

Plasma Homocysteine Level and Risk of Thrombosis

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ABSTRACT

Background and Objectives

Venous thrombosis is one of the important causes of morbidity and mortality all over the world. Elevated plasma homocysteine is known as a cause of vein morphology changes and endothelial dysfunction which lead to platelet activation, fibrinolysis inhibition and finally atherothrombosis. In this study, we evaluated the role of homocysteine in atherothrombosis as compared to the control group with no history of thrombosis.

Material and Methods

In this case control study, 100 patients with arterial thrombosis (54 men and 46 women) as the case group and 68 as control (40 men and 28 women) were involved. Blood samples were taken in the EDTA-located tube and transported to the laboratory for fasting plasma homocysteine to be measured by ELISA kits. Some data such as age, sex, thrombosis history and familial thrombosis history were taken from the patients through a questionnaire. We measured fasting plasma homocysteine in both case and control groups by ELISA method. The statistical analysis was performed by SPSS statistical software using T-Test and Chi-square, odds ratio was also calculated.

Results

The average rates of homocysteine in the case and control group were 23.85 ± 18.4 and 11.48 ± 3.4 $\mu\text{mol/lit}$ respectively showing statistical significance. The hyperhomocysteinemia frequency in the case group was 48% whereas 17.6% in the control. A significant difference was also observed in the frequency of hyperhomocysteinemia between male (70.4%) and female (21.7%) in the case group. There was a moderate correlation between homocysteine level and age in the case group.

Conclusions

According to the achieved odds ratio (2.27), hyperhomocysteinemia is an independent risk factor for thrombosis. It means that homocysteine measurement should be determined in thrombosis-affected or high risk patients. Dietary supplementation with low doses of folate and vitamin B12 should be considered in affected persons.

KEY WORDS: Homocysteine, Venous thrombosis, Risk factor

INTRODUCTION

Homocysteine is a sulfurous amino acid which is biosynthesized from methionine. In case of disorder in the metabolic pathway of homocysteine, its level thereof will elevate and homocysteinemia occurs. Homocysteinemia is one of the important causes of atherothrombosis (1). Although elevated Homocysteine, similar to smoking, high cholesterol level, and blood pressure, is one of the risk factors to cardiovascular diseases, it is more common in smokers and those with high levels of blood pressure (2). Hyperhomocysteinemia activates the coagulation and avoids fibrinolysis by changing the morphology of veins, as well as reducing or ruining the endothelial antithrombotic veins. Most of the damage to the veins is associated to homocysteine oxidation (3).

One of the most important causes that lead to hyperhomocysteinemia is deficiencies of vitamin B₁₂ and Folic acid as the cofactors in homocysteine metabolism. Deficiencies of vitamin B₁₂, folic acid and vitamin B₆, as well as reduction in enzyme activity prevent the homocysteine to be degraded. As a result, the homocysteine intercellular density increases. By exiting homocysteine from inside the cell and entering into plasma, hyperhomocysteinemia happens. Statistics released in Europe show that 4 million people lose their lives due to cardiovascular disease and thrombosis. It imposes a heavy financial burden on health and treatment department, particularly in developing countries (3).

In order for timely diagnosis of hyperhomocysteinemia in risky groups (those with family history of thrombosis, cardiovascular diseases, high blood pressure, high level of LDL- cholesterol, deficiency of vitamin B₁₂ and folic acid, and smokers) as well as in patients with venous diseases and thrombosis, it is of great importance to measure the level of homocysteine. It was shown that reduction in the level of homocysteine in hyperhomocysteinemia patients avoids venous thrombosis and cardiovascular diseases by 25 percent (3).

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Cardiovascular diseases and thrombosis are among the first causes of mortality and invalidity in Iran (4).

This study aims to investigate the role of homocysteine level in pathogenesis of thrombosis by measuring the level of this type of amino acid in patients with venous thrombosis. Adopting appropriate approaches to control the level of homocysteine led to reduction of thrombosis rate.

MATERIAL AND METHOD

The current study is a case control study. Data was collected using questionnaire and experiments. Individuals diagnosed to have arterial thrombosis were selected as the case group. From among our colleagues at the laboratory those with the same characteristics as the case group (with respect to age and sex) were selected as the control group. The control group had no self and/or family thrombosis history. Items on the designed questionnaire included individual characteristics such as age, sex, thrombosis history, thrombosis location, family thrombosis history, smoking, and history of thrombotic inherit disorder. Questionnaires were completed by the patients themselves contently. Then, blood samples were collected from the fasting patients and control group and transferred into the tubes contained anti-clot EDTA and transported to laboratory for plasma homocysteine measuring. Samples were kept in freezer at -20°C until the experiment. Axis Homocysteine EIA kit from German IBL Co. was used to measure the level of plasma homocysteine. The measurement was carried out on the basis of ELISA. Firstly, the homocysteine attached to protein was transformed to a free homocysteine using Dithiotetheritol. Then it transformed into S-Adenosyl-L-homocysteine using s-adenosylhomocysteine hydrolase (SAH). Through the next reaction, which was an Immunoassay one, plasma homocysteine and the homocysteine attached to ELISA Plate tries to join to homocysteine monoclonal antibody. In the next step, the activity reaction of peroxidase was measured by adding HRP substrate and antibody at 450 nm wave lengths, where the amount of light absorption showed to be reversely related. Data collated from the questionnaires and the results obtained from the experiments were processed using SPSS 11 Software. Chi Square and T-test were used to compare the background variables. Odd ratio was determined and confidence interval was estimated at 95% confidence level.

Findings

In the current study the case group consisted of 46 females with thrombosis aged 38 ± 13 and 54 males aged 35 ± 13 (total=100 people), were compared to the control group (people with no thrombosis history) consisted of 28 females aged 35 ± 13 and 68 males aged 36 ± 10 . 12 patients (13%) had thrombosis and 12 patients (12%) had thrombosis family history. The average homocysteine in case group was 23.85 ± 8.4 and 11.48 ± 3.4 in control group. The difference was statistically significant. There was a significant increase in homocysteine level in the case group compared to the one in control group ($p < 0.001$).

48 patients (48%) in case group (those with thrombosis) and 12 people in control group (17.6%) had hyperhomocysteinemia. 38 males (70.4%) and 10 females (21.7%) in case group had hyperhomocysteinemia. The difference between the males and females was statistically significant ($P < 0.001$). Results show that there is a moderate correlation between sex variable and increase in the level of homocysteine ($p = 0.05$, $r = 0.2$). Table 1 shows the homocysteine level in case and control groups.

20 patients (20%) had Deep Venous Thrombosis, 27 (27%) showed Cerebral Venous Thrombosis, 34 (34%) Retinal Venous Thrombosis and 15 (15%) patients showed lung, mesenteric and portal thrombosis. 19 patients with Retinal Venous Thrombosis (55.9%), 11 patients with Cerebral Venous Thrombosis, and 8 patients (40%) with DVT showed increase in the level of blood homocysteine (figure 1).

Odd ratio was determined at 2.72 ($CL_{95\%} = 1.56-4.73$). It suggests that increase in homocysteine level can be considered a risk factor to thrombosis.

DISCUSSION

Thromboembolism is one of the significant and common causes of mortality in world (3,5). Embolism can occur as the result of thrombosis in different organs. Cerebral Venous thrombosis and peripheral Venous Thrombosis are the commonest ones. Increase in homocysteine level can be a risk factor to venous diseases and thrombosis. It may occur due to a genetic disorder or acquisitive causes. Although it is not clear that whether the homocysteine itself is the cause of thrombosis or related metabolites and/or a co-factor can be involved too. While the association between thrombosis and homocysteine level is not thoroughly known, homocysteine composes 10% of risk factors in catching thrombosis (1-3, 6).

Our findings are in consistency with Lin et al (2002) which show males are in higher risk of thrombosis than females (7). Our findings also show that the increased level of homocysteine is directly related to aging in patients. In other words, older people are exposed to higher risk of thrombosis and hyperhomocysteinemia. Our findings suggest that 24.6% of patients with thrombosis and increased level of blood homocysteine were categorized under mild hyperhomocysteinemia. It shows that mild hyperhomocysteinemia is considered a risk factor causing thrombosis. It is one of the common causes that might be in association with deficiencies of vitamin B₁₂ and folic

acid (8). In presence of folic acid, B₁₂, and methionine synthesis, a huge part of homocysteine is transferred into methionine. Deficiencies of vitamins B₁₂ and B₉ inhibit homocysteine to be degraded. It leads to an increase in blood homocysteine and consequently hyperhomocysteinemia.

Our findings revealed that hyperhomocysteinemia can be viewed as a significant and independent risk factor to retinal venous thrombosis, Cerebral venous thrombosis, and deep venous thrombosis. This is consistent with findings of (9-12).

Increased homocysteine in individuals with damaged venous endothelial, increased lipids peroxidation, as well as changes in blood coagulation pathway are possible causes that can lead to venous thrombosis (3).

As the obtained odd ratio (2.72) suggests hyperhomocysteinemia is an independent risk factor to thrombosis. Odd ratio= 1.8 in Boushey et al (13) suggests that Coronary Venous diseases can be associated with increased level of homocysteine. It is critical to measure homocysteine level in patients with atherosclerosis, thrombosis or venous diseases or family history thereof. It is also suggested that taking vitamin B₁₂, folic acid, and B₆ and decreased homocysteine can be of great importance in preventing thrombosis in high risk individuals as well as in treating patients. Moghadasiyan et al (14) showed that a 5 μmol/l decrease in homocysteine decrease the risk of cardiovascular diseases by 15% in normal population and by 25% in high risk groups .Age, sex, and reason that lead to decrease in the level of vitamin B₁₂ and folic acid are all effective on homocysteine level (15).

Conclusion

Our findings show that an increase in the level of homocysteine can be considered an independent risk factor to venous thrombosis. Studying the association of frequency of thrombosis in family with homocysteine level, investigate the role of measuring folic acid and vitamin B₁₂ and how they are related with hyperhomocysteinemia in patients with thrombosis, and the role of deficiencies of folic acid and vitamin B₁₂ in thrombosis can be subject to further studies.

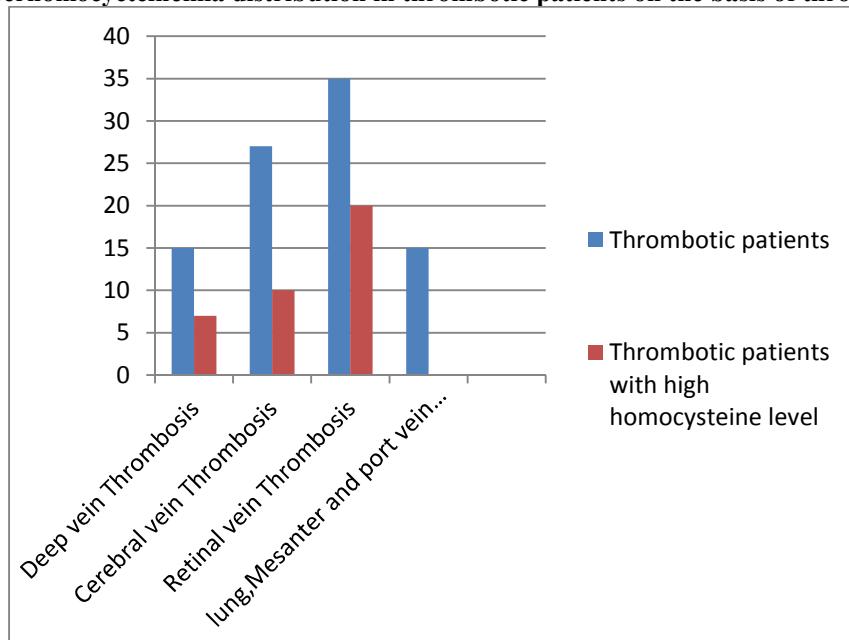
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Table 1 shows the homocysteine level in case and control groups.

	Normal homocysteine level (5-15 μmol/l)	Hyperhomocysteinemia (>15 μmol/l)
Case group		
Males	16 (29.6)	38 (70.4)
Females	36 (78.2)	10 (21.7)
Total	52 (52)	48 (48)
Control group		
Males	32 (80)	8 (20)
Females	24 (85.7)	4 (14.3)
Total	56 (82.4)	12 (17.6)

Figure 1: Hyperhomocysteinemia distribution in thrombotic patients on the basis of thrombosis location



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