The Effect of Cigarette Smoking on Serum Homocysteine, Folic Acid, and Vitamin B12 Concentrations in Patients with Cardiovascular Diseases

Running title: The Effect of Smoking on Cardiovascular Patients

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Abstract

Background: The purpose of this study was to investigate the impact of smoking on blood homocysteine, folic acid, and vitamin B12 levels in patients with cardiovascular diseases (CVD).
Methods: We collected 88 participants with cardiovascular complications who divided into two groups: Smokers (n=44) and non-smokers (n=44) groups. The serum levels of homocysteine, folic acid, and vitamin B12 were determined by the ELISA. Additionally, the systolic, diastolic blood pressure, and BMI were taken from patients.
Results: Our results illustrated that the serum level of folic acid significantly decreased in smoker patients (22.41±5.95) compared with the non-smokers group (28.05±4.13, p=0.000). In contrast, the serum level of homocysteine (p=0.958) and vitamin B12 (p=0.578) was not altered significantly between both groups. Our data also showed a negative correlation between folic acid and systolic blood pressure. No relationship was observed between folic acid and vitamin B12 and homocysteine in the smoking group.
Conclusion: Our study showed that the levels of folic acid significantly decreased in CVD patients with smokers compared with non-smokers. A significant negative relationship was observed between folic acid and systolic blood pressure in smoker group.

Keywords: cardiovascular disease, blood pressure, cigarette smoking, folic acid, vitamin B12, homocysteine.

Introduction

Cigarette smoking is considered a serious health concern that has adverse effects on human well-being. There is a strong link between smoking and the progression of heart-related diseases(1, 2). Smoking encompasses thousands of toxic compounds which may have detrimental effects on cardiovascular health. Interestingly, a significant number of preventable deaths in modern and prosperous areas of the world are attributed to smoking. While a robust epidemiological link exists between smoking and cardiovascular disease(CVD), the mechanisms for developing of CVD in smokers remain unclear(3). Vascular endothelial damage is considered a fundamental step in the case of atherosclerosis pathogenesis(3). Accumulation of reactive oxygen species(ROS) concomitant with oxidative events is a key sector in vascular endothelial dysfunction after exposure to smoking(4).

Homocysteine is a sulfur-containing metabolite, generated from methionine by the losing of a methyl group at the terminal end(5). Given the fact obtained from comprehensive research on European society, smoking is associated with elevated levels of plasma homocysteine. Moreover, higher homocysteine concentrations have been linked with an increased risk of CVD, possibly through mechanisms such as oxidative stress, inflammation, and endothelial dysfunction(1). On the other hand, smoking was correlated with an insufficient intake of vitamins. In this regard, a lack of folate and vitamin B12 is commonly observed in a significant number of hyperhomocysteinemia patients(6). Strikingly, the administration of B vitamins such as folic acid can reduce homocysteine levels and prevent the development of CVD(5). In fact, exposure to smoking can lead to diminished intake of folic acid and vitamin B12, which are necessary for the normal function of metabolic enzymes(7).

Given the harmful effect of smoking on a different aspect of human health, we aimed to evaluate the impact of cigarette smoking on serum homocysteine, folic acid, and vitamin B12 concentrations in patients with atherosclerosis. The findings of this study may provide valuable insights into the mechanisms underlying the association between cigarette smoking and CVD risk and may have important implications for developing of strategies to prevent and manage CVD in high-risk populations.

Methods

The current study was a case-control study conducted in November-April 2022-23 at the Metabolic Disorder Research Centre in Gorgan, Golestan Province, Iran. The study was approved by the Ethics Committee (No. IR.GOUMS.REC.1401.534) of the Golestan University of Medical Sciences.

Eighty-eight patients with cardiovascular complications were included in this cross-sectional study. All participants gave their informed consent. The non-smoker group (n= 44) was defined as patients reporting to be never smokers. The smoking group (n= 44) consumed 5-15 cigarettes daily. The number of cigarettes was an estimation of the patient at intake, confirmed by her partner. Exclusion criteria were chronic hypertension, familial dyslipidemia, chronic heart disease, and the use of folic acid or vitamin supplements. Blood samples were drawn between 8.30 and 9.30 a.m. after fasting overnight for 10 h and a resting period of 20 min and in the smoker's group at least 1 h after the last cigarette was smoked. The 5-mL blood samples were provided for all subjects after 12 h overnight fast. After the serum separation, it was used to determine biochemical parameters. Immediately after venesection, the plasma was separated, stored at -70º C in plastic tubes and thawed with tap water of 37º C for 5 min before serial analysis. Homocysteine (Zell Bio,

Germany), Vitamin B12, and folic acid (Monobind, USA) serum concentrations were determined with an enzyme-linked immunosorbent assay (ELIZA).
Weight was measured by digital weight balance; all heavy clothing has been lifted, and light clothing was restricted, with a loss of about 0.5 kg. Body mass index (BMI) was calculated by dividing the weight (kg) by square body height (m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) (mm Hg) were measured while taking into consideration resting approximately 5 min before measurement.
The student t-test was used to compare the differences between the mean values of the parameters in both groups at the P < 005 level. Normal distribution test was performed by Kolmogorov-Smirnov test using SPSS software, version 21. For the calculation of the coefficients of correlation, the Spearman rank test was used.

Results

A total of 44 cases and 44 age and gender matched control were analyzed. The mean age of smokers and non-smokers were 62.10 ± 9.97 and 65.46 ± 8.98 respectively (p= 0.096). As well, 31 patients (70.2%) and 24 controls (54.4%) were male (p=0.3). As shown in Table 1, the smoking group had a strong and significant reduction in serum folic acid relative to the non-smoking group (p-value <0.001). Smoking patients have significantly higher SBP and DBP than non-smokers. There was also a decrease in serum vitamin B12 levels in the smoker's group, although not significantly. In addition, a non-significant increase in body mass index and homocysteine levels were observed compared to the control group.
To examine the relationship between folic acid and study parameters, we also performed a correlation study. As Table 2 shows, there is a moderate, negative, and significant correlation (r=-0.293, p-value 0.048) between folic acid and systolic blood pressure in the smoking group. We also found no significant association between folic acid and study parameters in both groups.

Table 1. Demographic and biochemical characteristics of the study population

Parameter	Mean ± SD		P-value
	Smoker(n=44)	Non-smoker(n=44)	
Age (Years)	62.10 ± 9.97	65.46 ± 8.98	0.096
BMI (kg/m^2)	27.47 ± 2.70	27.28 ± 4.80	0.583
DBP (mmHg)	80.13 ± 10.74	70.77 ± 10.74	0.025*
SBP (mmHg)	130.54 ± 11.42	120.72 ± 11.40	0.009*
Homocysteine (nm/ml)	32.33±10.9	32.22±8.61	0.958
Folic acid(ng/ml)	22.41±5.95	28.05±4.13	P < 0.001*
Vitamin B12(ng/ml)	43.97±8.02	47±6.88	0.578

Data were presented as mean ± standard deviation. T student tests were applied. *P-value < 0.05 is highly significant. BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure. P- Value indicates the statistical difference between non-smoker and smokers.

Table 2. Correlations of folic acid with study parameters in smoking and non-smoking individual

Parameters	Smoking(n=44)		Non-smoking(n=44)	
	r	p-value	r	p-value
Age (Years)	0.133	0.214	-0.16	0.298
BMI (kg/m^2)	0.110	0.467	-0.018	0.907
DBP (mmHg)	-0.095	0.530	-0.149	0.335
SBP (mmHg)	-0.293*	0.048*	-0.104	0.502
Homocysteine (nm/ml)	0.089	0.557	0.094	0.542
Vitamin B12 (ng/ml)	-0.156	0.299	0.260	0.088

Correlation-Spearman tests were applied. *P-value < 0.05 is highly significant. BMI: Body Mass Index; DBP: Diastolic Blood Pressure; SBP: Systolic Blood Pressure.

Discussion

Research on the effects of cigarette smoking on human well-being is vital to demonstrate the dangers of smoking. In this study, we investigate the impact of cigarette smoking on serum homocysteine, folic acid, and vitamin B12 levels in smoking and non-smoking patients with CVD. Our results indicate a significant reduction in serum folic acid levels in smokers compared with non-smokers. This finding is consistent with previous studies that found a negative connection between cigarette smoking and serum folic acid levels(8, 9). The underlying reason for this association is not well understood, but it may be due to the fact that harmful chemicals present in cigarette smoke led to increased oxidative stress, which in turn can lead to the breakdown of folic acid in the body. In addition, smokers often consume less fruit and vegetables, which are the primary sources of these vitamins, which could lower serum levels(7). Based on a study conducted by Vardavas et al., smoking status is associated with lower consumption of fiber, fruit, and vegetables, and smoking status affects serum folic acid regardless of diet(10).

In addition, our data showed a reduction in serum vitamin B12 levels in smoking group, but not in a significant manner. This could be a result of the small number of participants. Our results also indicate a non-significant change in serum homocysteine levels between smokers and non-smokers. In contrast to our findings, several reports have demonstrated that smoking can increase serum homocysteine levels(11). Based on previous studies, besides the sample size, factors such as the number of cigarettes smoked per day, duration of smoking status, age, gender, and consumption of coffee and exercise may influence homocysteine levels in serum(7). On the other hand, patients with CVD often experience high serum homocysteine levels, which may mask any differences in homocysteine levels associated with smoking in our study, which can be considered another possible explanation.

The results from the correlation study indicate a negative significant association between serum folic acid and systolic blood pressure in smoking individuals. This result suggests that lower levels of folic acid may contribute to an increase in systolic blood pressure among smokers. However, we did not observe a significant correlation between folic acid levels and diastolic blood pressure. In order to confirm the correlation between folic acid and hypertension, further studies with a larger sample size are needed. The relationship between folate intake and hypertension has been studied extensively. The reason why low folic acid levels give rise to elevated blood pressure is often related to the augmented deposition of homoceysteine in blood vessels, which subsequently leads to endothelial dysfunction. Homocysteine has been linked to arteriolar constriction, increased sodium reabsorption, renal dysfunction, and increased arterial stiffness, all of which can lead to

elevated blood pressure(12, 13). Furthermore, in this study we did not observe any significant relationship between folic acid and vitamin B12, as well as homocysteine.

Conclusion

In conclusion, our findings suggest that smoking patients with a low level of folic acid may experience elevated blood pressure. This highlights the potential benefits of folic acid supplementation for heart failure patients. However, it is important to note that our study did not find significant changes in serum levels of homocysteine and vitamin B12 between the two groups. There may be some limitations to sample collection, technical methods, or variations in the amount of cigarette smoke exposure that can account for these results.

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Ethical statement

Ethical approval for this research study was granted by the Golestan University of Medical Sciences Ethics Committee (No. IR.GOUMS.REC.1401.534). All procedures were performed in accordance with the guidelines for studies involving human participants, considering the ethical standards of the institutional and/or national research committee, as well as the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. During data collection, the researchers obtained informed consent from the participants after explaining the purpose and objectives of the study.

Conflicts of interest

The authors declare that they have no conflict of interest.

Author contributions

All authors have accepted responsibility for the entire content of this submitted manuscript and approved the final version. HRJ and ShH conceptualized the study, provided the project design, and interpreted the data. NB analyzed the data. KhGh was a major contributor in collecting the serum and data. ZH and FF interpreted the data, drafted, and wrote the manuscript. N.H collected the data and contributed to writing the manuscript.

References

1. O'callaghan P, Meleady R, Fitzgerald T, Graham I. Smoking and plasma homocysteine. European heart journal. 2002;23(20):1580-6.
2. Kondo T, Nakano Y, Adachi S, Murohara T. Effects of tobacco smoking on cardiovascular disease. Circulation Journal. 2019;83(10):1980-5.

3. de Jongh S, Lilien MR, Bakker HD, Hutten BA, Kastelein JJ, Stroes ES. Family history of cardiovascular events and endothelial dysfunction in children with familial hypercholesterolemia. Atherosclerosis. 2002;163(1):193-7.
4. Özerol E, Özerol I, Gökdeniz R, Temel I, Akyol O. Effect of smoking on serum concentrations of total homocysteine, folate, vitamin B12, and nitric oxide in pregnancy: a preliminary study. Fetal diagnosis and therapy. 2004;19(2):145-8.
5. Shi Y, Wu Z, Wu J, Chen Z, Li P. Serum homocysteine level is positively correlated with serum uric acid level in US Adolescents: a cross sectional study. Frontiers in Nutrition. 2022;9:818836.
6. Al-Alawi KSS, Waly MI, Sadiq MA, Al Balushi RM, Ali A. Biochemical Assessment of Hyperhomocysteinemia-Mediated Oxidative Stress in Coronary Artery Disease Patients: A Hospital-Based Cross-Sectional Study. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2021;11(1):88-93.
7. Mouhamed DH, Ezzaher A, Neffati F, Douki W, Najjar MF. Effect of cigarette smoking on plasma homocysteine concentrations. Clinical chemistry and laboratory medicine. 2011;49(3):479-83.
8. Mansoor MA, Kristensen O, Hervig T, Drabløs PA, Stakkestad JA, Woie L, et al. Low concentrations of folate in serum and erythrocytes of smokers: methionine loading decreases folate concentrations in serum of smokers and nonsmokers. Clinical chemistry. 1997;43(11):2192-4.
9. McDonald SD, Perkins SL, Jodouin CA, Walker MC. Folate levels in pregnant women who smoke: an important gene/environment interaction. American journal of obstetrics and gynecology. 2002;187(3):620-5.
10. Vardavas CI, Linardakis MK, Hatzis CM, Malliaraki N, Saris WH, Kafatos AG. Smoking status in relation to serum folate and dietary vitamin intake. Tobacco induced diseases. 2008;4:1-7.
11. Dekou V, Gudnason V, Hawe E, Miller GJ, Stansbie D, Humphries SE. Gene-environment and gene-gene interaction in the determination of plasma homocysteine levels in healthy middle-aged men. Thrombosis and haemostasis. 2001;85(01):67-74.
12. Brandes RP. Endothelial dysfunction and hypertension. Hypertension. 2014;64(5):924-8.
13. Lee Y, Park S. Serum folate levels and hypertension. Scientific Reports. 2022;12(1):10071.