B12 and Folate Concentrations in Opium Addicts Compared to Healthy Subjects: A Case Control Study from Kerman Coronary Artery Disease Risk Study

Ahmad Gholamhossenian PhD1, Beydolah Shahouzehi PhD2, Mostafa Shokoohi PhD3, Hamid Najafipour PhD4

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 lifestyle, biochemical factors, and vitamins values, and also is considered as a risk for heart diseases. Folate and B12 levels are related to homocysteine and studies about their levels in opium addicts are controversial; therefore, we designed this study to evaluate B12 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 B12 and folate levels were measured.

Findings: Opium addiction did not change B12 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 B12 levels (P > 0.050). TG had significant effects on B12 and folate levels although opium addiction did not show any impact.

Conclusion: High TG levels were accompanied by low levels of B12 and folate. Reduced B12 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 B12 and folate levels.

Keywords: Opium addiction; Vitamin B12; Folate; Cardiovascular disease

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Opium and Serum B₁₂ and Folate Concentration

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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.¹ 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.² 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, opiate users are always at risk of malnutrition.³⁻⁷

Opium addiction can affect biochemical and hematological factors, lipid profile, and vitamins and hormones values which are very important for general health.⁸⁻¹⁰ 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 B₁₂ levels than healthy control group.¹¹ 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.⁸ Also, there are other studies which demonstrated that opium addiction was a risk factor for CVD.¹²⁻¹⁵ Masoomi et al. reported that drug abuse could be considered as an independent risk factor for coronary artery disease (CAD).¹² Najafi and Sheikhvatan described that opium addicts had lower level of B₁₂ and folate compared to non-addict controls.⁹

Both vitamins B₁₂ 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 B₁₂ as cofactor, is methylene-THF reductase (MTHFR). Thus, homocysteine level is correlated to B₁₂ and folate levels.¹⁴ 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.¹⁴⁻¹⁸

As we mentioned above, the data about serum B₁₂ and folate levels in opium addicts are controversial and inconsistent and therefore need more evaluations.⁵⁻⁹,¹⁰,¹⁹ After homocysteine assessment in opium addicts, we decided to evaluate vitamin B₁₂ and folate levels in this population in order to evaluate that whether homocysteine elevation is because of either B₁₂ 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.²⁰

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).²¹ Serum levels of vitamins B₁₂ 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 (m²)] was categorized into 3 groups: BMI < 25 kg/m² considered as normal, BMI between 25-30 kg/m² considered as overweight, and BMI > 30 kg/m² considered as obese. There were two categories for cigarette smoking which were smoker and non-smoker.

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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: 
\[ \text{LDL} = \text{total cholesterol} - (\text{HDL} + \frac{\text{TG}}{5}). \]
This study was approved by the Ethic Committee of Kerman University of Medical Sciences (permission No. 90.09). 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\(_{12}\) 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\(_{12}\) 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>
<thead>
<tr>
<th>Table 1. Demographic and baseline clinical parameters in two groups of addicts and non-addicts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable</strong></td>
</tr>
<tr>
<td>Age (year) (mean ± SD)</td>
</tr>
<tr>
<td>BMI (kg/m(^2)) (mean ± SD)</td>
</tr>
<tr>
<td>BMI categories</td>
</tr>
<tr>
<td>Normal[n (%)]</td>
</tr>
<tr>
<td>Overweight[n (%)]</td>
</tr>
<tr>
<td>Obese [n (%)]</td>
</tr>
<tr>
<td>SBP (mmHg) (mean ± SD)</td>
</tr>
<tr>
<td>DBP (mmHg) (mean ± SD)</td>
</tr>
<tr>
<td>Smoking (current) [n (%)]</td>
</tr>
<tr>
<td>Frequency of BP (mmHg) [n (%)]</td>
</tr>
<tr>
<td>DM (FBS ≥ 126 mg/dl) [n (%)]</td>
</tr>
<tr>
<td>Cholesterol (&gt; 240 mg/dl) [n (%)]</td>
</tr>
<tr>
<td>TG (&gt; 200 mg/dl) [n (%)]</td>
</tr>
<tr>
<td>HDL (&lt; 40 mg/dl) [n (%)]</td>
</tr>
<tr>
<td>LDL (&gt; 130 mg/dl) [n (%)]</td>
</tr>
<tr>
<td>Alcohol [n (%)]</td>
</tr>
<tr>
<td>CVD history [n (%)]</td>
</tr>
<tr>
<td>Waist circumference (inches) (mean ± SD)</td>
</tr>
<tr>
<td>Folate (µg/l) (mean ± SD)</td>
</tr>
<tr>
<td>Folate (log-transformed) (mean ± SD)</td>
</tr>
<tr>
<td>B(_{12}) (ng/l) (mean ± SD)</td>
</tr>
<tr>
<td>B(_{12}) (log-transformed) (mean ± SD)</td>
</tr>
</tbody>
</table>

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
addict healthy control subjects. This may be a consequence of the fact that some addicts who used opium daily continued to use this drug and their dietary habit was not affected. In addition, clinical and demographic variables did not influence vitamin B12 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 B12 (P = 0.900 and P = 0.750, respectively). Among the other clinical variables, only increased BP (β = 0.130, P = 0.030), TG (β = -0.130, P = 0.028), and the history of CVD (β = -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 B12 (β = -0.124, P = 0.038) and other variables did not affect the B12 variable.

### Table 2. Univariate linear regression analysis of the impact of opium and other variables on folate and B12

<table>
<thead>
<tr>
<th>Variable</th>
<th>Folate β Standard</th>
<th>SE</th>
<th>P</th>
<th>B12 β Standard</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opium</td>
<td>0.035</td>
<td>0.013</td>
<td>0.510</td>
<td>0.013</td>
<td>0.016</td>
<td>0.810</td>
</tr>
<tr>
<td>Age (year)</td>
<td>0.094</td>
<td>0.001</td>
<td>0.083</td>
<td>0.001</td>
<td>0.002</td>
<td>0.980</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.007</td>
<td>0.002</td>
<td>0.890</td>
<td>-0.061</td>
<td>0.002</td>
<td>0.260</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>0.160</td>
<td>0.081</td>
<td>0.003</td>
<td>-0.038</td>
<td>0.022</td>
<td>0.480</td>
</tr>
<tr>
<td>DM</td>
<td>0.130</td>
<td>0.027</td>
<td>0.018</td>
<td>0.050</td>
<td>0.022</td>
<td>0.360</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.012</td>
<td>0.014</td>
<td>0.022</td>
<td>-0.054</td>
<td>0.016</td>
<td>0.320</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>-0.044</td>
<td>0.020</td>
<td>0.410</td>
<td>0.077</td>
<td>0.024</td>
<td>0.150</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>-0.126</td>
<td>0.015</td>
<td>0.021</td>
<td>-0.092</td>
<td>0.018</td>
<td>0.093</td>
</tr>
<tr>
<td>HDL (&lt; 40 mg/dl)</td>
<td>0.027</td>
<td>0.014</td>
<td>0.610</td>
<td>0.022</td>
<td>0.016</td>
<td>0.690</td>
</tr>
<tr>
<td>LDL (&gt; 130 mg/dl)</td>
<td>-0.019</td>
<td>0.014</td>
<td>0.730</td>
<td>0.070</td>
<td>0.016</td>
<td>0.210</td>
</tr>
<tr>
<td>Alcohol</td>
<td>-0.128</td>
<td>0.048</td>
<td>0.029</td>
<td>-0.063</td>
<td>0.057</td>
<td>0.280</td>
</tr>
<tr>
<td>CVD History</td>
<td>-0.139</td>
<td>0.025</td>
<td>0.011</td>
<td>-0.011</td>
<td>0.030</td>
<td>0.840</td>
</tr>
<tr>
<td>Waist circumference (inches)</td>
<td>-0.072</td>
<td>0.001</td>
<td>0.180</td>
<td>-0.017</td>
<td>0.001</td>
<td>0.750</td>
</tr>
</tbody>
</table>

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

### Discussion

We evaluated the serum B12 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. Some people use opium as recreation and their dietary habits are not affected by opium usage. This may be a consequence of the fact that some addicts who used opium daily continued to use this drug and their dietary habit was not affected. Among the other clinical variables, only increased BP (β = 0.130, P = 0.030), TG (β = -0.130, P = 0.028), and the history of CVD (β = -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 B12 (β = -0.124, P = 0.038) and other variables did not affect the B12 variable.

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). The univariate linear regression analysis revealed that opium did not have a significant effect on folate (β = 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 B12 (β = 0.013, P = 0.810). Clinical and demographic variables did not influence vitamin B12 levels (P > 0.050) (Table 2).

Data about

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B_{12} and folate levels in opium addicts and drug abusers are inconsistent.\textsuperscript{9,11,24} Current study was the following of previous study by Masoomi et al.\textsuperscript{8} (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_{12} and folate serum levels would be decreased in opium addicts but unlike our expectation, the B_{12} and folate levels were not significantly different between addict and non-addict individuals. There are other studies which evaluated the B_{12} 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.\textsuperscript{9,11} Diaz-Flores Estevez et al. showed that B_{12} 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).\textsuperscript{11} 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.\textsuperscript{3,10} Absence of inverse relationship between B_{12} and folate against homocysteine levels in opium-addicted subjects shows that homocysteine elevation in opium addicts is independent of B_{12} 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.\textsuperscript{11}

Drug addiction can change GI system function and also cause anorexia, constipation, malnutrition, and malabsorption.\textsuperscript{4,6} 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.\textsuperscript{5,8,9,25} 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.\textsuperscript{10} Some opium-addicted subjects consume opium as recreation and this group of addicts commonly have a sufficient food intake; thus B_{12} and folate deficiency in these individuals is scarce.\textsuperscript{2,3}

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_{12} ($\beta = -0.124$, $P = 0.038$) and other variables did not affect the B_{12} level. Whatever, high TG levels were accompanied by low levels of B_{12} and folate. Also, it has been reported that TG levels were elevated in opium addicts who started drug abuse again after detoxification;\textsuperscript{10} on the other hand, opium addiction can affect B_{12} and folate levels by reducing eating pattern in these persons.\textsuperscript{8,9} 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.\textsuperscript{3,4,7,9} 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.\textsuperscript{3,10,25}

**Conclusion**

Serum levels of B_{12} 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_{12} and folate levels were not contributed to opium addiction; therefore, we conclude that homocysteine elevation in opium addicts is independent of B_{12} 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_{12} and folate levels. This phenomenon needs more assessments and TG value and its relationship with B_{12} and folate levels should be monitored in addicted subjects.

**Conflict of Interests**

The Authors have no conflict of interest.

**Acknowledgements**

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Opium and Serum B₁₂ and Folate Concentration

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References


گلفتاهی ویتامین B12 و فولات در افراد معتاد به تریاک در مقایسه با افراد سالم:
แมทیعه مورد— شاهدی از بررسی عوامل خطر بیماری‌های عروق کرونر کرمان

دکتر احمد غلامحسینیان، دکتر بیداله شاهوژه، دکتر مصطفی شکوهی، دکتر حمید نجفی‌پور

چکیده

مقامه: اعتیاد به تریاک مشکلی جهانی می‌باشد که تاکنون جوابی زیادی را در رفع نیازهای این فراموش کرده است. اعتیاد به تریاک و سوء استفاده‌های دارویی مرتبط با آن، بیماری‌های متعددی مانند کبدی، شایع‌ترین بیماری بوشیمی، ویتامین‌ها و مقادیر هومونی را تحت تأثیر قرار می‌دهد و همچنین، عامل خطری برای بیماری‌های متعدد مانند مشکلات قلبی است. در این پژوهش، نتایج بهبودی بسیاری در مقایسه با مصرف الکل و قهوه در افراد معتاد به تریاک نشان داده شد.

روش‌ها: 240 نمونه مرد و زن از افراد حاضر در مرکز تحقیقات و نیروی انتظامی کرمان در این استادیو انتخاب شدند. در هر 30 نفر، در 80 نفر طرح دوگانه را در این طرح انجام دادند.

نتیجه‌گیری: مقدار بازیگر این عوامل در افراد معتاد به تریاک کمتر از افراد غیر معتاد بود.

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

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