



DOI: 10.2478/rmlm-2014-0018

## Screening of the vitamin B<sub>12</sub> status in an urban population sample from Romania: a pilot study

### Screening al statusului vitaminei B<sub>12</sub> într-un eșantion al populației urbane din România: un studiu pilot

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#### Abstract

The aim of this study was to evaluate the vitamin B<sub>12</sub> status in a non-vegetarian sample of the adult urban population from Romania. The studied sample included 80 non vegetarian subjects aged between 19 and 74.6 years who did not use vitamin B<sub>12</sub> supplements or vitamin B<sub>12</sub> fortified food. The serum vitamin B<sub>12</sub> assay was performed using electrochemiluminescence immunoassay on a Roche Elecsys 2010 analyzer. Among all subjects, 93.75% (95% CI 86.2-97.3%) had serum vitamin B<sub>12</sub> concentrations in the reference range of the employed method (191-663 pg/ml). In these subjects, we found no statistically significant correlations between serum vitamin B<sub>12</sub> concentration and age or between serum vitamin B<sub>12</sub> concentration and hemoglobin level. The serum vitamin B<sub>12</sub> levels did not differ significantly ( $p > 0.05$ -Student's *t*-test) between women and men. Subnormal serum levels of vitamin B<sub>12</sub> were observed in 5% (95%CI 1.96-12.16%) of the investigated subjects, associated with irritable bowel syndrome and with long-term therapy with proton pump inhibitors or birth control pills. In the present study, we have identified a large number of subjects with marginal depletion of vitamin B<sub>12</sub> in the population aged below 50 years. These results suggest the need of a screening for vitamin B<sub>12</sub> status in people from all age groups, the importance of the identification of responsible causes for the high prevalence of vitamin B<sub>12</sub> marginal status, as well as the fact that monitoring the vitamin B<sub>12</sub> status is especially important in subjects undergoing long-term treatment with certain drugs.

**Keywords:** general population, vitamin B<sub>12</sub> deficiency, marginal status of vitamin B<sub>12</sub>.

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## Rezumat

Scopul acestui studiu a fost evaluarea statusului vitaminei  $B_{12}$  într-un eșantion al populației urbane adulte din România. În studiu au fost incluși 80 de subiecți non-vegetarieni, cu vârste cuprinse între 19 și 74,6 ani, care nu au utilizat suplimente de vitamina  $B_{12}$  sau alimente fortificate cu vitamina  $B_{12}$ . Dozarea vitaminei  $B_{12}$  serice s-a realizat prin metoda imunochimică cu detecție prin chemiluminiscență pe analizor Elecsys 2010 (Roche). Concentrația serică a vitaminei  $B_{12}$  s-a încadrat în intervalul de referință (191-663 pg/ml) la 93,75% (95% CI 86,2-97,3%) din subiecți. La acești subiecți nu s-au evidențiat corelații semnificative statistic între concentrația serică a vitaminei  $B_{12}$  și vârsta subiecților sau concentrația hemoglobinei. Nu s-au găsit nici diferențe semnificative ( $p > 0.05$ ) ale statusului vitaminic la femei față de bărbați. Concentrația serică a vitaminei  $B_{12}$  s-a situat sub limita inferioară a intervalului de referință la 5% (95% CI 1,96-12,16%) din subiecții investigați, fiind asociată tratamentului cu contraceptive orale, inhibitori ai pompei de protoni (IPP) respectiv sindromului de colon iritabil. La un număr mare de subiecți cu vârsta sub 50 de ani, concentrația serică a vitaminei  $B_{12}$  s-a încadrat în limitele corespunzătoare statusului marginal. Rezultatele studiului sugerează necesitatea efectuării screeningului statusului vitaminei  $B_{12}$  la întreaga populație, importanța identificării factorilor responsabili de prevalența crescută a statusului marginal și importanța monitorizării statusului vitaminei  $B_{12}$  la subiecții care fac tratament îndelungat cu unele medicamente.

**Cuvinte cheie:** populația generală, deficiența vitaminei  $B_{12}$ , status marginal al vitaminei  $B_{12}$ .

**Received:** 27<sup>th</sup> January 2014; **Accepted:** 18<sup>th</sup> May 2014; **Published:** 25<sup>th</sup> May 2014.

## Introduction

Maintaining an optimal vitamin  $B_{12}$  status is essential for human health. Deficiency of this vitamin causes megaloblastic anemia, leads to elevated levels of homocysteine and methylmalonic acid, and is associated with cognitive and neuropsychiatric disorders (1).

Vitamin  $B_{12}$  deficiency has been reported in certain population groups: in elderly people (2), in vegetarians and especially the vegans (3), in patients with intestinal diseases (4), in patients suffering from primary hypothyroidism (5). At risk are also subjects receiving for long periods certain medications such as: proton pump inhibitors, histamine  $H_2$ -receptor antagonists (6), and metformin (7).

Questions regarding the screening for vitamin  $B_{12}$  deficiency, the potential benefit of vitamin  $B_{12}$  fortification of flour and the identification of subjects with subtle vitamin  $B_{12}$  deficiency which need treatment with  $B_{12}$  vitamin are largely debated in the scientific literature (8-11).

Data regarding the prevalence of vitamin  $B_{12}$  deficiency in the Romanian population have not been reported to date. Therefore the aim of this study was to screen for the vitamin  $B_{12}$  status in a non-vegetarian sample of the urban adult population from North-Western Romania.

## Material and methods

### Subjects

The study was approved by our University Ethics Committee and all participants signed an informed written consent before being included in the study, according to the World Medical Association Declaration of Helsinki, revised in 2013, in Fortaleza, Brazil. The study was carried out over a period of two month, from January to February 2012.

A convenience sample of 80 urban residents from the city of Cluj-Napoca was included in our pilot study (35 male and 45 female). The age of subjects ranged between 19 and 74.6 years, with a mean age of 31.8 years. All participants were non-vegetarians and declared that they did

not take vitamin B<sub>12</sub> supplements or vitamin B<sub>12</sub> fortified food and that their serum concentration of vitamin B<sub>12</sub> had never been measured prior to this study. Information regarding the use of medication such as: proton pump inhibitors (PPI), histamine H<sub>2</sub>-receptor antagonists, metformin and oral contraceptives was obtained, from all participants, using a self-administered questionnaire.

### ***Blood sampling and biochemical methods***

#### ***Vitamin B<sub>12</sub> assay***

Venous blood samples were collected, after an overnight fast, into tubes without anticoagulant. The samples were placed at room temperature for 1h and then they were centrifuged for 10 minutes at 590g. The serum was separated and assayed for vitamin B<sub>12</sub>. The assays were performed at the SC Synevo Romania SRL, Laboratory from Cluj-Napoca, using electrochemiluminescence immunoassay (Roche Diagnostics GMBH Mannheim, Germany) on a Roche Elecsys 2010 analyzer. The laboratory uses the reference range recommended by Roche Diagnostics GMBH, for adults from the European population: 191-663 pg/ml (12, 13). The intra-assay coefficient of variation (CV) determined by the laboratory, for this method, was: CV=3.25% for a target value of 25.6 pg/ml and CV=5.5% for a target value of 1920 pg/ml.

Vitamin B<sub>12</sub> deficiency was defined at serum vitamin levels under 191 pg/ml and a concentration between 191 and 350pg/ml was defined as marginal level (2, 14).

#### ***Hemoglobin and mean cell volume determination***

A fasting blood sample was collected from all subjects into tubes containing the anticoagulant K3-EDTA. The hemoglobin concentration was determined by Drabkin's method, in all studied subjects (15). The mean cell volume (MCV) and the hemoglobin concentration (sodi-

um lauryl sulfate-hemoglobin method) were determined on an automated hematology analyzer XT-4000i (Sysmex, Japan), only in vitamin B<sub>12</sub> deficient subjects (16).

### ***Statistical methods***

Description of categorical data has been performed by computing frequencies and their 95% confidence intervals. Descriptive statistics for central tendency and spread of quantitative variables have been computed. Normality of quantitative variables was tested using a Kolmogorov-Smirnov test followed by Q-Q plots. Since all investigated variables seemed to have originated from normal distributions, Student's t-test for independent groups was used for hypothesis testing. Statistical significance was defined for  $p < 0.05$ . Correlation between quantitative variables has been investigated using Pearson's correlation coefficient ( $r$ ) and evaluated for statistical significance at a level  $\alpha = 0.05$ .

Data description and analysis were performed using Microsoft Excel 2003, PSPP 0.7.10 and R 2.15.1.

### ***Results***

Among all subjects, 93.75% (95% CI 86.2-97.3%) had serum vitamin B<sub>12</sub> concentrations in the reference range of the employed method (191-663 pg/ml). In these subjects, we found no statistically significant correlations between serum vitamin B<sub>12</sub> concentration and age or between serum vitamin B<sub>12</sub> concentration and hemoglobin level.

The serum level of vitamin B<sub>12</sub> did not differ significantly between female and male subjects (*Table I*).

A marginal status of vitamin B<sub>12</sub> (serum levels between 191 and 350 pg/ml) was observed in 50.67% (95% CI 39.6-61.67%) of subjects (female  $n=22$ , male  $n=16$ ) who exhibited vitamin B<sub>12</sub> levels within the reference interval ( $n=75$ ).

**Table I. Age and serum vitamin B<sub>12</sub> concentrations in females and males**

	Females (n=43)	Males (n=32)	p-value
Age (years)	31.1±10.4	32.7±12.8	>0.05
Serum vitamin B <sub>12</sub> concentration (pg/ml)	361.7±131.1	372.3±88.5	>0.05

Values are mean±SD

**Table II. Characteristics of the four vitamin B<sub>12</sub> deficient patients**

Subjects			Measured parameters		
Age (years)	Gender	Medication/Disease	Vitamin B <sub>12</sub> (pg/ml)	Hemoglobin (g/dl)	MCV (fL)
69	male	Irritable bowel syndrome and Antihypertensive medication	175	14.4	84.3
43	male	PPI	183	14.3	89.4
59	male	PPI	133	16.7	89.6
22	female	Birth control pills	156	13.3	87.6

Reference intervals for hemoglobin concentration:

Male: 13.2-17.3 g/dl (18-44 years); 13.1-17.2 g/dl (45-64 years); 12.6-17.4 (>65 years). Female: 11.7-15.5 g/dl (18-44 years)

Reference intervals for MCV:

Male: 80-99 fL (18-44.9 years); 81-101 fL (45-64.9 years); 81-103 fL (>65 years). Female: 81-100 fL (18-44.9 years).

In order to achieve a precision of 5% for the 95% CI of a comparable prevalence determined by a future study, a necessary sample size of 384 subjects has been calculated.

Among the 80 evaluated subjects, 5% (95% CI 1.96-12.16%) (n=4) had vitamin B<sub>12</sub> levels below 191 pg/ml. In all these subjects, both MCV and hemoglobin concentration were within the reference range (*Table II*). In one subject, the serum vitamin B<sub>12</sub> concentration exceeded the upper threshold of the reference interval (663 pg/ml).

## Discussions

Vitamin B<sub>12</sub> status is not routinely evaluated by physicians. For instance, in 2012, the Synevo laboratory from Cluj-Napoca performed a mean number of only 56 vitamin B<sub>12</sub> assays per month.

The sample size of 80 subjects has been chosen in our study because a pilot study of more than 80 subjects is likely to be unrealistic in terms of time and costs (17).

In this pilot sample we observed that only 5% of the investigated subjects had vitamin B<sub>12</sub> deficiency (<191 pg/ml). All these subjects were at risk to develop vitamin B<sub>12</sub> deficiency. Two subjects declared to use proton pump inhibitors (2 years of treatment), and one subject declared to use birth control pills (4 years of treatment). Unfortunately, no data was available regarding the serum vitamin B<sub>12</sub> concentration prior to the commencement of treatment with PPI and birth control pills. Nevertheless, the existence of a relationship between such medication and serum vitamin B<sub>12</sub> concentrations is plausible. Previous studies reported significantly lower serum vitamin B<sub>12</sub> concentrations in healthy female

using oral contraceptives compared to controls, (18, 19). Vitamin B<sub>12</sub> deficiency without clinical symptoms has also been described in such cases (18). Our results also suggest that the evaluation of serum vitamin B<sub>12</sub> concentration at the beginning of treatment and a periodic reassessment of vitamin B<sub>12</sub> concentration are important to identify progressive depletion of vitamin B<sub>12</sub> serum levels in subjects treated with PPI or birth control pills for long periods of time.

In one subject from our study vitamin B<sub>12</sub> deficiency was associated with irritable bowel syndrome. Vitamin B<sub>12</sub> deficiency has been reported to be associated with intestinal disorders (10, 20). Therefore, a regular screening for vitamin B<sub>12</sub> deficiency is important in patients with medical conditions affecting intestinal vitamin B<sub>12</sub> absorption.

Once the cut-off value for vitamin B<sub>12</sub> deficiency was raised to 350 pg/ml, 52.5% of all investigated subjects were found to be vitamin B<sub>12</sub> deficient in our study.

For an urban North Indian population (with an age range between 16 and 74 years, close to the one investigated in our study), Arora et al. reported a prevalence of 43% for vitamin B<sub>12</sub> deficiency (levels <200 pg/ml) and 77.7% when the cut-off was raised to 350 pg/ml. The study group included vegetarians and non-vegetarians and the vegetarian lifestyle was found to be a major cause for vitamin B<sub>12</sub> deficiency (14). Given the fact that our studied sample was constituted only by non-vegetarian subjects, future research needs to be performed in order to elucidate the causes of the high prevalence of marginal vitamin B<sub>12</sub> depletion observed in this study.

A study conducted in the US population showed that marginal depletion of vitamin B<sub>12</sub> (serum level: 200-298 pg/ml) was present in ≈14-16% of subjects aged 20-59 years (9). Had we used the same threshold levels to identify subjects with marginal vitamin B<sub>12</sub> depletion for subjects from our study falling within the same

age range (20-59 years), the prevalence in our sample would have been higher (43.66%) compared to the above mentioned study.

Qatatsheh et al. found low vitamin B<sub>12</sub> concentrations (<207 pg/ml) in 25.6% of investigated healthy Jordanian subjects. The study group included 511 participants aged between 15 and 80 years, and 1.2% of them were vegetarians (21). Compared to the results of this study, the prevalence of low vitamin B<sub>12</sub> concentration was lower (5%) in our study group (n=80). The mean concentration of vitamin B<sub>12</sub> was somewhat higher in our study group (360.78±123 pg/ml versus 340±197.2 pg/ml), possibly because our sample included only non-vegetarians.

Scientific literature suggests that a screening for vitamin B<sub>12</sub> is recommended at age 50, and after 65 years this test must be repeated annually (22, 23). In our study, marginal status of vitamin B<sub>12</sub> was observed in 45.33% of subjects below 50 years. Since all subjects from the study group were non-vegetarians, further studies are necessary to identify the factors that could be responsible for such a high prevalence of vitamin B<sub>12</sub> marginal status.

Data from literature revealed that vitamin B<sub>12</sub> deficiency symptoms may appear even in individuals who have vitamin B<sub>12</sub> levels considered as normal (24). In this context, the results of the present study suggest, in our opinion, the need for a periodic screening of vitamin B<sub>12</sub> status, even before the age of 50, in all age groups.

A limitation of our study resided in the relatively low number of subjects that agreed to participate in this pilot study. Further research studies are needed to evaluate the status of vitamin B<sub>12</sub> in subjects from Romania and to identify the responsible causes for the high prevalence of the marginal status of vitamin B<sub>12</sub> observed in this study. These studies should be performed on a larger, representative sample of healthy subjects, by coupling the measurement of vitamin B<sub>12</sub> levels with the assay of other biomarkers that are



recognized to be more reliable indicators of the vitamin B<sub>12</sub> status such as: holotranscobalamine, methyl malonic acid and homocysteine (10).

## Conclusions

This is the first study to report data on vitamin B<sub>12</sub> status in a Romanian population. Vitamin B<sub>12</sub> deficiency was found in 3 subjects treated for long timespans with certain drugs (proton pump inhibitors, oral contraceptives) and in a subject with irritable bowel syndrome. Marginal status of serum vitamin B<sub>12</sub> levels was observed in a substantial number of subjects aged below 50 years. Further studies, using more specific indicators for assessing vitamin B<sub>12</sub> status, are needed in order to obtain further information regarding the prevalence of vitamin B<sub>12</sub> deficiency in Romania.

## Acknowledgements

We express our thanks to all the volunteers who accepted to take part in the present study. We are grateful to Teodora Tudoran Sârbu and her laboratory colleagues at Synevo Laboratory, Cluj-Napoca, Romania for their help in this study. This research was partially supported by the "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania (Grant no.22714/6/06.10.2011).

## Abbreviations

PPI - proton pump inhibitors

CV - coefficient of variation

MCV - mean cell volume

## Conflict of interest

None of the authors had a personal or financial conflict of interest.

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