

QUESTIONABLE RELIABILITY OF HOMOCYSTEINE AS THE METABOLIC MARKER FOR FOLATE AND VITAMIN B₁₂ DEFICIENCY IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

NEIZVESNA POUZDANOST HOMOCISTEINA KAO METABOLIČKOG MARKERA ZA NEDOSTATAK FOLATA I VITAMINA B₁₂ KOD PACIJENATA SA HRONIČNOM OPSTRUKTIVNOM BOLEŠĆU PLUĆA

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Summary

Background: An increased homocysteine (Hcy) concentration may represent a metabolic marker of folate and vitamin B₁₂ deficiency, both significant public health problems. For different reasons, patients with chronic obstructive pulmonary disease (COPD) are prone to these deficiencies. The study evaluates the reliability of Hcy concentration in predicting folate or vitamin B₁₂ deficiency in these patients.

Methods: A group of 50 COPD patients (28 males/22 females, age ($\bar{x} \pm SD = 49.0 \pm 14.5$) years) was enrolled. A chemiluminescent microparticle immunoassay was applied for homocysteine, folate and vitamin B₁₂ concentration. Kolmogorov-Smirnov, Mann-Whitney U and χ^2 tests, Spearman's correlation and ROC analysis were included in the statistical analysis, with the level of significance set at 0.05.

Results: Average (SD) concentrations of folate and vitamin B₁₂ were 4.13 (2.16) $\mu\text{g/L}$ and 463.6 (271.0) ng/L , whereas only vitamin B₁₂ correlated with the Hcy level ($P = -0.310$ ($R = 0.029$)). Gender related differences were not significant and only a borderline significant correlation

Kratak sadržaj

Uvod: Povišena koncentracija homocisteina (Hcy) može predstavljati metabolički marker nedostatka folata i vitamina B₁₂, značajnih problema javnog zdravlja. Bolesnici sa hroničnom opstruktivnom bolešću pluća (HOBP) skloni su nedostatku ovih vitamina usled različitih razloga. Prikazana studija procenjuje pouzdanost koncentracije Hcy kao prediktora nedostatka folata i vitamina B₁₂ kod ovih bolesnika.

Metode: Studija je sprovedena u grupi od 50 osoba obolelih od HOBP (28 muškaraca/22 žene, starosti ($\bar{x} \pm SD = 49,0 \pm 14,5$) godina. Koncentracije Hcy, folata i vitamina B₁₂ su određivane hemiluminiscentnim imunoodređivanjem na mikročesticama. Statistička analiza je uključila testove Kolmogorov-Smirnov, Mann-Whitney U and χ^2 , Spearman-ovu korelaciju i ROC analizu, uz nivo značajnosti od 0,05.

Rezultati: Prosečne (SD) koncentracije folata i vitamina B₁₂ su iznosile 4,13 (2,16) $\mu\text{g/L}$ i 463,6 (271,0) ng/L , pri čemu je samo kod vitamina B₁₂ uočena korelacija sa nivoom Hcy ($R = -0,310$ ($P = 0,029$)). Koncentracije vita-

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List of abbreviations: Hcy, homocysteine; Met, methionine; HHcy, hyperhomocysteinemia; COPD, chronic obstructive pulmonary disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease; CMLA, chemiluminescent microparticle immunoassay; SD, standard deviation; Q₁, 1st quartile; Q₃, 3rd quartile; AUC, area under the curve; SE, standard error; CI, confidence interval.

between age and folate was confirmed ($R=0.279$ ($P=0.047$)). The incidence of folate and vitamin B₁₂ deficiency differed significantly ($P=0.000$ and $P<0.000$ for folate and vitamin B₁₂ respectively), depending on the cut-off used for classification (4.4, 6.6 and 8.0 $\mu\text{g/L}$ – folate; 203 and 473 ng/L – vitamin B₁₂). ROC analyses failed to show any significance of hyperhomocysteinemia as a predictor of folate or vitamin B₁₂ deficiency.

Conclusion: Reliability of the Hcy concentration as a biomarker of folate or vitamin B₁₂ depletion in COPD patients is not satisfactory, so their deficiency cannot be predicted by the occurrence of HHcy.

Keywords: homocysteine, folate, vitamin B₁₂, COPD

Introduction

Folate is essential for »one carbon units' metabolism«, which generates intermediates necessary for nucleic acid synthesis and methylation of various biomolecules (1). Vitamin B₁₂ is required as the coenzyme to methylmalonyl-CoA mutase (EC 5.4.99.2) and methionine synthase (EC 2.1.1.13), whereby the second enzyme links the metabolism of these two »B complex« vitamins (2). It catalyzes the conversion of homocysteine (Hcy) to methionine (Met) by virtue of a methyl group transfer from the methyltetrahydrofolate. Deficiency of either vitamin can slow the reaction and lead to deficient DNA synthesis, hyperhomocysteinemia (HHcy) and compromised methylation potential (3).

World Health Organization (WHO) estimated that folate and vitamin B₁₂ deficiencies could be encountered in millions of people throughout various populations (3–5). Either of them may occur in subclinical form, when vitamin depletion can be indicated only through laboratory tests, and severe form in which clinical symptoms become overt (3). In a majority of cases, macrocytic anemia can be attributed to severe folate and vitamin B₁₂ deficiencies (6). A strong causative relation has been established between severe folate deficiency and neural tube defects, adverse pregnancy outcomes, cognitive disorders in elderly etc. Severe deficiency of vitamin B₁₂ is evidenced as a significant risk factor for developmental delay in childhood, neuropathy in adults and memory and cognitive impairments in all ages (4, 7). Although associated with cardiovascular disease, cancers and osteoporosis, the significance of subclinical deficiencies is still not reliably evaluated (5, 8). Inadequate folate intake is usually responsible for folate deficiency and malabsorption for vitamin B₁₂ deficiency (5).

Reliable laboratory assessment of folate and vitamin B₁₂ status is necessary to detect a deficiency, develop a strategy for supplementation and monitor its efficacy. Folate status is usually evaluated through measurement of the folate concentration in serum/plasma, indicating dietary intake, or erythrocytes, reflecting long-term status. Vitamin B₁₂ concentrations in serum/plasma are considered a screen-

ing test in suspected deficiency, with holotranscobalamin measurement being recommended to improve sensitivity (5). The utilization of metabolic markers in assessment has been recommended by the WHO since 2005 (6). HHcy may serve as an indicator of both deficiencies. An increased level of methylmalonic acid is specific for vitamin B₁₂ deficiency, while the fraction of »unmetabolized folic acid« might also be associated with impairment in the pathways depending on both vitamins (3, 5). The initially proposed cut-off values for the general population, based upon the occurrence of HHcy (defined as a Hcy concentration above 10 or 12 $\mu\text{mol/L}$) as an indicator of vitamin depletion, have undergone significant changes. For folate, 4.0 $\mu\text{g/L}$ (4) was increased to 6.6 $\mu\text{g/L}$ (3, 9, 10) or even 8.0 $\mu\text{g/L}$ (11), while to consider vitamin B₁₂ status as inadequate, cut-offs of 203 ng/L (3, 4) and 473 ng/L (9, 10) were used.

Zaključak: Pouzdanost koncentracije Hcy kao markera nedostatka folata ili vitamina B₁₂ kod bolesnika sa HOBP nije zadovoljavajuća, pa se deficijencija ovih vitamina ne može predvideti na osnovu pojave hiperhomocisteinije.

Ključne reči: homocistein, folat, vitamin B₁₂, HOBP

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Patients with chronic obstructive pulmonary disease (COPD) are evidenced to be prone to folate and vitamin B₁₂ deficiency, supposedly due to the severe nutritional impairments like increased basal metabolic rate, altered caloric intake, decreased body mass index accompanied with skeletal muscle loss. Furthermore, in certain populations an inverse relationship between folate food intake and COPD risk was observed. In studies investigating HHcy in these patients the relationship between Hcy concentration and vitamin intake or blood correlation was evaluated. Nevertheless, the ability of HHcy occurrence to predict folate or vitamin B₁₂ deficiency was not addressed (12–15).

The study aims to provide an initial insight into the reliability of Hcy concentration measurement in COPD patients for the purpose of detecting folate or vitamin B₁₂ deficiency. To achieve this, vitamin levels in this group were correlated with the Hcy concentration as well as with age and gender, the major physiological Hcy determinants (16). In addition, the possibility of various Hcy concentrations to predict folate or vitamin B₁₂ deficiency in COPD patients was analyzed.

Materials and Methods

This cross-sectional study, approved by a competent Ethics Committee and conducted at the Clinic for Lung Diseases and the Center for Medical Biochemistry of the Clinical Center of Serbia, enrolled 50 COPD patients (28 males/22 females, age $(\bar{x} \pm SD = 49.0 \pm 14.5)$ years, diagnosed according to the GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria (17). Among them, 15 attended the outpatient department of the Clinic, while 35 were hospitalized and enrolled upon remission accomplishment. Exclusion criteria were smoking, alcohol consumption and supplementation with folic acid and/or vitamin B₁₂, as well as the presence of coronary heart disease, cerebrovascular and gastrointestinal disorders, renal insufficiency, diabetes, malignant or autoimmune disease.

Blood samples were collected into SST[®] II Advance tubes (BD Vacutainer, Franklin Lakes, NJ, USA) following 12 hours of overnight fasting after a light meal and serum was separated by centrifugation for 15 minutes at 1500 g (16, 18). The chemiluminescent microparticle immunoassays (CMIA) on the ARCHITECT[®] ci8200 Integrated System (Abbott Diagnostics, Wiesbaden, Germany) was applied for measurement of homocysteine, folate and vitamin B₁₂ concentrations.

Kolmogorov-Smirnov, Mann-Whitney U tests and χ^2 tests, Spearman's correlation and ROC (Receiving Operator Characteristic) analysis were included in the statistical analysis, performed with SPSS[®] Statistic software version 22.0 (IBM[®], New York, USA). Deficiencies of both vitamins were defined using the abovementioned different cut-offs from the literature. To test the possibility that these may be predicted by the occurrence of HHcy, ROC analyses were performed for HHcy defined as a Hcy concentration above 10, 12 and 15 $\mu\text{mol/L}$. These cut-offs were chosen because they are applied to distinguish tolerable from increased Hcy concentrations (19). P values below 0.05 were considered statistically significant.

Results

The Gaussian mode of distribution was observed for folate (P=0.200) and vitamin B₁₂ (P=0.200), while this was not the case for Hcy (P=0.041). The following concentrations (mean (SD) /median (Q1–Q3)) were measured in patients enrolled: folate 4.13 (2.16) $\mu\text{g/L}$, vitamin B₁₂ 463.6 (271.0) ng/L, and Hcy 13.22 (11.48–16.08) $\mu\text{mol/L}$. Correlation analyses of the variables investigated (Table I) revealed an inverse relationship between vitamin B₁₂ and Hcy concentration as the only one significant.

Table I Correlation coefficients (R) between the investigated variables.

| | | |
|--------------------------------|-------------------------|--------------------------------|
| | Folate, $\mu\text{g/L}$ | Vitamin B ₁₂ , ng/L |
| Hcy, $\mu\text{mol/L}$ | - 0.138 (0.340) | - 0.310 (0.029) |
| Vitamin B ₁₂ , ng/L | 0.008 (0.955) | / |

P values are given in brackets

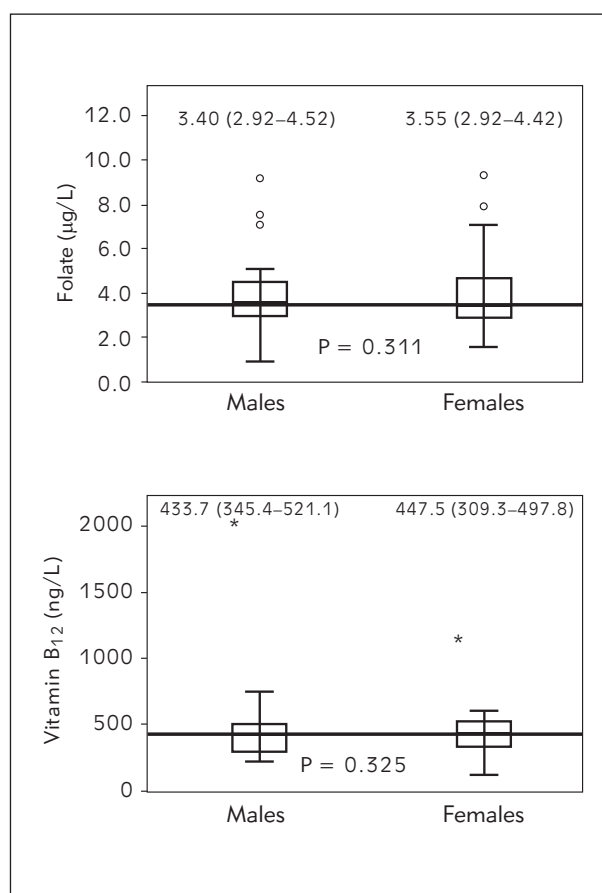


Figure 1 Gender associated differences in concentrations of folate and vitamin B₁₂.

Table II Incidence of vitamin deficiencies and the corresponding cut-offs.

| Folate | | | |
|-----------------------------|-------|-------|-------|
| Cut-off ($\mu\text{g/L}$) | 4.0 | 6.6 | 8.0 |
| Ratio | 16/50 | 42/50 | 45/50 |
| Vitamin B ₁₂ | | | |
| Cut-off (ng/L) | 203 | | 473 |
| Ratio | 2/50 | | 29/50 |

Table III ROC analysis for prediction of folate deficiency.

| Vitamin cut-off | 4.0 µg/L | | | | 6.6 µg/L | | | | 8.0 µg/L | | | |
|-----------------|----------|-------|-------------|-------|----------|-------|-------------|-------|----------|-------|-------------|-------|
| HHcy cut-off | AUC | SE | 95% CI | P | AUC | SE | 95% CI | P | AUC | SE | 95% CI | P |
| 10 µmol/L | 0.514 | 0.101 | 0.317–0.711 | 0.721 | 0.552 | 0.112 | 0.332–0.773 | 0.651 | 0.686 | 0.109 | 0.472–0.900 | 0.229 |
| 12 µmol/L | 0.556 | 0.103 | 0.354–0.757 | 0.584 | 0.603 | 0.113 | 0.382–0.824 | 0.375 | 0.661 | 0.139 | 0.389–0.933 | 0.298 |
| 15 µmol/L | 0.620 | 0.094 | 0.435–0.805 | 0.235 | 0.615 | 0.104 | 0.410–0.819 | 0.322 | 0.671 | 0.113 | 0.451–0.892 | 0.267 |

Table IV ROC analysis for prediction of vitamin B₁₂ deficiency.

| Vitamin cut-off | 203 ng/L | | | | 473 ng/L | | | |
|-----------------|----------|-------|-------------|-------|----------|-------|-------------|-------|
| HHcy cut-off | AUC | SE | 95% CI | P | AUC | SE | 95% CI | P |
| 10 µmol/L | 0.553 | 0.267 | 0.029–1.000 | 0.859 | 0.633 | 0.097 | 0.443–0.824 | 0.166 |
| 12 µmol/L | 0.632 | 0.224 | 0.193–1.000 | 0.657 | 0.671 | 0.094 | 0.487–0.854 | 0.076 |
| 15 µmol/L | 0.842 | 0.108 | 0.631–1.000 | 0.248 | 0.504 | 0.096 | 0.316–0.693 | 0.965 |

Gender associated differences in concentration (Figure 1) were not of significance for either vitamin. Correlation between patients' age and vitamin concentration was characterized with the $R=0.279$ ($P=0.047$) for folate and $R=-0.103$ ($P=0.477$) in case of vitamin B₁₂.

The calculated incidence of folate and vitamin B₁₂ deficiencies differed significantly ($P=0.000$ and $P<0.000$ for folate and vitamin B₁₂ respectively), depending on which cut-off was used for classification (Table II).

ROC analyses failed to show any significance of HHcy (defined by various Hcy concentrations which should be exceeded) as a predictor of folate or vitamin B₁₂ deficiency, regardless of the cut-off used to define vitamin deficiency. Tables III and IV contain details about the ROC analysis for folate and vitamin B₁₂ deficiency, respectively.

Discussion

In the COPD patients enrolled, an inverse relationship between the concentrations of vitamins and Hcy was significant only for vitamin B₁₂. This finding is opposite to the one reported on the basis of values measured in a group of Italian patients (15) and partially concordant with the results in a group of patients from North America in whom no significant association was observed between HHcy and these vitamins' intake as estimated from a food frequency questionnaire (14). Although higher concentrations

of folate were observed in healthy women (6, 10), gender related differences in COPD patients were not evaluated previously. Results from this study indicate that male and female patients have comparable values of both vitamins. Furthermore, a positive correlation between folate (6, 10) and vitamin B₁₂ (10) levels and age was shown in a healthy population. However, only a borderline significant correlation with folate was confirmed in this study.

Average folate and vitamin B₁₂ concentrations in this study were lower than those measured in a group of Swedish patients (7.0 µg/L and 540.0 ng/L for folate and vitamin B₁₂ respectively) (12) and higher in comparison with Italian patients (2.5 µg/L and 324.5 ng/L) (15). Nevertheless, significant caution is recommended when interpreting these differences. Elderly patients were enrolled in the Swedish study and information about the methods applied for measurement was not included in the article reporting the results. In the study conducted in Italy, although the concentrations were also measured using CMIA, only patients with no COPD exacerbations 6 months prior to blood sampling were included.

Prevalence of deficiency in the present study was highly dependent on the cut-off values used. In the case of folate, it increased from 32.3%, when defined with the cut-off of 4.0 µg/L, to 84.0% or even 90.0% with the cut-off set at 6.6 µg/L and 8.0 µg/L respectively. For vitamin B₁₂, the change of cut-off from 203 ng/L to 473 ng/L was accompanied by an increase in the percentage of patients considered deficient from 4.0% to 58.0%. To consider Swedish

patients as folate deficient, the cut-off was set at 2.64 µg/L and yielded the prevalence of approximately 17%, while a vitamin B₁₂ concentration of 203 ng/L classified almost 6% as deficient (12).

The ROC analyses, using different vitamin and Hcy cut-offs, clearly demonstrated that folate or vitamin B₁₂ deficiency in the investigated group cannot be predicted by the occurrence of HHcy. Although this finding might significantly neglect the clinical utility of Hcy measurement in COPD patients, further consideration could offer additional standpoints. It may support the hypothesis that intensive methylation of DNA, RNA and different proteins during lung tissue repair generates high amounts of S-adenosyl-Hcy and Hcy in the lung tissue and plasma respectively (20). Despite the fact that this Hcy influx might be regarded as an epiphenomenon in COPD, not influenced by any traditional determinant of Hcy concentration (20), certain attention should be focused on the molecular and cellular oxidative damage caused by HHcy (21). Taken together with the report about the presence of increased oxidative stress at the moment of remission accomplishment after COPD exacerbation (22), it can be assumed that a certain

time span is needed for the vitamins' depletion, predictable by HHcy, to develop in these patients.

A rather small number of participants is a limitation of the present study. Although the other studies addressing »B-vitamins complex« deficiency and its relationship with the Hcy level included a comparable number of patients, the results presented should be interpreted as »pilot« and further evaluation of their confidence in larger studies is recommended.

The results presented support the conclusion that the reliability of Hcy concentration as a biomarker of folate or vitamin B₁₂ depletion in COPD is not satisfactory and that their deficiency cannot be predicted by the occurrence of HHcy.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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