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Serum Homocysteine in Deep Venous Thrombosis, Peripheral Atherosclerosis and Healthy Iramians: A Case-Control Study

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Abstract: This study was aimed to evaluate the association of serum homocysteine with peripheral atherosclerosis and deep vein thrombosis in an Iramian population complaining from vascular symptoms in lower limbs referred to a university general hospital in the capital of Iran. The study design was case-control. Deep vein thrombosis and atherosclerosis groups were, respectively consisted of 25 patients presenting with signs and symptoms of deep vein thrombosis whom disease was confirmed by duplex ultrasonography and 25 patients presenting with signs and symptoms of chronic arterial insufficiency who were candidate for arterial reconstruction whom disease was confirmed by angiography. The control group was consisted of 25 persons selected among relatives accompanying the traumatic patients admitted in the general surgery ward of the same hospital. The age of atherosclerosis, DVT and control group were 61±14, 47±16 and 40±14, respectively. The serum level of homocysteine was higher in males ($p<0.01$) except for atherosclerotic patients. The prevalence of high homocysteine was 15% (control), 36% (DVT) and 56% (atherosclerosis) among females and 75% (control), 73% (DVT) and 56% (atherosclerosis) among males. The serum homocysteine in the control group which was representative of Tehran population who do not take vitamin B supplements was unexpectedly high. It seems that fortification of popular foodstuffs should be considered for Tehran. The association between homocysteine and atherosclerosis and deep vein thrombosis was not confirmed in this study especially for men who had higher serum homocysteine than women. It is possible that this association fades away in populations with high prevalence of hyperhomocysteinema.

Key words: Homocysteine, DVT, atherosclerosis, fortification, Iran

INTRODUCTION

Homocysteine (Hcy) is a product of intracellular demethylation of methionine. Hyperhomocysteinaemia is a disorder of methionine metabolism (Wilcken and Wilcken, 1976). Hyperhomocysteinaemia defined as Hcy more than 15 μmol L^{-1} (Omar *et al*., 2007) is proposed to be a modifiable risk factor of atherosclerosis (Cattaneo, 1999; Graham *et al*., 1997), myocardial infarction (Nygård *et al*., 1997; Whincup *et al*., 1999) peripheral arterial thrombosis (Cattaneo, 1999) as well as venous thromboembolism (Den-Heijer *et al*., 1996, 1998). Homocysteine-related damage to intimal cells has been attributed to oxidative stress, production of hydrogen peroxide and superoxide, inactivation of nitric oxide and inhibition of glutathione peroxidase activity and synthesis (Welch *et al*., 1997). By stimulating the procoagulant mechanism or by inhibiting anticoagulant mechanism, Hcy is supposed to exert its effect on thrombus formation (Cattaneo, 1999).

However, some studies designed to validate the association between atherosclerosis or DVT and serum Hcy do not support the association reported by Valentine *et al*. (1996), Lonn (2007) and Ray *et al*. (2007). This association may vary in different ethnicities due to gene polymorphisms (Nakai *et al*., 2001), considering the discrepancy in studies conducted in different countries. This study aimed to evaluate the association of serum Hcy with peripheral atherosclerosis and Deep Vein Thrombosis (DVT) in an Iranian population complaining from vascular symptoms in lower limbs referred to a university general hospital in the capital of Iran.

MATERIALS AND METHODS

The study had a case-control design. Sampling method was convenient sampling among patients referred to Vascular Surgery Department of Sina Hospital from June 2007 to July 2008, complaining from vascular

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symptoms in lower limbs and they were consecutively included in the study.

Deep vein thrombosis group consisted of 25 patients with signs and symptoms of DVT whose disease was confirmed by duplex ultrasonography.

Atherosclerosis group consisted of 25 patients with signs and symptoms of chronic arterial insufficiency and were candidates for arterial reconstruction whose disease was confirmed by angiography.

The control group was consisted of prevalent controls i.e., they were disease free at the time of study, regardless of time of disease onset among cases. They were consisted of 25 individuals selected among relatives accompanying the traumatic patients admitted in the general surgery ward of the same hospital.

Sina Hospital is a level III University health center. Patients are referred to this hospital from the similar routes; therefore, cases and controls selected from this hospital are from the same source population i.e., residents of Tehran Province.

The study design was approved by the ethics committee of the Sina trauma research center affiliated to Tehran University of Medical Sciences; informed consent was obtained from all the participants.

All subjects were screened for thrombophilia. Participants with abnormality in any of the following indices were excluded from the study: antithrombin III, protein C, protein S, activated protein C resistance, lupus anticoagulant, prothrombin time, activated partial thromboplastin time, bleeding time, platelet count and anti-phospholipid antibodies. The subjects who took vitamin B supplements within the last year or folic acid supplements within the last month were excluded as well.

Laboratory test: Blood samples were collected from the antecubital vein at 8 am; after an overnight 12 h fasting. Participants were asked to refrain from smoking and from taking vigorous exercise prior to blood sampling. The samples were placed on ice immediately and centrifuged within 15 min of venipuncture. The plasma was separated and stored at -4°C until analysis.

Axis shield diagnostics Ltd., homocysteine Enzyme Immunoassay (EIA) kit was used to measure total L-homocysteine. Concentration of homocysteine was measured and reported in μmol L^{-1}.

Statistical analysis: Data were analyzed using SPSS 13 software. A double data entry was arranged by different operators to prevent data entry errors. Continuous data are represented as mean values with standard deviations. The t-student test was used to compare age and homocyteine level among genders. The one way analysis of variance was used to compare age of the three study groups. The Kruskal-Wallis H test (Norman and Streiner, 1994) was used to compare the homocysteine serum level among the study groups. Chi square test was used to compare distribution of nominal variables among the study groups. The p-value less than 0.05 was considered as statistically significant.

To control the simultaneous effect of age and gender on the association between serum Hcy and the disease, two logistic regression models were fitted. In both models age, sex and Hcy were considered as independent variables, while DVT in one model and atherosclerosis in the other model were considered as independant variables.

RESULTS

The mean age of the normal group was 40±14 (Table 1). The atherosclerotic patients were older (61±14) than both DVT patients (47±16) and normal persons (Table 1), the difference was statistically significant ($p<0.001$). The DVT group patients were older than normal individuals(Table 1), however, the difference was not statistically significant ($p = 0.165$).

Male patients were older than females (Fig. 1) though the difference was not statistically significant ($p = 0.11$).

The serum level of Hcy was higher in males (Fig. 2) except for atherosclerotic patients; the difference was statistically significant for normal and DVT groups ($p<0.01$).

The difference of serum Hcy level among the study groups (Table 2) was not statistically significant ($p = 0.52$). The Hcy serum level in normal women was lower than DVT and atherosclerotic women although the difference did not achieve statistical significance. This pattern was not the same with men (Fig. 2).

The serum Hcy concentration up to 15 was considered normal according to the laboratory kits used

Table 1: The characteristics of normal, DVT and atherosclerotic groups

			Age			
Study groups	Gender	Count	Minimum	Maximum	Mean	SD
Atherosclerotic	Male	16	45	89	64	13
	Female	9	38	78	57	14
	Total	25	38	89	61	14
DVT	Male	11	21	77	49	18
	Female	14	13	72	46	16
	Total	25	13	77	47	16
Normal	Male	12	24	67	40	14
	Female	13	24	70	40	14
	Total	25	24	70	40	14
Total	Male	39	21	89	52	18
	Female	36	13	78	46	16
	Total	75	13	89	49	17

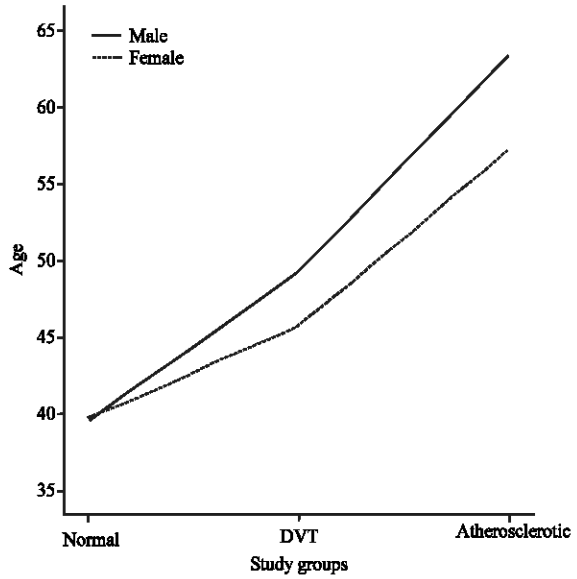

Fig. 1: The mean age of patients according to the gender

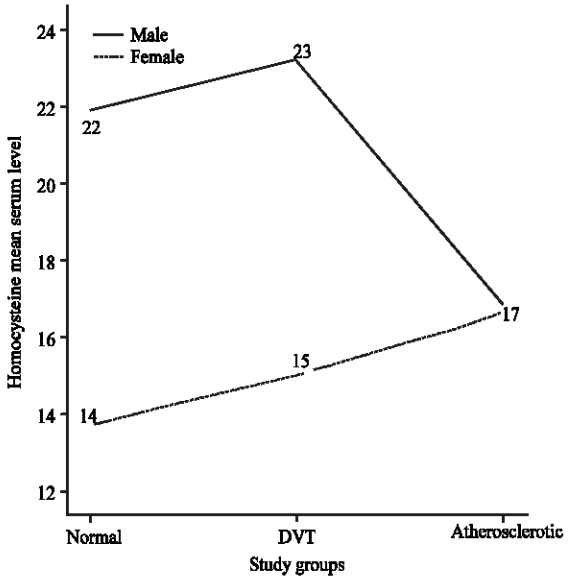

Fig. 2: The mean serum homocysteine level in study groups according to the gender

to measure it. In women, the individuals with Hcy concentrations higher than 15 were more frequent in atherosclerotic and DVT groups compared to the normal group (Fig. 3a, b) though the difference was not statistically significant (p-values: 0.07 and 0.39, respectively). The power of this study to detect the above differences was 0.34 and 0.11, respectively. This pattern was different in men as the frequency of individuals with Hcy more than 15 was higher in the normal group to

Table 2: Homocysteine serum concentration among study groups

		Homocysteine serum concentration				
Study groups	Gender	Minimum	Maximum	Mean	SD	Median
Atherosclerotic	Male	8.0	35.3	16.8	7.3	15.2
	Female	9.1	30.6	16.6	7.6	15.9
	Total	8.0	35.3	16.8	7.2	15.2
DVT	Male	8.7	50.0	23.1	11.8	21.9
	Female	9.3	33.4	15.0	6.6	12.8
	Total	8.7	50.0	18.6	9.9	15.3
Normal	Male	12.3	50.0	21.9	10.5	18.8
	Female	7.9	21.2	13.7	3.8	13.3
	Total	7.9	50.0	17.6	8.7	14.6

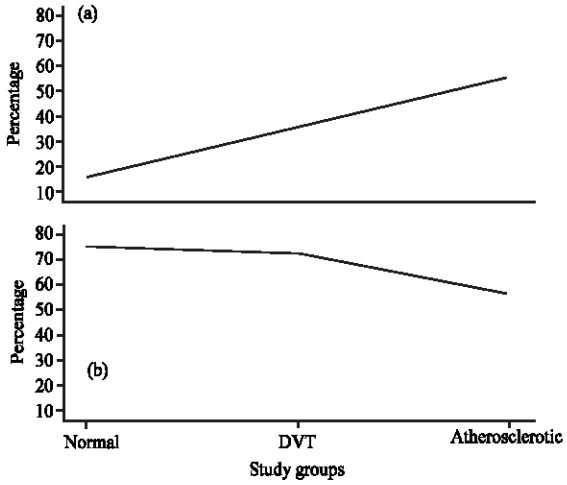

Fig. 3: Percentage of homocysteine >15 in study groups according to the gender, (a) female and (b) male

compared to atherosclerosis and DVT groups though the difference was not statistically significant (p-values: 0.43 and 0.99, respectively).

In the logistic regression analysis, the association between DVT and age, sex, serum Hcy did not appear to be statistically significant. The association between Atherosclerosis and sex, serum Hcy did not appear to be statistically significant either. Atherosclerosis was associated with increasing age of patients ($p<0.001$).

DISCUSSION

This study was conducted to compare the serum Hcy of patients admitted for treatment of DVT or peripheral atherosclerosis with that of the control group in an Iranian population. We did not detect statistically significant difference aomng study groups. The serum level of Hcy in control group was higher than its normal range mentioned for western populations (Angelova *et al*., 2005; Ganji and Kafai, 2006). This may be indicative of higher serum Hcy level in Tehranian people compared to Western population. There is another published study

that reported high Hcy level in Tehran in a population based survey (Fakhrzadeh *et al*., 2006). The high Hcy level in healthy population may mask the association of Hcy with atherosclerosis and DVT reported in some studies. In other words, the association of Hcy with atherosclerosis and DVT may be valid only in populations which hyperhomocystinemia is not common. This is an incidental finding which was not in agreement with the study hypothesis and needs to be evaluated more in later studies.

In female subjects, the high serum Hcy was more frequent in atherosclerotic and DVT patients compared to the control group. High serum Hcy is reported to be associated with these conditions (Hsu *et al*., 2001; Bhargava *et al*., 2007; Naushad, 2008). The power of this study was not enough to detect a significant difference between female cases and control groups; therefore, failure to detect a statistically significant difference does not indicate lack of such a difference. The higher prevalence of hyperhomocysteinemia in cases compared to controls was seen in females only. This pattern was different in men (Fig. 3). There is another study that has reported a relationship in women only (Folsom *et al*., 1998). The reason for this is not clear but it could be discussed as follows; the serum Hcy was already high in healthy (control) men. Three out of four men in the control group had abnormal Hcy which was much more than healthy women. Again, the higher serum Hcy level in normal (control) men population compared to normal (control) women population might masked the association between Hcy and atherosclerosis and DVT reported in some other studies. However, this hypothesis needs to be evaluated in furthur studies.

The serum level of Hcy in the control group could be considered as its level in general population in Tehran who do not take vitamin B supplements as this group consisted of healthy individuals accompanying patients who were admitted due to trauma.

The high Hcy serum level in Tehranian normal people who do not take vitamin B supplements, is considered as an accidental finding and needs to be evaluated in future population based studies. Some explanations to this finding may be as follows: Low serum folic acid and cobalamin are known to be associated with high Hcy (Rasmussen *et al*., 1996; Hao *et al*., 2007). There is a report of low serum folic acid and Vit B12 in healthy Tehranian population (Fakhrzadeh *et al*., 2006). Prolonged cooking of vegetables and meat which is quite common in Persian cooking can destroy the folate and cobalamin of the food (Dawson and Waters, 1994) and result in low intake of these vitamins which are associated with increased Hcy. Low dietary meat content in Tehranian population may be another explanation for high serum Hcy level. There are some reports that indicate high Hcy in vegetarian (Hung *et al*., 2002). Genetic factors which are associated with high Hcy (Gellekink *et al*., 2005; Bathum *et al*., 2007) may play a role in high Hcy level seen in this study as pointed out in some other studies (Vermaak *et al*., 1991; Cortese and Motti, 2001).

In this study, the age distribution of DVT and atherosclerotic patients reflects the mean age of these patients in the general population because the study sample was a random sample of people referred to a university general hospital in Tehran. The hospital was among the first points of referral for general population with vascular problems. This is not the case with the control group as they consisted of people accompanying patients admitted to the hospital due to a traumatic event. The mean age of the control group is expected to be more than normal population which was 28 years according to the national census data. The mean age of atherosclerotic patients was higher than DVT patients. The control group participants were younger than both DVT and atherosclerotic patients since no matching was performed on age.

As expected, the mean age of the control group was the same for both genders. The reason for higher age of males in DVT and atherosclerotic groups is not clear and more studies are needed to clarify the reason. It may be due to earlier medical care seeking in women compared to men. It may also reflect earlier mortality of affected women which prevents them to appear in the hospital in older ages.

Overall, the serum level of HCY in men was higher than women; this is in accordance with other studies (Refsum *et al*., 1996; Chou *et al*., 2000). This was not the case with atherosclerotic patients in whom the mean Hcy was the same for male and female patients. The reason for this finding is not known. This may be due to survival bias: atherosclerotic men with high serum Hcy may not live as long as atherosclerotic men with low serum Hcy. This phenomena may bias the estimation of serum Hcy in atherosclerotic men in a cross sectional study.

This study was subject to limitations. We failed to adjust the effect of potential confounders as smoking. The power of this study was not enough to detect statistically significant difference in female cases and controls. Further studies with more sample size are needed to evluate the association of serum Hcy and peripheral vasclar disease in Tehranian population. Future studies might address the cause-effect relationship of serum Hcy and peripheral vascular diseases.

CONCLUSION

The association between Hcy and atherosclerosis and DVT was not confirmed in this study especially for men who had higher serum Hcy than women. It is possible that this association fades away in populations with a high prevalence of hyperhomocysteinema however; this needs to be evaluated in further studies. The high serum Hcy in the individuals of the control group who were representative of Tehranian population and did not take vitamin B supplements was an incidental finding. It seems that fortification of popular foodstuffs should be considered for Tehran.

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