ORİJİNAL ARAŞTIRMA / *ORIGINAL RESEARCH*

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

CİNSİYET, SİGARA İÇİMİ, FOLAT VE VİTAMİN B12'NİN PLAZMA HOMOSİSTEİN 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 fleurescence 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

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Özet

Amaç: Total homosistein metabolizması folat, B12, B6 ve B2 gibi çeşitli vitaminlere bağımlıdır. Plazma total homosistein konsantrasyonunu etkileyen diğer faktörler hakkında çelişkili görüşler vardır. Bu faktörler yaş, cinsiyet ve sigara içimidir. Çalışmamızın amacı, cinsiyet, sigara içimi, folat ve vitamin B12 düzeylerinin plazma total homosistein konsantrasyonlarını etkileyip etkilemediğini araştırmaktır.

Gereç ve Yöntemler: 48 sağlıklı kişide plazma total homosistein konsantrasyonlarını, cinsiyet ve sigara içimine göre eşit sayıda vaka içeren dört grupta çalıştık. Total homosistein ve vitamin düzeyleri florösans polarizasyon immünanaliz ve elektrokemiluminisans yöntemleriyle çalışıldı.

Bulgular: Total homosistein konsantrasyonları ile cinsiyet ve sigara içimi arasında anlamlı bir farklılık yoktu. Erkekler arasında total homosistein, folat (rho=-0.597, p=0.003) ve vitamin B12 ile (rho=-0.472, p=0.023) negatif korelasyon gösterdi. Her iki cinsin sigara içen gruplarında total homosistein herhangibir vitamin ile korelasyon göstermedi.

Sonuç: Sigara içimi direkt olarak total homosistein metabolizmasını etkilememektedir. Bu farklı sonuçlar diyete bağlı olarak değişebilen vitamin düzeylerinden kaynaklanmış olabilir.

Anahtar Kelimeler: Homosistein, vitamin B12, folat, sigara içimi

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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

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studies showed that, tHcy inhibits inactivation of factor 5-a via modification of activated protein C by free cysteine.[8] 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.[9] Trombolic tendency observed in patients with hyperhomocysteinemia may be explained by these mechanisms.

Physiological and pathophysiological factors that modulate plasma tHcy concentrations are life style, age, gender, genetic factors, renal functions, chronic inflammatory and intestinal diseases and vitamin deficiencies.[10] tHcy metabolism is principally dependent on four vitamins: folate, vitamin B12, vitamin B6 and riboflavin (vitamin B2).[11] Plasma tHcy levels are modulated by the rate of remethylation to methionine involving vitamin B12 and folate as the methyl donor or transsulphuration to form cystathionine involving vitamin B6.[12] It has been shown that supplementation of these vitamins decrease tHcy levels and restore flow-mediated vasodilation.[13] This result has been attributed to the response of NO by especially folate supplementation independent of tHcy levels. So, plasma folate and vitamin B12 levels should be interpreted with the concentrations of plasma tHcy.[1,6] Because of the effects on vitamin levels and oxidative status, smoking may be attributed with tHcy concentrations. Therefore, we undertook this investigation to evaluate the major determinants of tHcy levels regarding to age, gender, smoking, vitamin B12, and folate in our healthy population.

Material and Methods

The study population included healthy laboratory coworkers, 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 electrochemiluminescens 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 standart 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 manifacturers (<125 pmol/L). According to plasma folate cut-off values determined by Brouwer et al,[14] 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-

Table 1. tHcy, vitamin B12 and folate concentrations in smoker and nonsmoker study groups pertaining to both gender

	Male		Female	
	Smoker	Nonsmoker	Smoker	Nonsmoker
n (%)	12 (25)	12 (25)	12 (25)	12 (25)
Age years (median)	21-47 (34)	16-65 (36)	23-51 (38)	17-51 (38)
tHcy (µmol/L)	9.65±2.51	11.26±3.95	12.01±6.75	9.33±2.43
Vitamin B12 (nmol/L)	248.84±100.14	268.66±146.11	251.58±135.25	264.91±86.24
Folate (pmol/L)	24.46±13.51	22.17±8.63	19.61±9.33	19.88.8.54

tween 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.[15-20] 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.[15,19,20] One of that has also significantly increased levels of reduced form of Hcy in smokers.[20] 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.[15] found unsignificant differences in tHcy levels between smokers and nonsmokers in midpregnancy. In the Framingham Study, Bostom et al. demonstrated a small and unsignificant reduction in tHcy values with smoking in their earlier report,[17] but in recent study, they obtained a significant increase[18] They concluded that these elevated levels could be related with heavy smoking (≥26 cigarettes/day). It seems that smoking is not directly affecting tHcy metabolism. These controversial results may be due to altered vitamin status.

Other researchers also found that age and gender predict tHcy concentrations.[21,22] These reports are in contrast to the present study, in which we found no correlation. This lack of association was explained by narrow limitation of age in our study compared to these studies. In another study, Vecchi et al[23] found similar results to ours. These findings may be resulting from the great availability of fresh vegetables in our country and Italy. All valunteers in our study had normal folate values probably for this reason. In the present study, 25% of the subjects showed decreased vitamin B12 levels. These findings could be explained by low consumption of meat and high consumption of green leaf vegetables. For this reason, in our study, tHcy levels were found significantly correlated with vitamin B12 and folate, but in women, they only correlated with vitamin B12. These significant correlations disappeared by smoking. We suggest that this effect could be depending on the influence of smoking on vitamin levels. But these effect can not reach significant values. These conflicting results could be explained by the genetic background of different populations, metabolic or social factors. Especially dietary vitamin consumption might play a major role in maintaining tHcy concentrations.

REFERENCES

1. Miner SES, Evrovski J, Cole DEC. Clinical chemistry and molecular biology of homocysteine metabolism: an update. Clin Biochem 1997;30:189-201.
2. Rauh M, Verwied S, Knerr I, Dörr HG, Sönnichsen A, Koletzko B. Homocysteine concentrations in a German cohort of 500 individuals: reference ranges and determinants of plasma levels in healthy children and their parents. Amino Acids 2001;30:409-18.
3. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998;338:1042-50.
4. Robinson K, Arheart K, Refsum H, et al. Low circulating folate and vitamin B6 concentrations. Risk factor for stroke, peripheral vascular disease, and coronary artery disease. Circulation 1998;97:437-43.
5. Cleophas TJ, Hornstra N, Hoogstraten B, Meulen J. Homocysteine, a risk factor for coronary artery disease or not? A meta-analysis. Am J Cardiol 2000;86:1005-8.
6. Knekt P, Reunanen A, Alfthan G, et al. Hyperhomocysteinemia a risk factor or a consequence of coronary heart disease? Arch Intern Med 2001;161:1589-94.
7. Dudman NPB. An alternative view of homocysteine. Lancet 1999;354:2072-4.
8. Undas A, Williams B, Butenas S, Orfeo S, Mann K. Homocysteine inhibits inactivation of factor Va by activated protein C. J Biol Chem 2001;276:4389-97.
9. Bellamy MF, McDowell IFW. Putative mechanisms for vascular damage by homocysteine. J Inher Metab Dis 1997;20:307-15.
10. Schneede J, Refsum H, Ueland PM. Biological and environmental determinants of plasma homocysteine. Clin Chem 2000;26:263-79.
11. McKinley MC, Strain JJ, McPartlin J, Scott JM, McNulty H. Plasma homocysteine is not a subject to seasonal variation. Clin Chem 2001;47:1430-6.
12. Blacher J, Safar ME. Homocysteine, folic acid, B vitamins and cardiovascular risk. J Nutr Health Aging 2001;5(3):196-9.
13. Usui M, Matsuoka H, Miyazaki H, Ueda S, Okuda S, Imaizumi T. Endothelial dysfunction by acute hyperhomocyst(e)inemia: restoration by folic acid. Clin Sci (Colch) 1999;96:235-9.
14. Brouwer DAJ, Welten HTME, Reijngoud D, Doormaal JJ, Muskiet FAJ. Plasma olic acid cutoff value, derived from its relationship with homocysteine. Clin Chem 1998;44:1545-50.
15. Pagan K, Hou J, Goldenberg RL, Cliver SP, Tamura T. Effect of smoking on serum concentrations of total homocysteine and B vitamins in mid-pregnancy. Chimica Acta 2001; 306: 103-9.
16. Rasmussen LB, Ovesen L, Bülow I, Knudsen N, Laurberg P, Perrild H. Folate intake, lifestyl factors, and homocysteine concentrations in younger and older women. Am J Clin Nutr 2000; 72: 1156-63.
17. Bostom AG, Rosenberg IH, SilBershatz H. Nonfasting plasma total homocysteine levels and stroke incidence in elderly persons: the Framingham Study. Ann Intern Med 1999;131:352-5.
18. Jacques PF, Bostom AG, Wilson PWF, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 2001;73:613-21.
19. Mansoor MA, Kristensen O, Hervig T, et al. Low concentrationa of folate in serum and erythrocytes of smokers: methionine loading decreases folate concentrations in serum of smokers and nonsmokers. Clin Chem 1997;43:2192-4.
20. Bergmark C, Mansoor MA, Svardal A, Faire U. Redox status of plasma homocysteine and related aminothiols in smoking and nonsmoking young adults. Clin Chem 1997;43:1997-9.
21. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12 and serum total homocysteine levels in confirmed alzheimer disease. Arch Neurol 1998;1449-55.
22. Herrmann W, Schorr H, Bodis M, Knapp JP, Müller A, Stein G, Geisel J. Role of homocysteine, cystathionine and methylmalonic acid measurement for diagnosis of vitamin deficiency in high-aged subjects. Eur J Clin Invest 2000;30:1083-9.
23. De Vecchi AF, Bamonti-Catena F, Finazzi S, et al. Homocysteine, vitamin B12 and serum and erythrocyte folate in peritoneal dialysis and hemodialysis patients. Perit Dial Int 2000;20(2);169-73.