FSAI (2006) – 1 mg (4).

The Guideline for Supplementation of Folic Acid in Intermittent Hemodialysis & Peritoneal Dialysis, Beamond Hospital, Dublin Department of Nephrology, Dialysis and Transplantation (19) recommends: The medical team should, when initiating dialysis, prescribe 5 mg folic acid once a week to the patient (Sunday – non-dialysis day). If the serum level falls below the reference range of 2.3 ng/ml, despite administering 5 mg weekly, increase the dose to 5 mg once a day or increase the level above 3.0 ng/ml.

Nurses should monitor serum folate levels on a quarterly basis to allow for dose adjustment.

Nutritionist should regularly monitor serum FA levels to allow for dose adjustment.

There is currently no guide in Bulgaria (Guideline for Supplementation of Folic Acid in Intermittent Hemodialysis & Peritoneal Dialysis). This provokes the interest of checking whether FA and Vitamin B12 taken orally maintain sufficiently high FA and Vitamin B12 values in patient serum.

ABOPHOLIC META is available in Bulgaria since 2011. Since then it has been used in the Dialysis Department, UMHAT “Sveta Anna”, Sofia. It is noteworthy that during the years of use of this preparation, using additional amounts of Vitamin B12 is becoming rarer and in fewer patients.

ABOPHOLIC META composition, % Recommended daily intake: Methyl folic acid (calcium L methyl folate) 400 μg 200; B1 (thiamine) 1.4 mg 127; B2 (riboflavin) 1.6 mg 114; Niacin (nicotinamide) 18 mg NE 113; Pantothenic acid (Potassium D-pantothenate) 6 mg 100; Vitamin B6 (pyridoxine) 2 mg 143; Biotin (D-biotin) 150 μg 300; Vitamin B12 (cyanocobalamin) 1 μg 40

The purpose of this follow-up is to:

Determine whether patients maintain stable (within the in reference range) Vitamin B12 and Folic acid levels over a 6-month period of administration of ABOPHOLIC META. Confirm the hypothesis of a real difference (association) between the outcomes in collaborative (taking the medication on a regular basis) patients of both sexes.

Material and methods:

57 dialysis patients were followed. The methods of prospective follow-up and the following Data analyses were used: t-Test: Two-Sample Assuming Unequal Variances, Descriptive statistics: point estimates of parameter-finding of averages, deductive statistics, parametric analysis.

The results of this follow-up indicated a stable maintenance of Vitamin B12 and FA within the reference range.

Abbott Diagnostics' CLIA Aliniy ci-series was used as the method of testing of serum Vitamin B12 and FA levels.

Hemoglobin was tested by a colorimetric method.

Outcomes:

57 dialysis patients from the Dialysis Department, UMHAT "Sveta Anna", Sofia were followed up for a period of 6 months, from December 2018 to June 2019. Patients: 18 female and 39 male patients.

Vitamin B12 and FA test results were compared at the baseline and at the end of the period. 57 patients were assessed in December 2018. All patients had previously taken ABOPHOLIC META1 tablet/day. Of those, 18 were women with an average age 64.42±4.3. The youngest patient was 31 years old, the oldest was 83 years old. The men were 39, with an average age of 61±2.15. The youngest patient was 29 years old and the oldest patient was 80 years old. The results obtained were discussed with the patients and in those with Vitamin B12 results over the reference range the medication was suspended. The results are presented in Table 1. At the beginning of the follow-up, there were no patients with results above the FA reference range.

2018	**Average age (years)**	**Mean Hb level g/l**	**Mean Vitamin B12 value pg/ml** (Reference values 187-883)	**Mean folic acid value pg/ml** (Reference values 1.87-8.83)
Female n 18	**64.47±3.85** min. 31 max. 83	**94.70±70** min. 52 max. 132	**847.11±156.1** min. 197 max. 2000	**6.6±0.9** min.1.8 max.12.8
Male n 39	**61±2.15** min. 29 max. 80	**98.64±2.8** min.62 max. 144	**652±87.66** min.203 max. 2000	**4.34±0.44** min.1,6 max. 12.2

Table 1. Baseline data at the start of follow-up in December 2018

Data analysis showed that 6 women and 9 men had Vitamin B12 results above the reference range. Those patients were advised not to take any form of Vitamin B12 other than Abofolic meta and if Vitamin B12 prescribed by other colleagues was required, do so after testing the serum Vitamin B12 level. Pure folic acid was temporary given to those patients (for 2 months) to normalize their serum Vitamin B12 level. Despite the recommendation, however, in the last month, one man used Milgamma and one woman used Neurobex.

At the beginning of the follow-up, there were no patients with Vitamin B12 levels below the reference range.

At the beginning of the follow-up, there were no patients with FA level above the reference range; 5 female and 18 male patients had FA deficiency.

Data following the 6-month follow-up (June 2019) are presented in Table 2.

During that 6-month period, 12 patients were lost for follow-up for various reasons, e.g. transfer to another dialysis facility, death, etc.

2019	**Average age**	**Mean Hb level**	**Mean Vitamin B12 value**	**Mean folic acid value**
	(years)	g/l	pg/ml	pg/ml
Female n 15	**64.47±3.85** min. 31 max. 83	**80.53±6.5** min. 33 max. 118	**517.92±75.99** min. 194 max. 1196	**7.19±1.36** min. 2.2 max. 19.1
Male n 30	**61±2.15** min. 29 max. 80	**93.20±3.35** min. 64 max. 141	**444±53.5** min. 173 max. 1323	**4.54±0.44** min. 2.2 max. 11.2

Table 2. Data following the 6-month follow-up (June 2019)

From the analysis of data from each patient's results and the open, careful conversation with the patients, it became clear that one female patient started taking Neurobex for 10-day herniated disc without preliminary testing of Vitamin B12 level. One man did the same; he took Milgamma for diabetic polyneuropathy.

Following the 6-month follow-up, one male patient had Vitamin B12 hypovitaminosis. There were no female patients with Vitamin B12 hypovitaminosis.

Five female patients had FA levels below the reference range (3 of them reported irregular Abopholic meta administration for various reasons, e.g. forgetting, financial reasons, etc.). Nine male had FA levels below the reference range. Six of them did not receive the medication regularly.

Mean values from collaborative patients with good compliance at the baseline (December 2018) are presented in the following Table 3, and mean values at the end of the follow-up (June 2019) are presented in Table 4.

2018	**Average age** /years/	**Mean Hb level** g/l	**Mean Vitamin B12 value** pg/ml	**Mean folic acid value** pg/ml
Female n 11	**72±4.2** min. 37 max. 83	**98±5.44** min. 69 max. 132	**566±171.11** min. 197 max. 2000	**8.9±1.25** min. 1.8 max. 12.8
Male n 21	**67.5±2.7** min. 36 max. 78	**106±3.66** min. 75 max. 144	**332±55.7** min. 203 max. 1183	**3.4±0.7** min. 1.8 max. 12.2

Table 3. Average values from collaborative patients with good compliance at the baseline (December 2018)

2019	**Average age** /years/	**Mean Hb level** g/l	**Mean Vitamin B12 value** pg/ml	**Mean folic acid value** pg/ml
Female n 11	**72±4.2** min. 37 max.83	**76±6.9** min. 33 max.107	**493±84.9** min. 286 max.1198	**8.5±1.4** min. 2.3 max.19.1
Male n 21	**67.5±2.7** min. 36 max. 78	**95.5±2.9** min. 72 max. 124	**335±46.3** min. 173 max. 984	**5.15±0.5** min. 2.2 max. 11.2

Table 4. Average values from collaborative patients with good compliance at the end of the foolow-up (June 2019)

DISCUSSION: The results obtained show: During a 6-month period of ABOPHOLIC META 1 tab. administration, the patients maintained stable (within the reference range) levels of Vitamin B12 and folic acid. The hypothesis of a real difference (association) between the outcomes of patients with good compliance in both sexes was confirmed. There was a statistically significant difference between the two sexes in terms of Folic acid. T-stat = 2.07; t-critical = 1.78; p-value = 0.030 at alpha level of significance = 0.05 were calculated using t-Test: Two-Sample Assuming Unequal Variances.

There was also a statistically significant difference between serum Vitamin B12 levels in women and men. T-stat = 1.98; t-critical = 1.74; p -value = 0.032 at alpha level of significance = 0.05 were calculated using t-Test: Two-Sample Assuming Unequal Variances.

No similar data on available sex differences have been reported in the scientific literature. This is the first time this has been proven in this type of patients. There are also differences in the different standards for taking 1 milligram of folic acid per day, as well as taking 5 mg once on Sunday. THE AUTHORS' VISION/OPINION: DAILY ADMINISTRATION OF FA 400 MICROGRAMS TO MAINTAIN A STABLE SERUM FOLIC ACID LEVEL INSTEAD OF 1 MILLIGRAM OR 5 MILLIGRAMS ONCE A WEEK. IT IS KNOWN THAT VITAMIN B12 AND FOLIC ACID MOLECULES ARE ELIMINATED THROUGH DIALYSIS. VITAMIN B12 CLEARANCE VARIES WITHIN A DIFFERENT RANGE (DEPENDING ON THE DIALYZER MEMBRANE) 107-146 for Fx8 and Fx10 dialyzers and 118-131 for F8HPS and F10HPS dialyzers (such type of Low Flux dialyzers are used for hemodialysis of the followed-up patients).

Discussion and summary of results: After the follow-up of the paraclinic results and clinical status of the patients receiving periodic hemodialysis treatment for a period of 6 months, it may be summarized that MINOR/MAJOR SYMPTOMS OF FOLIC ACID DEFICIENCY IN SOME PATIENTS MAY BE DUE TO /OVERLAPPING WITH/ THE ANEMIC SYNDROME THAT IN SOME PATIENTS IS CAUSED BY the administration of insufficient amounts of Erythropoietin Stimulating Agents (ESA), or by ESA resistance.

CONCLUSIONS: THE RESULTS FROM THIS FOLLOW-UP INDICATE STABLE MAINTENANCE OF VITAMIN B12 AND FOLIC ACID SERUM LEVELS WITH THE ADMINISTRATION OF ABOPHOLIC META 1 TAB/DAY. There were no patients with FA level above the reference range. There was a statistically significant difference between the two sexes in terms of Folic acid. There was also a statistically significant difference between Vitamin B12 serum levels in women and men.

IN WHAT DIRECTION MAY THIS THESIS BE EXTENDED: FOR EXAMPLE, JUXTAPOSE/COMPARE THE RESULTS IN 1 MG/D AND 5 MG ONCE WEEK ADMINISTRATION. Our next follow-up will be probably focused in that direction.

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