

## **B<sub>12</sub> and Folate Concentrations in Opium Addicts Compared to Healthy Subjects: A Case Control Study from Kerman Coronary Artery Disease Risk Study**

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### Short Communication

#### Abstract

**Background:** Opium addiction is a global problem which has implicated many societies. Opium addiction and drug abuse is related to harmful consequences which affect life style, biochemical factors, and vitamins values, and also is considered as a risk for heart diseases. Folate and B<sub>12</sub> levels are related to homocysteine and studies about their levels in opium addicts are controversial; therefore, we designed this study to evaluate B<sub>12</sub> and folate values in opium addicts.

**Methods:** From the Kerman Coronary Artery Disease Risk Study (KERCADRS) which is a population-based study, we randomly selected 340 men and entered them into two groups: case (n = 170) and control group (n = 170). Then vitamin B<sub>12</sub> and folate levels were measured.

**Findings:** Opium addiction did not change B<sub>12</sub> and folate levels significantly in opium addicts compared to non-addict control subjects. However, only some variables including blood pressure (BP) and diabetes positively and cigarette smoking, triglyceride (TG), alcohol, and cardiovascular disease (CVD) history negatively affected folate, and none of clinical and demographic variables influenced the B<sub>12</sub> levels (P > 0.050). TG had significant effects on B<sub>12</sub> and folate levels although opium addiction did not show any impact.

**Conclusion:** High TG levels were accompanied by low levels of B<sub>12</sub> and folate. Reduced B<sub>12</sub> and folate values are accompanied by serum homocysteine elevation. As TG elevates in opium addicts, it can be considered as an important factor which affects vitamins levels and reduces their absorption. Opium addiction elevates homocysteine level, since we can conclude that homocysteine elevation in opium addicts is independent of B<sub>12</sub> and folate levels.

**Keywords:** Opium addiction; Vitamin B<sub>12</sub>; Folate; Cardiovascular disease

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## Introduction

Opium addiction is a major socioeconomic problem which has implicated many societies globally and is more common in Middle Eastern countries, especially Afghanistan, Iran, and Pakistan.<sup>1</sup> According to previous studies, the opium addiction rate in Iran is about 3-9 percent of adult population and it is also higher in rural regions.<sup>2</sup> Drug abuse can cause damage to digestive tract and also it has been reported that drug addict subjects showed problems which involved gastrointestinal (GI) system such as constipation, diarrhea, and reduced appetite. Opium addiction can also result in changed dietary pattern and habits, anorexia, poor food intake, and weight loss; therefore, opiates users are always at risk of malnutrition.<sup>3-7</sup>

Opium addiction can affect biochemical and hematological factors, lipid profile, and vitamins and hormones values which are very important for general health.<sup>8-10</sup> Diaz-Flores Estevez et al. showed that vitamin A, vitamin E, and folate levels were higher in healthy control subjects compared to opium addicts which had higher B<sub>12</sub> levels than healthy control group.<sup>11</sup> Also, we previously showed that opium addiction increased homocysteine levels in opium-addicted individuals and thus increased the risk of cardiovascular diseases (CVDs) in this population.<sup>8</sup> Also, there are other studies which demonstrated that opium addiction was a risk factor for CVD.<sup>9,12,13</sup> Masoomi et al. reported that drug abuse could be considered as an independent risk factor for coronary artery disease (CAD).<sup>12</sup> Najafi and Sheikhatan described that opium addicts had lower level of B<sub>12</sub> and folate compared to non-addict controls.<sup>9</sup>

Both vitamins B<sub>12</sub> and folate have pivotal role in homocysteine metabolism. Homocysteine undergoes trans-sulfuration and re-methylation to produce amino acids Cysteine and Methionine, respectively. The enzyme responsible for 5,10-methylenetetrahydrofolate (5, 10-methylene-THF) conversion to 5-methyl-THF which needs B<sub>12</sub> as cofactor, is methylene-THF reductase (MTHFR). Thus, homocysteine level is correlated to B<sub>12</sub> and folate levels.<sup>14</sup> It has been described previously that reduced serum folate level is strongly associated with elevated homocysteine level and also related to increased risk of CVD. It has been

suggested that low plasma levels of B group vitamins are associated with high homocysteine levels which directly relate to CVD.<sup>14-18</sup>

As we mentioned above, the data about serum B<sub>12</sub> and folate levels in opium addicts are controversial and inconsistent and therefore need more evaluations.<sup>5,9,10,19</sup> After homocysteine assessment in opium addicts, we decided to evaluate vitamin B<sub>12</sub> and folate levels in this population in order to evaluate that whether homocysteine elevation is because of either B<sub>12</sub> or folate changes or not.

## Methods

The subjects that enrolled in the current study were selected from people who participated in Kerman Coronary Artery Disease Risk Study (KERCADRS) which was conducted in Kerman Province, Iran (5900 of 15-75-year-old residents were recruited in this study). Sample size for this study was 340 men that were equally divided into two groups, opium addicts and healthy subjects as control group. Opium addiction prevalence in male subjects is higher than female subjects, thus only male participants were included in our study. Exclusion criteria for this study were subjects with acute infectious or inflammatory disorders, liver infectious and viral diseases, cirrhosis, malignant disease, and severe renal disease.<sup>20</sup>

The definition of opium addiction was according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (American Psychiatric Association, 2000).<sup>21</sup> Serum levels of vitamins B<sub>12</sub> and folate were measured by specific kits that were based on chemiluminescence. Demographic and clinical variables including age, body mass index (BMI), cigarette smoking, family history of CAD, waist circumference, systolic blood pressure (SBP) and diastolic blood pressure (DBP), fasting blood sugar (FBS), cholesterol, triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and alcohol drinking were obtained from KERCADRS and used in this study.

BMI [calculated as weight (kg) divided by the square of height (m<sup>2</sup>)] was categorized into 3 groups: BMI < 25 kg/m<sup>2</sup> considered as normal, BMI between 25-30 kg/m<sup>2</sup> considered as overweight, and BMI > 30 kg/m<sup>2</sup> considered as obese. There were two categories for cigarette smoking which were smoker and non-smoker.

Other variables were family history of CAD (first degree relatives before the age of 55), waist circumference (more than 40 inches), hypertension (BP > 140/90 mmHg), type 2 diabetes mellitus (DM) (FBS ≥ 126 mg/dl), serum cholesterol > 240 mg/dl, HDL < 35 mg/dl, LDL > 130 mg/dl, and serum TG > 200 mg/dl. LDL was calculated based on Friedewald formula: LDL = total cholesterol-(HDL + TG/5).<sup>8,20</sup> This study was approved by the Ethic Committee of Kerman University of Medical Sciences (permission No. 90.09 P). Informed consent was obtained from individuals that participated in the study.

Absolute and relative frequencies were calculated and presented for qualitative variables and mean accompanied with standard deviation (SD) for quantitative/continuous variables. To compare the distribution of the variables of interest between addicts (cases) and non-addicts (controls), we applied the chi-square test (and Fisher's exact test if necessary) for categorical variables and the student's t-test (and the Mann-Whitney U test when the variables were not normally distributed) for continuous ones.

To determine the association between addiction with serum folate and B<sub>12</sub> levels, the univariate linear regression was used. For adjusting potential confounding variables which had significant level less than 0.200 in univariate analysis, we entered them in multivariate linear regression model. The two dependent variables, serum folate and B<sub>12</sub> levels, were not normally distributed; therefore, they logarithmically transformed. Thus we used log-transformed variables in the linear regression models. Statistical analyses were conducted by SPSS software (version 16, SPSS Inc., Chicago, IL, USA). P-value less than 0.050 was considered significant.

### Results

Baseline demographic and clinical characteristics of both groups are presented in table 1. As it is shown, two groups did not have significant difference in terms of mean age and BMI (P > 0.050). Although mean SBP and DBP among control group was slightly greater than addicts, it was statistically significant (P < 0.050).

**Table 1.** Demographic and baseline clinical parameters in two groups of addicts and non-addicts

Variable	Groups		P
	Control (non-addict) (n = 170)	Case (addict) (n = 170)	
Age (year) (mean ± SD)	42.4 ± 4.9	43.2 ± 4.5	0.091
BMI (kg/m <sup>2</sup> ) (mean ± SD)	25.3 ± 4.1	24.9 ± 4.2	0.410
BMI categories			0.840
Normal[n (%)]	78 (45.3)	79 (46.5)	
Overweight[n (%)]	74 (44.0)	76 (44.7)	
Obese [n (%)]	18 (10.7)	15 (8.8)	
SBP (mmHg) (mean ± SD)	116.5 ± 15.5	112.8 ± 14.2	0.023
DBP (mmHg) (mean ± SD)	78.9 ± 9.7	76.7 ± 9.8	0.035
Smoking (current) [n (%)]	68 (40.0)	77 (45.3)	0.320
Frequency of BP (mmHg) [n (%)]	32 (18.5)	23 (13.5)	0.185
DM (FBS ≥ 126 mg/dl) [n (%)]	24 (14.2)	30 (17.6)	0.370
Cholesterol (> 240 mg/dl) [n (%)]	22 (13.1)	22 (13.1)	0.990
TG (> 200 mg/dl) [n (%)]	48 (28.6)	45 (26.8)	0.710
HDL (< 40 mg/dl) [n (%)]	95 (56.5)	96 (56.8)	0.990
LDL (> 130 mg/dl) [n (%)]	77 (47.2)	72 (46.2)	0.840
Alcohol [n (%)]	4 (2.7)	3 (2.1)	0.990
CVD history [n (%)]	14 (8.4)	12 (7.2)	0.680
Waist circumference (inches) (mean ± SD)	89.1 ± 10.5	88.2 ± 11.2	0.040
Folate (µg/l) (mean ± SD)	9.9 ± 2.8	10.1 ± 2.9	0.450
Folate (log-transformed) (mean ± SD)	0.98 ± 0.12	0.99 ± 0.12	0.510
B <sub>12</sub> (ng/l) (mean ± SD)	327.0 ± 121.1	328.7 ± 115.5	0.890
B <sub>12</sub> (log-transformed) (mean ± SD)	2.48 ± 0.15	2.49 ± 0.14	0.810

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; DM: Diabetes mellitus; FBS: Fast blood sugar; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; CVD: Cardiovascular disease; SD: Standard deviation

**Table 2.** Univariate linear regression analysis of the impact of opium and other variables on folate and B<sub>12</sub>

Variable	Folate		P	B <sub>12</sub>		P
	β Standard	SE		β Standard	SE	
Opium	0.035	0.013	0.510	0.013	0.016	0.810
Age (year)	0.094	0.001	0.083	0.001	0.002	0.980
BMI (kg/m <sup>2</sup> )	-0.007	0.002	0.890	-0.061	0.002	0.260
BP (mmHg)	0.160	0.081	0.003	-0.038	0.022	0.480
DM	0.130	0.027	0.018	0.050	0.022	0.360
Smoking	-0.012	0.014	0.022	-0.054	0.016	0.320
Cholesterol (mg/dl)	-0.044	0.020	0.410	0.077	0.024	0.150
TG (mg/dl)	-0.126	0.015	0.021	-0.092	0.018	0.093
HDL (< 40 mg/dl)	0.027	0.014	0.610	0.022	0.016	0.690
LDL (> 130 mg/dl)	-0.019	0.014	0.730	0.070	0.016	0.210
Alcohol	-0.128	0.048	0.029	-0.063	0.057	0.280
CVD History	-0.139	0.025	0.011	-0.011	0.030	0.840
Waist circumference (inches)	-0.072	0.001	0.180	-0.017	0.001	0.750

BP: Blood pressure; DM: Diabetes mellitus; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; CVD: Cardiovascular disease; SE: Standard error

When the prevalence of BP was compared between the groups, 18.5% of non-addicts versus 13.5% of addicts had hypertension ( $P = 0.180$ ). Waist circumference among non-addicts was a little but significantly higher than addicts ( $P < 0.050$ ). Other variables were not statistically varied in two groups of the study. The comparison of both forms of non-transformed and transformed levels of folate and B<sub>12</sub> between two groups was not statistically significant.

The univariate linear regression analysis revealed that opium did not have a significant effect on folate ( $\beta = 0.030$ ,  $P = 0.510$ ). However, only some other variables including BP and DM positively and cigarette smoking, TG, alcohol, and CVD history negatively had a significant effect on the levels of folate ( $P < 0.050$ ). Also, opium addiction did not affect the levels of B<sub>12</sub> ( $\beta = 0.013$ ,  $P = 0.810$ ). Clinical and demographic variables did not influence vitamin B<sub>12</sub> levels ( $P > 0.050$ ) (Table 2).

When the variables which had the significant level less than 0.150 entered in the multivariate analysis in order to control the confounding factors, the results showed that opium did not have still a significant effect on the levels of either folate or B<sub>12</sub> ( $P = 0.900$  and  $P = 0.750$ , respectively). Among the other clinical variables, only increased BP ( $\beta = 0.130$ ,  $P = 0.030$ ), TG ( $\beta = -0.130$ ,  $P = 0.028$ ), and the history of CVD ( $\beta = -0.140$ ,  $P = 0.016$ ) were found as independent variables influencing the level of folate variable. Likewise, only the TG had an inversely significant effect on the level of B<sub>12</sub> ( $\beta = -0.124$ ,  $P = 0.038$ ) and other variables did not affect the B<sub>12</sub> variable.

## Discussion

We evaluated the serum B<sub>12</sub> and folate levels in opium addicts and non-addict healthy control subjects. Opium addiction is considered as a biological disease followed by critical consequences. Some nutritional deficiencies were reported in opium addicts which include minerals, vitamins, and protein deficiencies that affect their health.<sup>3</sup> Some people use opium as recreation and their dietary habit is not affected by opium usage. This people may have normal levels of nutrients and vitamins in their serum,<sup>3</sup> but individuals that were strongly dependent on opium and probably concomitantly were addicted to alcohol showed deficiency of vitamins and other nutrients.<sup>3</sup> Our data showed that there were no significant differences between opium addicts and non-addicts considering serum B<sub>12</sub> and folate levels. B<sub>12</sub> and folate are associated with homocysteine metabolism. There is a direct inverse correlation between group B vitamins such as B<sub>12</sub> and folate with homocysteine concentration.<sup>21</sup> Homocysteine is an independent and strong risk factor for CVD.<sup>21,22</sup> It has been showed that opium addiction increases homocysteine levels and is associated with high CVD risk.<sup>8,19,23,24</sup>

Masoomi et al. showed that opium addiction could cause significant increase of homocysteine level compared to non-addict healthy group. They assumed that probably group B vitamins deficiency could be involved in homocysteine elevation in opium-addicted subjects.<sup>8</sup> Data about

B<sub>12</sub> and folate levels in opium addicts and drug abusers are inconsistent.<sup>9-11,24</sup> Current study was the following of previous study by Masoomi et al.<sup>8</sup> (subjects of that study are included in our study) which assessed homocysteine in addict and non-addict individuals. Considering that homocysteine level significantly increased in opium addicts, we expected that probably the B<sub>12</sub> and folate serum levels would be decreased in opium addicts but unlike our expectation, the B<sub>12</sub> and folate levels were not significantly different between addict and non-addict individuals. There are other studies which evaluated the B<sub>12</sub> and folate in opium addicts and non-addicts and data in some cases are the same as our data and in some cases are totally different.<sup>9-11</sup> Diaz-Flores Estevez et al. showed that B<sub>12</sub> level was higher in opium-addicted subjects compared to healthy age-matched control group (582 ± 196 vs. 523 ± 295); on the other hand, they found that folate level was higher in healthy control group compared with addicted individuals (8.13 ± 2.37 vs. 3.23 ± 1.76).<sup>11</sup> This discrepancy was not fully understood yet, but it was connected to changed dietary habits, reduced usage of vegetables and other foods, and drinking alcohol.<sup>3,10</sup> Absence of inverse relationship between B<sub>12</sub> and folate against homocysteine levels in opium-addicted subjects shows that homocysteine elevation in opium addicts is independent of B<sub>12</sub> and folate levels in these subjects. It has been described that opiate addicts had decreased levels of folate compared to the control group, which is inconsistent with our results, and this may explain high homocysteine levels in opium addicts.<sup>11</sup>

Drug addiction can change GI system function and also cause anorexia, constipation, malnutrition, and malabsorption.<sup>4-6</sup> It is expected that opium-addicted subjects must have lack of some nutrients, this has been proved by previous studies, and some studies provided evidence that opium-addicted subjects had some vitamin and mineral deficiencies and altered biochemical factors.<sup>4,8,9,25</sup> It has been reported that vitamin A and E levels were significantly reduced in opiate users, also their hepatic transaminases activity increased compared with healthy control group.<sup>10</sup> Some opium-addicted subjects consume opium as recreation and this group of addicts commonly have a sufficient food intake; thus B<sub>12</sub> and folate

deficiency in these individuals is scarce.<sup>2,3</sup>

We showed that alcohol drinking, CVD history, smoking, and TG levels resulted in reduced folate level. On the other hand, only the TG levels had an inverse significant effect on the level of B<sub>12</sub> ( $\beta = -0.124$ ,  $P = 0.038$ ) and other variables did not affect the B<sub>12</sub> level. Whatsoever, high TG levels were accompanied by low levels of B<sub>12</sub> and folate. Also, it has been reported that TG levels were elevated in opium addicts who started drug abuse again after detoxification;<sup>10</sup> on the other hand, opium addiction can affect B<sub>12</sub> and folate levels by reducing eating pattern in these persons.<sup>8,9</sup> It also has been reported that opium addicts tend to drink alcohol which can affect vitamin absorption, and their daily consumption of macronutrients is less than healthy non-addict individuals.<sup>3,4,7,9</sup> Opium has many compounds, about 70-80 substances, more of which are alkaloids and there is no evidence about their effect on homeostasis, biological function, hormonal variations, and enzyme activity which need to be defined.<sup>1,10,25</sup>

## Conclusion

Serum levels of B<sub>12</sub> and folate are inversely correlated with homocysteine values. In our previous study, we found that there was a direct correlation between opium addiction and elevated serum homocysteine concentrations. In this study, we showed that B<sub>12</sub> and folate levels were not contributed to opium addiction; therefore, we conclude that homocysteine elevation in opium addicts is independent of B<sub>12</sub> and folate levels. Other factors related to opium addiction such as opium components and opium effect on dietary intake and food tendency can be involved in opium side effects in addicted persons and need more evaluation. Moreover, we found that hypertriglyceridemia was significantly contributed to reduced B<sub>12</sub> and folate levels. This phenomenon needs more assessments and TG value and its relationship with B<sub>12</sub> and folate levels should be monitored in addicted subjects.

## Conflict of Interests

The Authors have no conflict of interest.

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## غلظت‌های ویتامین B12 و فولات در افراد معتاد به تریاک در مقایسه با افراد سالم: مطالعه مورد- شاهدی از بررسی عوامل خطر بیماری‌های عروق کرونر کرمان

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### مقاله کوتاه

### چکیده

**مقدمه:** اعتیاد به تریاک مشکلی جهانی می‌باشد که تاکنون جوامع زیادی را درگیر نموده است. اعتیاد به تریاک و سوء استفاده‌های دارویی مرتبط با آن، پیامدهای مضرى هستند که نحوه زندگی فرد، شاخص‌های بیوشیمیایی، ویتامین‌ها و مقادیر هورمون‌ها را تحت تأثیر قرار می‌دهد و همچنین، عامل خطری برای بیماری‌های مرتبط با عملکرد قلب به شمار می‌رود. فولات و ویتامین B12 نقش مهمی در متابولیسم هموسیستئین دارند، اما اطلاعات در مورد مقادیر آن‌ها در افراد معتاد بحث‌برانگیز است. پژوهش حاضر با هدف بررسی سطوح فولات و ویتامین B12 در افراد معتاد به تریاک انجام شد.

**روش‌ها:** ۳۴۰ نمونه سرم مرد به صورت تصادفی از نمونه‌های سرمی طرح هم‌گروهی قلب سالم بررسی عوامل خطر بیماری‌های قلبی در کرمان انتخاب شدند و در دو گروه شاهد (۱۷۰ نفر) و معتاد (۱۷۰ نفر) قرار گرفتند. سپس مقادیر فولات و ویتامین B12 در نمونه‌ها اندازه‌گیری گردید.

**یافته‌ها:** اعتیاد به تریاک، منجر به تغییر معنی‌دار مقادیر سرمی فولات و ویتامین B12 در گروه معتاد در مقایسه با گروه سالم نشد. با این وجود، بعضی شاخص‌ها همچون پرفشاری خون و دیابت به صورت مثبت و سیگار کشیدن، تری‌گلیسرید بالا، مصرف الکل و تاریخچه بیماری‌های قلبی-عروقی به طور منفی مقادیر فولات را تحت تأثیر قرار داد. از طرف دیگر، مقادیر ویتامین B12 تحت تأثیر هیچ کدام از شاخص‌های دموگرافیک قرار نداشت. میزان تری‌گلیسرید اثر معنی‌داری بر روی مقادیر فولات و ویتامین B12 نشان داد؛ در حالی که مصرف تریاک هیچ‌گونه اثری را نشان نداد.

**نتیجه‌گیری:** مقادیر بالای تری‌گلیسرید با کاهش ویتامین B12 و فولات و کاهش ویتامین B12 و فولات نیز با افزایش سرمی هموسیستئین همراه می‌باشد. هنگامی که تری‌گلیسرید در افراد معتاد افزایش می‌یابد، می‌تواند به عنوان عامل تعیین‌کننده‌ای که مقادیر ویتامین‌ها را تحت تأثیر قرار می‌دهد و منجر به کاهش جذب آن‌ها می‌شود، در نظر گرفته شود. اعتیاد به تریاک، مقدار هموسیستئین را افزایش می‌دهد و از این‌رو، می‌توان نتیجه گرفت که افزایش هموسیستئین در افراد معتاد به تریاک مستقل از فولات و ویتامین B12 است.

**واژگان کلیدی:** اعتیاد به تریاک، ویتامین B12، فولات، بیماری‌های قلبی-عروقی

**ارجاع:** غلامحسینیان احمد، شاه‌وزهی بیداله، شکوهی مصطفی، نجفی‌پور حمید. غلظت‌های ویتامین B12 و فولات در افراد معتاد به تریاک در مقایسه با افراد سالم: مطالعه مورد- شاهدی از بررسی عوامل خطر بیماری‌های عروق کرونر کرمان. مجله اعتیاد و سلامت ۱۳۹۷؛ ۱۰ (۳): ۱۹۸-۲۰۴.

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