

The role of folic acid on the hyperhomocysteinemia in the Buerger's disease (Thromboangiitis Obliterans)

Ali Akbar Beigi, Mohammad Ali Hoghoughi¹, Afrooz Eshaghian², Akbar Hassan Zade³, Hassan Masoudpour⁴

Departments of Vascular Surgery, ¹Surgery and ²Head and Neck Surgery, Saint Al-Zahra Hospital, ³Department of Epidemiology and Biostatistics, Isfahan University of Medical Sciences, Isfahan, Iran, ⁴Molecular Structure and Function, Research Institute, The Hospital for Sick Children, University of Toronto, Ontario, Canada

Background: The mechanism underlying Buerger's disease (BD) is still unknown. Recently, thrombophilic conditions predisposing to a hypercoagulable state have been hypothesized as triggers for BD. The aim of the study is to evaluate the prevalence of the hyperhomocysteinemia and level of the anticardiolipin antibodies, and the role of folic acid on the hyperhomocysteinemia and on the rate of the amputations in the patients with BD. **Materials and Methods:** In an experimental placebo-controlled double-blinded study, between 2004 and 2010, thirty patients with BD were randomly assigned into two groups (14 patients in a drug group and 16 patients in the placebo group). Drug or placebo was administered, and they were followed in 2 and 6 months for homocysteine, Anticardiolipin antibodies and the risk of amputations. **Results:** At the beginning of the study homocysteine level was higher than normal in 19 patients (63%). There was a significant decrease in homocysteine level during 6 months in folic acid group ($P < 0.001$), but there was no change in the placebo group. None of our patients had elevated Anticardiolipin antibodies, and there was no change in the level of Anticardiolipin antibody during study. High level of homocysteine did not associate with more amputations during 6 months of study ($P > 0.05$). **Conclusion:** This study shows the hyperhomocysteinemia in BD, and the benefit of folic acid treatment in homocysteine lowering, but folic acid doesn't inhibit the risk of major and minor amputation during 6 months of follow-up. Longer follow-up may reveal the role of folic acid in these patients

Key words: Anticardiolipin antibodies, Buerger's disease, folic acid, hyperhomocysteinemia, thromboangiitis obliterans

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INTRODUCTION

Thromboangiitis Obliterans or Buerger's disease (BD) is a chronic nonatherosclerotic inflammatory obliterative disease which affects predominantly small and medium arteries and veins in the infra-popliteal and infra-brachial regions. Although BD has a worldwide distribution, nowadays it is more prevalent in the middle east and far east than in North America and Western Europe.^[1-4] It is seen more common in the young smoker men and in a low socioeconomic status.^[5] BD is established on the basis of the clinical presentation and physical findings, according to the following criteria; history of smoking (current or recent), onset of disease before 50 years old, infra-popliteal obliterative arterial involvement, upper limb involvement or phlebitis migrans, and absence of atherosclerosis risk factors other than smoking.^[1-4,6-8] There is no specific diagnostic laboratory indicator, so in those countries where the BD is common, the clinical criteria of Shionoya are accepted.^[3] However,

in countries with a rare incidence of BD, the diagnosis is based on imaging and by excluding other vasculitis, using expensive laboratory tests.

Etiology and pathogenesis of BD is not completely understood, but environmental predisposing factors such as genetic and nicotine was supposed.^[1-3] After smoking and tobacco cessation, other medical or surgical therapies have limited results.^[2,3]

Another possible pathogenesis observed in the patients with BD is a hemostatic disturbance. Hyperhomocysteinemia is common in BD, and may have an important effect on BD pathogenesis irrelevant to Nicotine.^[5] Preliminary data have been shown hyperhomocysteinemia is a risk factor for venous thrombosis^[9,10] aggravates the prognosis, and may reveal that the patients undergo amputation earlier.^[8]

Unfortunately, prevalence of smoking is very high in our country, and the prevalence of the BD is high as well;

Address for correspondence: Dr. Mohammad Ali Hoghoughi, Department of Surgery, Saint Al-Zahra Hospital, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: hoghoughima@yahoo.com

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in contrast with other Asian countries which encounter one or two new patients per year.^[1,3] The patients with BD take two in every 20 vascular surgery beds at St. Zahra Hospital. About 23% of the ischemic patients <50 years old who referred to our vascular clinic are diagnosed with Burger's disease. Also, we see many complications related to disease as a result of late diagnosis and treatment, but many of our patients could not stop smoking, and have no ideal treatment response.

It has been hypothesized that hypercoagulability state (like hyperhomocysteinemia, increased anticardiolipin, and genetic factors) has a role in the pathophysiology of BD.^[8,9,11-14] It has been established that Folic acid could improve hyperhomocysteinemia;^[15,16] Also Folic acid could improve endothelial function as a result of lowering homocysteine, and might have a beneficial effect on BD. This study was done to evaluate the role of homocysteine lowering effect of Folic acid on limb amputation, the major complication of BD.

MATERIALS AND METHODS

This was an experimental placebo controlled double-blinded study. The study group was 30 identified Buerger's patients based on the criteria who were randomly allocated in drug or placebo groups (14 patients in drug group and 16 patients in placebo group) from January 2004 to January 2010. All of them were advised stop smoking and were on standard routine treatment protocol of BD. To confirm the smoking cessation, urine check was done. All participants signed informed consent. The study was approved by Isfahan University of Medical Sciences Ethics Committee, Isfahan, Iran. It was registered in Iranian Registry of Clinical trials (IRCT201108012106N2).

The inclusion criteria contained negative history of hypertension, diabetes mellitus, ischemic heart disease, cerebrovascular disease or collagen vascular disease as well as negative vasculitis workup. The exclusion criteria were surgical sympathectomy or any sort of vascular bypass; using aspirin, calcium channel blocker, B6 or B12;

stop smoking during the study and have not compliance of being treated. Also, patients with hypercoagulable state due to inherited thrombophilia (factor V Leiden, A202120G prothrombin variant, acquired activated protein C resistance, protein C and S deficiency, antithrombin deficiency) were excluded from the study.

Medication was one oral 5 mg folic acid tablet (Jallinus Pharmacy, Tehran, Iran) and placebo (Amin Pharmacy, Isfahan, Iran) with the same as the color, size, weight, and box.

At the start of the study, we measured demographic data, homocysteine and Anticardiolipin level in the form. Following administration of drug and placebo, they were followed 2 and 6 months later. Lab data, minor or major amputations were recorded during follow-up.

At the end of the study, data were analyzed by SPSS version 17 (SPSS Inc., Chicago, USA) by Chi-square, *t*-test, covariance analysis, repeated measure ANOVA, Friedman, Wilkoxon and Mann-Whitney tests.

RESULTS

This study contained 30 patients (14 in Folic acid group and 16 in the placebo group) [Table 1]. All of them were male. None of the patients had a history of diabetes mellitus, chronic renal failure, ischemic heart disease, and hypertension. Only two patients had a history of hyperlipidemia. Except years of education, other baseline characteristics didn't have a difference between two groups ($P > 0.05$).

At the beginning of the study, 19 patients (63%) had homocysteine level higher than normal (normal value was 15 mg/dl). None of our patients had elevated anticardiolipin antibody. By one sample *t*-test, baseline homocysteine level at baseline was more than normal value in Buerger's patients ($P = 0.002$). The Baseline level of homocysteine and anticardiolipin antibody was not different between two groups ($P > 0.05$) [Tables 2 and 3].

Table 1: Demographic parameters in two groups

Feature	Folic acid group (n = 14)	Placebo group (n = 16)	P
Age (years)	39.42±5.7	36.06±7.76	Not significant
Sex (male/female)	Male	Male	Not significant
Duration of symptoms (months)	34.80±12.2	36.71±16.4	Not significant
History of smoking (pack/year)	25	23	Not significant
Years of education (years)	5.8±3.5	8.8±2.9	P<0.05
Average of pain based on the VAS			
0 month	7.07±2.82	5.9±2.21	Not significant
2 nd month	5.45±2.75	5.75±1.99	Not significant
6 th month	3.46±2.57	4.82±2.46	Not significant

VAS = Visual analogue scale

Table 2: The mean of homocysteine level at the baseline, 2nd and 6th month of study

Homocysteine level ($\mu\text{mol/L}$)	Folic acid group Normal <15 ($\mu\text{mol/L}$)	Placebo group Normal <15 ($\mu\text{mol/L}$)	P
Baseline, 0 month	21.37 (± 8.39)	17.93 (± 5.57)	Not significant
2 nd month	12.87 (± 2.68)	17.33 (± 4.68)	0.004 [¶]
6 th months	9.71 (± 2.90)	17.18 (± 5.25)	<0.001 [§]

[¶]By ANOVA, the difference between groups were significant after the 2nd month of the study and although in the 6th month; [§]By repeated measure ANOVA test, there was significant decrease in homocysteine level during 6 months in folic acid group ($P < 0.001$), but no change was seen in placebo group

Table 3: The mean of anticardiolipin level at the baseline, 2nd and 6th months

Anticardiolipin antibody	Folic acid group	Placebo group	P
Baseline, 0 month	3.90 (± 1.06)	4.58 (± 2.86)	Not significant
2 nd month	3.83 (± 1.28)	4.44 (± 2.38)	Not significant
6 th months	3.62 (± 1.02)	4.31 (± 2.23)	Not significant

Because the baseline of the homocysteine level was higher in folic acid group (that was not significant), covariance analysis was performed, and it demonstrated that the folic acid was more effective from the 2nd month of study.

We divided the patients into two normal and high level of homocysteine groups; and compared the major and minor limb amputations in each placebo and folic acid groups at the beginning, 2nd and 6th month. High level of homocysteine did not increase amputations during 6 months of study ($P > 0.05$) [Table 4]. Anticardiolipin antibody did not change in level during 6 months of study in none of the folic acid and placebo groups ($P > 0.05$).

DISCUSSION

This study revealed 63% prevalence of hyperhomocysteinemia in our Buerger's patients that was higher than previous reports, which was 23% in Stammers study^[3] and 60% in Deiham study.^[8]

This study have been shown the benefit of folic acid on homocysteine lowering in Buerger's disease but not on Anticardiolipin level. The studies show 10 mg folic acid for 4 weeks in patients with hyperhomocysteinemia (more than 75 percentile) and without cardiovascular risk factors could improve arterial endothelial function and decrease homocysteine.^[9] There was no change for independent endothelial vasodilatation. No side effect had been reported.^[1,9]

Two studies had been shown that endothelial function didn't improve with homocysteine lowering;^[3,14] but another

Table 4: The number of major and minor amputation in both groups

Amputation	Folic acid group	Placebo group	P
Minor amputation			
0 month	5/14	4/16	Not significant
2 nd month	5/14	4/16	Not significant
6 th month	5/14	4/16	Not significant
Major amputation			
0 month	1/14	0/16	Not significant
2 nd month	1/14	0/16	Not significant
6 th month	1/14	0/16	Not significant

one revealed the lower homocysteine is a protective vascular factor irrelevant to folate.^[8,10,15] Impaired endothelial function is assumed as a predisposing factor in BD^[1,2] and we hypothesized that the folic acid may improve vasculature endothelium and could decrease amputation. In present study, high level of homocysteine did not associate with more amputations after 6 months of study in either placebo or folic acid group; and we could not follow our supposition. This pilot result can be attributed to short follow-up or small sample size.

As in our results, previous studied revealed folic acid, Vitamin B6 and B12 could not inhibit major cardiovascular complications and recurrent venous thrombosis.^[1,8,11,12,15,17]

CONCLUSION

This study shows hyperhomocysteinemia in BD, and the benefit of folic acid in homocysteine lowering; folic acid could not inhibit major and minor amputation during 6 months of follow-up. Longer follow-up may reveal the benefit of folic acid in these patients.

This study emphasized the role of homocysteine in the pathogenesis of BD, especially in smoker patients. More detailed immunological and endothelial surveys can clarify this hypothesis.

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AUTHOR'S CONTRIBUTION

All authors have contributed in designing and conducting the study. All authors have assisted in preparation of the first draft of the manuscript or revising it critically for important intellectual content. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

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