Smoking Discriminately Changes the Serum Active and Non-Active Forms of Vitamin B12

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Received: 20 May 2016; Accepted: 01 Feb. 2017

Abstract- Smoking may modify the appetite, and consequently affect nutrient intake and serum micronutrients. The effect of smoking on vitamin B12 status has been considered in several studies. The research proposed that organic nitrites, nitro oxide, cyanides, and isocyanides of cigarette smoke interfere with vitamin B12 metabolism, and convert it to inactive forms. This research was carried out to determine the serum level of active and inactive forms of vitamin B12 in male smokers in comparison with male nonsmokers. This is a case-control study, in which the participants were 85 male smokers and 85 male nonsmokers. The serum levels of total and active form of vitamin B12 were measured. Dietary intake was recorded by a quantitative food frequency questionnaire and one-day 24-hour dietary recall method. Independent two sample T test was used to compare quantitative variables between the case and control groups. The serum level of total vitamin B12 was not significantly different between two groups, but serum level of active form of vitamin B12 in the smoking group was significantly lower than non-smoking group (P<0.001). This is one of the first studies that evaluated the serum level of active form of vitamin B12 in smokers in the Iranian community. The results of this study identified that serum level of total vitamin B12 might be not different between smoking and non-smoking people, but the function of this vitamin is disturbed in the body of smokers through the reduction of serum level of active form of vitamin B12.

Acta Med Iran 2017;55(6): 389-394.

Keywords: Smoking; Active form; Non-active form; Vitamin B12

Introduction

Smoking is one of the underlying causes of many diseases and health-related problems in the world (1). The World Health Organization (WHO) ranked tobacco smoking among the 10 major risks for health. The WHO estimated that each year almost 6 million premature deaths were attributed to smoking-related diseases worldwide, with the majority of deaths occurring in middle-income and low-income countries (2). Smoking is a major risk factor for cancer, heart diseases, lung disease, and other diseases (3).

Recent studies have reported that the intake of some micronutrients such as carotene, vitamin C, vitamin B6, vitamin B12, and folate is reduced in smokers (4). The mechanism of the effect of smoking on non-antioxidant nutrients is by destroying or disabling them by nitro oxide, cyanide, and isocyanides in cigarette smoking (5).

The effect of smoking on vitamin B12 status has been considered in a few studies. It is proposed that organic nitrites, nitro oxide, cyanide, and isocyanides in cigarette smoke interfere with vitamin B12 metabolism, and converts it to inactive forms (3,6). Cyanide should be detoxified in different ways. Cyanide and thiocyanate can be metabolized by major and several minor pathways. One of the minor routes includes the combination of cyanide with hydroxocobalamin (the active form of vitamin B12) to yield cyanocobalamin (the non-active form of vitamin B12) (7).

Considering the role of vitamin B12 in detoxification of cyanide, this hypothesis arose that the total amount of vitamin B12 in smokers may not be different compared with nonsmokers. However, due to the reduction of the active form of vitamin B12 in smokers, its biological activity would be disturbed, and side effects of vitamin B12 deficiency might arise in smokers. The present study was carried out with to determine the serum active and nonactive forms of vitamin B12 in male smokers in

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comparison with male nonsmokers.

Materials and Methods

Subject

This case-control study consisted of 85 male smokers and 85 male nonsmokers who referred voluntarily to Tehran University of Medical Sciences following notice. The sample size for this study was calculated based on the formula, N=2 [(Z1-α/2 Z1-β) 2×S2]/d2 where α=0.05 (type 1 error) and β=0.20 (type 2 error). Vitamin B12 was determined as the main variable. The variance of serum vitamin B12 was 139 (8), and the difference in mean of this variable was 60 pmol/l. The formula estimated that 84 participants were required for each group. The exclusion criteria included diverticulosis, ileum or stomach surgery, inflammatory bowel diseases, intestinal absorptive disorders, diabetes, cardiovascular diseases, uses of antacids, metformin, aminosalicylic acid, colchicines, neomycin, alcohol, and vegetarian diets. Subjects who were smoking more than 5 cigarettes per day for the last six consecutive months were categorized as smokers. Current smokers were asked about the amount and duration of smoking. Also, subjects were asked education level. All the participants had been informed about the aims and processes of the project and signed informed consent from Tehran University of medical sciences. The protocol was approved by the local ethical committee at Tehran University of Medical Sciences.

Anthropometric assessment

Subjects were weighed without shoes and in their light clothing with portable electronic digital scale (seca, clara-803, Hamburg, Germany), with an accuracy of ±100 g, while body height was measured without shoes to the nearest 0.1 cm by a stadiometer (seca 206, Hamburg, Germany). Body mass index (BMI) was calculated as body weight in kilograms (kg) divided by the square of the body height in meters (m^2).

Dietary assessment

Dietary intake was recorded by a quantitative food frequency questionnaire (FFQ) consisting of a list of foods with a standard serving size and one-day 24-hour dietary recall method. All participants were asked about the amount and frequency of consuming of each food item during the past year on a daily (e.g., bread), weekly (e.g., rice, meat), or monthly (e.g., fish) basis. Household measures were used to convert all the portion sizes of consumed foods to grams (9). The validity and reliability of the FFQ have been shown previously (10). In one-day 24-hour dietary recall method, detailed descriptions of every foods and beverage consumed on the day before the interview including the quantity and cooking method were recorded. Nutrient analysis of diets was done using Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods. Finally, the daily intakes of all food items were calculated and used in statistical analyses. All data were collected by trained and certified staff. Energy intake calculated from any 24-hour dietary recall or ffq which was below 3350 kJ/d (800 Kcal/d) or above 17570 kJ/d (4200 Kcal/d) denoted misreporting and were excluded eventually (11).

Laboratory measurements

Venous blood samples were drawn by trained staff from all participants, for biological screening tests in the morning between 7:00 and 9:00 AM following an overnight fast. Sera were separated, and stored at -70˚ c. Bio safety principles were observed for this collection. Quantitative determination of serum total vitamin B12 was performed by radioimmunoassay kit (simulTRAC-SNB), and the concentration of active form of serum vitamin B12 was determined by ELISA kit (Axis-shield). A 10 mL sample of blood was drawn from each subject into EDTA-containing evacuated tubes. Uncoagulated blood was used for measurement of hematological parameters [hemoglobin (Hb), hematocrit, mean corpuscular volume (MCV) and mean corpuscular Hb (MCH)], using an automated cell counter (K1000, Sysmex, Kobe, Japan).

Assessment of other variables

Data on demographic and background characteristics, including education, occupation, current special diet, medication usage, and using supplement were collected by an interviewer administered the questionnaires.

Statistical analysis

All statistical tests were performed by the Statistical Package for Social Science (SPSS) software, version 22, (SPSSInc, Chicago, IL, USA), and $P<0.05$ was considered statistically significant. Normality of variables was examined by Kolmogorov-Simonov test. All values were reported as mean±standard error. Independent two sample T test was used to compare quantitative variables between the case and control groups. In the case of nominal or categorized

characteristics, chi-square test has been utilized. The correlation between two characteristics has been evaluated by Pearson correlation.

Results

This study was conducted on 85 current smoker men and 85 non-smoker men. Their characteristics are reported in Table 1. The average age of participants in the group of smokers was 39.90±0.93 years, and in the group of non-smokers were 38.86±1.08 years. Mean age, height, weight, and BMI in both groups was not significantly different. Education levels between two groups were statistically significant (P<0.008). Non-smoking men had higher levels of education compared with smoking men (Table 1). The mean number of cigarettes smoked in the smoking group was 13.33±0.86 cigarettes per day, and the mean duration of smoking was 13.73±0.79 years.

Table 1. Characteristics of study subjects by smoking status

Parameters		Smokers (n=85)	Non-smokers (n=85)	*P*
Age (years)		39.90±0.93	38.86±1.08	0.17
Height (cm)		175.51±0.69	175.17±1.36	0.82
Body weight (kg)		81.20±1.33	82.54±1.73	0.54
Body mass index (kg/m^2)		26.32±0.40	26.35±0.51	0.96
educational levels	**0-5 year**	32.5%	22.6%	0.008
	5-8 year	30%	22.6%	--
	8-12 year	38.8%	21.4%	--
	>12year	8.8%	21.4%	--

Data are expressed as means ±standard error.
**P* from independent t test
P from chi-square test.

The energy and nutrient intakes of two groups from FFQ were compared (Table 2). The smoking group had lower consumption of iron, vitamin A, vitamin B3, folate, calcium, and copper, but just consumption of vitamin B1 (P<0.04) and copper (P<0.02) were significantly lower than the non-smoking group.

Table 2. Daily intake of nutrients by smoking status

Parameters	Smokers (n=85)	Non-smokers (n=85)	*P*
Total energy(kcal/d)	2316.66±59.11	2234.22±54.39	0.40
Protein(g/d)	84.41±2.16	80.72±2.08	0.22
VitaminB1(mg/d)	2.33±0.05	2.49±0.06	0.04
VitaminB3(mg/d)	25.91±0.66	27.46±0.71	0.11
VitaminA(RE/d)	1064.11±56.80	1072.12±60.38	0.92
VitaminC(mg/d)	162.32±9.63	174.61±9.56	0.19
VitaminE(mg/d)	3.92±0.14	3.19±0.13	0.23
Folate(µg/d)	205.16±7.86	221.66±8.04	0.15
VitaminB6 (mg/d)	1.34±0.04	1.24±0.04	0.10
Zinc(mg/d)	6.07±0.24	6.64±0.20	0.32
Copper(mg/d)	0.95±0.03	0.99±0.03	0.02
Calcium(mg/d)	1009.56±39.76	1042.42±38.54	0.55
Iron(mg/d)	14.84±0.40	15.81±0.44	0.11
Vitamin B12(mg/d)	3.89±0.17	3.51±0.19	0.15

Data are expressed as means±standard error.* *P* from independent t test

Hematological parameters of smokers and non-smokers are reported in Table 3. Hemoglobin and hematocrit (packed red cell volume) in smokers were slightly and significantly lower than nonsmokers (P<0.03 and P<0.001, respectively). MCV and MCH of smokers were not statistically significantly different from those of non-smokers.

Table 3. Hematological parameters in smokers and non-smokers.

Parameters	Smokers (n=85)	Non-smokers (n=85)	*P**
Heamoglob(g/dl)	14.84±0.32	15.91±0.10	0.03
Heamatocrit (%)	44.73±0.82	49.30±0.35	<0.001
MCH (pg)	28.37±0.27	28.51±0.18	0.68
MCV (FL)	95.37±10.24	87.59±0.44	0.42

Data are expressed as means ±standard error.**P* from independent t test
MCH=mean corpuscular hemoglobin, MCV=mean corpuscular volume

Mean serum total, active and non-active forms of vitamin B12 are reported in Table 4. Mean serum total vitamin B12 was significantly different between two groups (P<0.03). Mean serum inactive forms of vitamin B12 were not significantly different between two groups. But mean serum active form of vitamin B12 was significantly lower in smokers than non-smokers (P<0.001). Also, the ratio of the active form of vitamin B12 to total form of vitamin B12 was significantly lower in smokers than non-smokers (P=0.04).

Table 4. Serum vitamin B12 in smokers and non-smokers.

Parameters	Smokers (n=85)	Non-smokers (n=85)	*P**
Total vitamin B12(pmol/l)	14.84±0.32	15.91±0.10	0.03
Active form of vitamin B12 (pmol/l)	44.73±0.82	49.30±0.35	<0.001
Non-active form of vitamin B12 (pmol/l)	28.37±0.27	28.51±0.18	0.68
Active form of vitamin B12/ Total vitamin B12	95.37±10.24	87.59±0.44	0.04

Data are expressed as means±standard error.**P* from independent t test

Discussion

A hypothesis was that a total amount of vitamin B12 in smokers might not be different in comparison with non-smokers, but due to the reduction of the active form of vitamin B12 in smokers, the biological function of this vitamin is disturbed in the body. Although there is a wide variety of a study about the adverse effects of cigarette smoking on different diseases, the effect of smoking on nutrient concentrations is less well studied. Effect of smoking on serum vitamin B12 has been known for decades, but many of these studies have not considered other factors, including dietary intake, which might explain the differences in vitamin B12 status between smokers and nonsmokers.

This study was undertaken with regard to specific criteria, consisting of individuals without liver disease, kidney disease, and thyroid problem. Also, some drugs such as metformin, methotrexate, etc. that affect studied factors were not considered, and participants did not use any vitamin and mineral supplements for at least three months ago.

Comparison of the two groups in terms of the education level indicated that smokers had lower education level than nonsmokers. However, because of the low frequency of people in the each education groups, more research is needed in this area to confirm the result.

The serum active form of vitamin B12 was significantly lower in smokers than in non-smokers. Serum total vitamin B12 was not different between two groups. But the ratio of the active form of vitamin B12 to total vitamin B12 was significantly lower in smokers than non-smokers. The result about a total amount of vitamin B12 is in accordance with the result of studies by Iqbal *et al*., (12), Kim *et al*., (13), Gariballa *et al*., (14), Vardavas *et al*., (15), and Ozerol *et al*., (16). In these studies, a total vitamin B12 was not different between smokers and non-smokers.

In studies by Pagan *et al*., (8) and Mouhamed *et al*., (17), it was reported that serum vitamin B12 concentration was significantly lower in smokers than in non-smokers. However, a study by Tungtrongchitr *et al*. reported that serum vitamin B12 concentration was significantly higher in smokers than non-smokers. It may be because cigarette smokers have poorer diets than nonsmokers (18).

Tungtrongchitr *et al*., concluded that smokers were more likely to choose white bread, sugar, meat, butter, whole milk, and egg, and less likely to consume whole-

wheat bread, high fiber breakfast cereal, fruits, and vegetable than nonsmokers. The usual source of vitamin B12 is meat and meat products (18). What is clear from the studies is that changes in the serum level of vitamin B12 are important in smokers. Some ingredients in cigarettes may affect serum vitamin B12 through different mechanisms. Another study by Lindstrand *et al*., reported that plasma vitamin B12 was not different between smokers and non-smokers in the presence (a total form of vitamin B12) and absence (the inactive form of vitamin B12) of cyanide (19). DM. Matthews identified that serum vitamin B12 in the absence of cyanide (the inactive form of vitamin B12) in smokers was more than non-smokers. In these studies, they indicated that the high level of cyanide in the serum of smokers impairs normal ratio of cyanocobalamin and hydroxocobalamin (20).

The study has some limitations. Smoking status in this study was assessed by the questionnaire only. Biochemical markers such as serum cotinine were not available to determine the accuracy of the reported levels (21). Since there were no female cigarette smokers in this study, the results only could be applied to males. It is revealed the fact that smoking by females is less frequent in the country. Therefore, the possibility that some of the female smokers preferred not to accept this fact cannot be ruled out.

Few studies have been conducted on the effect of smoking on different forms of vitamin B12. In the present study, it was assumed that serum total vitamin B12 in smokers is not a valid criterion for determination of the biological function of this vitamin in the body. As a result, for determination of the function of this vitamin in the body, other forms of vitamin B12 along with a total form of vitamin B12 should be measured.

Acknowledgments

This article is a part of MSc thesis supported by Tehran University of Medical Sciences. We would like to thank the Deputy of Research and Technology of Tehran University of Medical Sciences for financial support of this study. We are particularly grateful to all participants in the study for their dedication and contribution to the research.

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