DE GRUYTER OPEN

Research article

DOI: 10.2478/rrlm-2014-0018

Screening of the vitamin B_{12} status in an urban population sample from Romania: a pilot study

Screening al statusului vitaminei B_{12} într-un eşantion al populaţiei urbane din România: un studiu pilot

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Abstract

The aim of this study was to evaluate the vitamin B_{12} 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_{12} supplements or vitamin B_{12} fortified food. The serum vitamin B_{12} 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_{12} 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_{12} concentration and age or between serum vitamin B_{12} concentration and hemoglobin level. The serum vitamin B_{12} levels did not differ significantly ($p>0.05$-Student's t-test) between women and men. Subnormal serum levels of vitamin B_{12} 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 pomp inhibitors or birth control pills. In the present study, we have identified a large number of subjects with marginal depletion of vitamin B_{12} in the population aged below 50 years. These results suggest the need of a screening for vitamin B_{12} status in people from all age groups, the importance of the identification of responsible causes for the high prevalence of vitamin B_{12} marginal status, as well as the fact that monitoring the vitamin B_{12} status is especially important in subjects undergoing long-term treatment with certain drugs.

***Keywords**: general population, vitamin B_{12} deficiency, marginal status of vitamin B_{12}.*

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Rezumat

Scopul acestui studiu a fost evaluarea statusului vitaminei B_{12} într-un eşantion al populaţiei urbane adulte din România. În studiu au fost incluşi 80 de subiecţi non-vegetarieni, cu vârste cuprinse între 19 şi 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 detecţie prin chemiluminiscenţă pe analizor Elecsys 2010 (Roche). Concentraţia serică a vitaminei B_{12} s-a încadrat în intervalul de referinţă (191-663 pg/ml) la 93,75% (95% CI 86,2-97,3%) din subiecţi. La aceşti subiecţi nu s-au evidenţiat corelaţii seminificative statistic între concentraţia serică a vitaminei B_{12} şi vârsta subiecţilor sau concentraţia hemoglobinei. Nu s-au găsit nici diferenţe semnificative ($p>0.05$) ale statusului vitaminic la femei faţă de bărbaţi. Concentraţia serică a vitaminei B_{12} s-a situat sub limita inferioară a intervalului de referinţă la 5% (95%CI 1,96-12,16%) din subiecţii investigaţi, fiind asociată tratamentului cu contraceptive orale, inhibitori ai pompei de protoni (IPP) respectiv sindromului de colon iritabil. La un număr mare de subiecţi cu vârsta sub 50 de ani, concentraţia 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 populaţie, importanţa identificării factorilor responsabili de prevalenţa crescută a statusului marginal şi importanţa monitorizării statusului vitaminei B_{12} la subiecţii care fac tratament îndelungat cu unele medicamente.

***Cuvinte cheie**: populaţia generală, deficienţa vitaminei B_{12}, status marginal al vitaminei B_{12}.*

Received: 27th January 2014; Accepted: 18th May 2014; Published: 25th 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 H2-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_{12} supplements or vitamin B_{12} fortified food and that their serum concentration of vitamin B_{12} had never been measured prior to this study. Information regarding the use of medication such as: proton pump inhibitors (PPI), histamine H2-receptor antagonists, metformin and oral contraceptives was obtained, from all participants, using a self-administered questionnaire.

Blood sampling and biochemical methods

Vitamin B_{12} 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_{12}. 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_{12} 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 (sodium lauryl sulfate-hemoglobin method) were determined on an automated hematology analyzer XT-4000i (Sysmex, Japan), only in vitamin B_{12} 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_{12} 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_{12} concentration and age or between serum vitamin B_{12} concentration and hemoglobin level.

The serum level of vitamin B_{12} did not differ significantly between female and male subjects (*Table I*).

A marginal status of vitamin B_{12} (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_{12} levels within the reference interval (n=75).

Table I. Age and serum vitamin B_{12} 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_{12} 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_{12} deficient patients

Subjects			**Measured parameters**		
Age (years)	**Gender**	**Medication/Disease**	**Vitamin B_{12}** (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_{12} 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_{12} concentration exceeded the upper threshold of the reference interval (663 pg/ml).

Discussions

Vitamin B_{12} 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_{12} 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_{12} deficiency (<191 pg/ml). All these subjects were at risk to develop vitamin B_{12} deficiency. Two subjects declared to use proton pomp 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_{12} 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_{12} concentrations is plausible. Previous studies reported significantly lower serum vitamin B_{12} concentrations in healthy female

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

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

Once the cut-off value for vitamin B_{12} deficiency was raised to 350 pg/ml, 52.5% of all investigated subjects were found to be vitamin B_{12} 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_{12} 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_{12} 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_{12} depletion observed in this study.

A study conducted in the US population showed that marginal depletion of vitamin B_{12} (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_{12} 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_{12} 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_{12} concentration was lower (5%) in our study group (n=80). The mean concentration of vitamin B_{12} 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_{12} 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_{12} 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 identity the factors that could be responsible for such a high prevalence of vitamin B_{12} marginal status.

Data from literature revealed that vitamin B_{12} deficiency symptoms may appear even in individuals who have vitamin B_{12} 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_{12} 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_{12} in subjects from Romania and to identify the responsible causes for the high prevalence of the marginal status of vitamin B_{12} observed in this study. These studies should be performed on a larger, representative sample of healthy subjects, by coupling the measurement of vitamin B_{12} levels with the assay of other biomarkers that are

recognized to be more reliable indicators of the vitamin B_{12} status such as: holotranscobalamine, methyl malonic acid and homocysteine (10).

Conclusions

This is the first study to report data on vitamin B_{12} status in a Romanian population. Vitamin B_{12} 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_{12} levels was observed in a substantial number of subjects aged below 50 years. Further studies, using more specific indicators for assessing vitamin B_{12} status, are needed in order to obtain further information regarding the prevalence of vitamin B_{12} 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 Haţieganu" 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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