Effects of Gender, Smoking, Folate and Vitamin B12 on Plasma Homocysteine Concentration

CİNSİYET, SİGARA İÇİMİ, FOLAT VE VİTAMİN B12’NİN PLAZMA HOMOSİSTEİNE KONSANTRASYONU ÜZERİNE ETKİLERİ

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Abstract

Objective: Total homocysteine (tHcy) metabolism is dependent on several vitamins; (e.g., folate and the vitamins B12, B6 and B2). There have been, however, conflicting results about other factors such as age, gender and smoking that may influence plasma total homocysteine concentrations. The aim of this study is to investigate whether and how gender, smoking and levels of vitamin B12 and folate affect the concentrations of plasma tHcy.

Material and Methods: We measured plasma tHcy concentrations in 48 healthy subjects who were divided into four equal groups according to sex and smoking habits. tHcy and vitamin levels were measured, respectively, by fluorescence polarization immunoassay and electrochemiluminescence.

Results: There was no difference in tHcy concentrations between the groups with regard to either gender and smoking habits. Among men, tHcy significantly correlated with folate (rho= -0.597, p=0.003) and B12 (rho= -0.472, p=0.023). tHcy levels correlated with vitamin B12. In smoker subgroups of both genders, tHcy concentrations correlated with none of the vitamins in question.

Conclusion: It appears that smoking does not directly affect tHcy metabolism. These controversial results may be due to altered vitamin status as a consequence of dietary factors.

Key Words: Homocysteine, vitamin B12, folate, smoking


Homocysteine (Hcy) is a nonessential sulfur-containing aminoacid produced by demethylation of methionine and eliminated through one of the two vitamin-dependent pathways, remethylation to methionine or transsulphuration to cysteine via cystationine.¹ Homocysteine has attracted much interest during recent years because an elevated total Hcy (tHcy) concentration is thought to be an independent risk factor for cardiovascular disease.²,³ It has been reported that causal association of hyperhomocysteinemia and vascular disease would be a marker or consequence of other factors such as lifestyle or a primary risk factor.⁴,⁷

Experimental evidences suggest that an increased concentration of tHcy may result in vascular changes through several mechanisms. Recent
studies showed that, tHcy inhibits inactivation of factor 5a via modification of activated protein C by free cysteine. Another aspect of the effect of tHcy in endothelial injury is that tHcy may decrease the bioavailability of nitric oxide (NO) by increasing its degradation via interactions between its free thiol moiety and NO; and consequently, autooxidation causes to the generation of tHcy-related oxygen free radicals. Trombolic tendency observed in patients with hyperhomocysteinemia may be explained by these mechanisms.

Physiological and pathophysiological factors that modulate plasma tHcy concentrations are lifestyle, age, gender, genetic factors, renal functions, chronic inflammatory and intestinal diseases and vitamin deficiencies. tHcy metabolism is principally dependent on four vitamins: folate, vitamin B12, vitamin B6 and riboflavin (vitamin B2). Plasma tHcy levels are modulated by the rate of remethylation to methionine involving vitamin B12 and folate as the methyl donor or transsulfuration to form cystathionine involving vitamin B6. It has been shown that supplementation of these vitamins decrease tHcy levels and restore flow-mediated vasodilation. This result has been attributed to the response of NO by especially folate supplementation independent of tHcy levels.

Material and Methods

The study population included healthy laboratory coworker, 24 men, ages 16-65 years (mean: 34±4.3 SD); and 24 women, ages 17-51 years (mean: 38±6.8 SD). Twentyfour were nonsmokers who had never smoked and 24 were current smokers consumed at least 10 cigarettes/day. Informed consent was obtained in accordance with the Helsinki Declaration. We used a questionnaire form for all probands. The subjects were healthy without using any therapeutic agents, not having diabetes, renal or cardiac diseases.

Blood samples were drawn in EDTA containing tubes, in the morning after an overnight fasting period; put on crushed ice and centrifuged immediately at 1500 g for 10 min under 4°C. Plasma was stored at –70°C up to 15 days. Plasma tHcy analysis was performed by fluorescence polarization immunoassay (FPIA) with Abbott IMx analyzer. Vitamin B12 and folate were measured simultaneously in the plasma using Roche Elecsys 2010 electrochemiluminescence analyzer.

Statistical Analysis

Results had a normal distribution and thus were expressed as mean ±SD. SPSS 10.0 program was used for statistical analysis. Comparison between means was performed using Mann-Whitney U test. Correlations were calculated using Spearman correlation coefficient and regression analysis was performed using the linear model. p<0.05 was considered statistically significant.

Results

In Table 1, means and standard deviations for male and female regarding to smoking were presented for tHcy, vitamin B12 and folate. Plasma levels of tHcy did not significantly differ among four groups, regarding to gender and smoking. Vitamin B12 and folate did not show statistically significant differences too. In the majority of our groups, tHcy and plasma folate levels were within the reference intervals. Elevated plasma tHcy levels above 15 μmol/L were found in four (8.3%) of participants in all groups, one in nonsmoker females and three in smoker females. Twelve (25%) of participants had vitamin B12 deficiency using the cut-off value for vitamin B12 suggested by the test kit manufacturers (<125 pmol/L). According to plasma folate cut-off values determined by Brouwer et al., all results were above this values.

In the whole study group, there was a significant negative correlation between tHcy and vitamin B12 (rho=-0.552, p=0.000) and folate (rho=-0.349, p=0.016). There was no association be-
between tHcy and age, gender and smoking. Comparison between means was performed using Mann-Whitney U test. In all comparisons, there was no significant difference at the level of p<0.05. Among men tHcy significantly correlated with folate (rho= -0.597, p=0.003) and B12 (rho= -0.472, p=0.023), but among women correlated only with B12 (rho= -0.631, p=0.001). In smoker subgroups of both genders, tHcy had no correlations with any vitamins. Smokers did not show any significant correlation with the parameters of vitamin B12, folate and tHcy.

To determine the independent predictors of tHcy, a multipl regression model was constructed that included age, gender, smoking, vitamin B12, and folate. Analysis revealed that only vitamin B12 (p=0.001) and folate (p=0.044) were variables that influenced the tHcy concentration significantly.

**Discussion**

There are conflicting results in the literature on the association of smoking with tHcy and vitamin status. In our study smokers had slightly higher concentrations of tHcy than nonsmokers especially in women, but the difference was not statistically significant. In the literature, there are three studies having similar results to ours. One of that has also significantly increased levels of reduced form of Hcy in smokers. The authors concluded that these findings can be explain by displacement of disulfide bound of Hcy in plasma by compounds in the cigarette smoke. Bagan et al. found unsignificant differences in tHcy levels between smokers and nonsmokers in mid-pregnancy. In the Framingham Study, Bostom et al. demonstrated a small and unsignificant reduc-
REFERENCES


