Original Research Article

Serum homocysteine as a predictor of severity of peripheral arterial disease in association with Doppler ultrasonography

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ABSTRACT

Background: To evaluate association between raised serum homocysteine levels and severity of peripheral vascular disease (PVD) and to assess the role of homocysteine as a prognostic marker in PVD and thereby aid in early referrals to prevent cerebral and coronary events in those patients and introduce homocysteine lowering with vitamin therapy as part of medical management in patients diagnosed with PVD.
Methods: The serum homocysteine levels was measured in all patients admitted for peripheral vascular disease of upper and/or lower limb and to correlate the above values with Doppler ultrasonography (USG) in the duration between 2016-2018 (18 months).
Results: The comparison of homocysteine levels with Doppler shows corresponding significant increase in Serum Hcy levels only in cases of moderate PVD. Statistical analysis with binary logistic regression does show a significant association with moderate severity of PVD and serum homocysteine levels with 66.7% predictability.
Conclusions: There was significant association of homocysteinemia only in Moderate PVD with no statistically significant correlation with mild, severe cases of peripheral arterial disease (PAD) and radiological normal cases, further large randomised trials are required to elucidate its clinical relevance in PAD as a prognostic marker of severity.

Keywords: Homocysteine, Peripheral arterial disease, Peripheral vascular disease

INTRODUCTION

Peripheral arterial disease (PAD) is the most common condition affecting the arteries of lower extremity. Compromise of arterial flow due to stenosis and occlusions can result in limb ischemia, which may manifest as claudication, rest pain, local tissue loss (ulceration), and, potentially, amputation.[1] Patients with PAD may have symptoms but can also be asymptomatic.

These patients have an increased risk of mortality, myocardial infarction and stroke. It adversely affects the functional status of the limb and is associated with poor quality of life. The most common cause in the lower limb arterial occlusive disease is atherosclerosis.[1] Less common causes include thromboembolism, acute thrombotic occlusion, micro embolism, trauma and vasculitis including vasospastic disorders and Beurger's disease.[2] Atherosclerosis, though typically asymptomatic for decades, eventually produces two main problems: First, the atheromatous plaques, though long compensated for by artery enlargement, eventually lead to plaque ruptures and clots inside the artery lumen over the ruptures.[3] The clots heal and usually shrink but leave behind stenosis (narrowing) of the artery (both locally and in smaller downstream branches), or worse, complete closure, and, therefore, an insufficient blood supply to the tissues and organ it feeds. Second, if the compensating artery enlargement process is excessive, then a net aneurysm result. Not only we can save the limb but

improve the function of the limb by diagnosing and treating this condition. Treatment options include medical therapy, bypass surgery, and various percutaneous interventions such as angioplasty, atherectomy, stent placement, and thrombolysis.[4]

First recognized in patients with rare inborn errors of metabolism, the association of elevated plasma homocysteine concentrations with atherosclerosis and thrombosis now seems relevant to the general population as well.[1,2]

Homocysteine is prothrombotic, atherogenic agent, it promotes endothelial cell damage, platelet hyperactivity, and the production of abnormal clotting factors leading to the development of thromboembolic plaques in the coronary, carotid, and peripheral vascular systems and confers an independent risk of vascular disease similar to that of smoking or hyperlipidaemia.[5,6]

The association as stated above is independent of other factors, is fairly consistent across many studies, is strong and dose-related, and is biologically plausible. However, the evidence needs to be strengthened by a systematic review of all comparable studies and the demonstration, in randomised trials, that lowering serum homocysteine is followed by a significant reduction in atherothrombotic vascular disease.[3]

Hyperhomocysteinaemia can be shown in 30% of patients with premature peripheral vascular disease (PVD).[6] Long-term survival in patients with lower extremity PVD is greatly diminished as a result of atherosclerotic complications in the coronary and cerebrovascular beds, hence primary therapy should be directed at treating the generalized atherosclerotic process i.e. the management of lipids, blood sugar, and blood pressure and therefore monitoring of homocysteine (Hcy) levels in such patients and primary treatment to lower the raised titers along with early referrals aid in improving the prognosis of those with PVD.[4,7]

Objectives of the study was to evaluate association between raised serum homocysteine levels and severity of peripheral vascular disease and to assess the role of homocysteine as a prognostic marker in PVD and thereby aid in early referrals to prevent cerebral and coronary events in those patients and introduce homocysteine lowering with vitamin therapy as part of medical management in patients diagnosed with PVD.

METHODS

This research was as an observational study.

Study place and duration

Patients diagnosed or suspected to have PVD in JSS Hospital out-patient, surgery and emergency department between September 2016 to September 2018 were included in the study.

Selection criteria

Inclusion criteria

Adults of both sexes with clinical diagnosis of peripheral vascular disease of upper and/or lower limbs in whom Doppler USG will be done.

Exclusion criteria

It was ongoing or previous treatment for hyperhomocystinemia.

Procedure

Identifying patients with symptoms and/or signs of peripheral arterial disease to the JSS OPD and Emergency Department. Sample size was established as 75 patients based on the prevalence of PAD among males at our hospital which was found to be 84 patients out of 1582 total admissions in the department of surgery in one year. Serum homocysteine was sent in these patients along with preliminary Doppler USG. Correlation of Hcy levels with Doppler USG was done to establish a correlation.

Classification of homocysteine levels give in points-

- Mild: 15-30 μmol/l
- Moderate: 30-100 μmol/l
- Severe: Above 100 μmol/l

Ethical approval approved by the scientific committee after due diligence in September 2016.

Statistical methods

Descriptive statistics was done measuring mean and proportions. Kruskal-Wallis test was done for logistic regression analysis. All statistical calculations documented in this report were done using SPSS version 18.0 and the graphical presentations were done by using Microsoft Excel.

RESULTS

The study included 75 patients. The prevalence of PAD increases with age affecting up to 50% of patients over the age of 40 years.

The age and sex distribution are in accordance with the general demographics of PAD, the incidence of which increases with age, from approximately 0.3%/year for men aged 40 to 55 years to approximately 1%/year for men older than 75 years.

Table 1: Age distribution in study subjects.

Age (in years)	N	%
<30	10	13.3
31-40	15	20
41-50	19	25.3
51-60	19	25.3
>60	12	16

Table 2: Gender wise distribution in study subjects.

Gender	N	%
Female	16	21.3
Male	59	78.7

These results are consistent with the overall distribution of PAD in general population.

Table 3: Limb wise distribution in study subjects.

Limb	N	%
Bilateral	30	38.7
Left	21	28

Atherosclerosis is a systemic progressive condition that affects all major vascular systems and in our study majority of patients studied had bilateral involvement at presentation.

Table 4: Distribution of peripheral pulsations in study wise subjects.

Pulsations	N	%
Present	43	57.3
Absent	9	12.0
Feeble	23	30.6

57.3% of patients had normal pulsations (premature PVD) with 30% having feeble pulses.

Table 5: Distribution of diabetes, hypertension and smoking among study subjects.

Variable	No		Yes	
	N	%	N	%
Diabetes mellitus	47	62.7	28	37.3
Hypertension	60	80	15	20
Smoking	44	58.7	31	41.3

In this study, Hcy levels were not raised in smokers or in those with co-morbid status of diabetes or hypertension.

Normal limits of serum Hcy values was reported in majority of the cases, i.e. in 34 out of 75 with prevalence of 45.3%. Mild and moderate homocystenemia have 26.7% and 28% prevalence in this study while severe levels were not recorded at all.

Table 6: Distribution of Hcy levels in study subjects.

Serum homocysteine levels	N	%
Normal (<15 umol/l)	34	45.3
Mild (15-30 umol/l)	20	26.7
Moderate (30-100 umol/l)	21	28
Severe (100 umol/l)	0	0

Table 7: Intervention in study subjects.

Any intervention	N	%
Nil	55	71.3
Above knee amputation	3	1.3
Below knee amputation	6	2.7
Auto amputation	1	2.7
Debridement	4	3.3
Disarticulation	9	8.6
Trans metatarsal amputation	1	1.3
Thrombectomy	1	1.3
Angioplasty	1	1.3
Aorto-femoral bypass	1	1.3

Table 8: Indication for arterial doppler in study subjects.

Indication for doppler	N	%
	9	12
Chronic smoker	31	41.3
Cold peripheries	2	2.6
Discolouration	4	6
Wound over foot	4	13.3
Nonhealing ulcer	8	18.6
Feeble pulsations	23	30.6
Recurrent cellulitis	1	4
Gangrene	6	20

This table reflects on the various surgical modalities of intervention that was warranted in our study subjects after due investigation and consent.

The common indications for seeking or advising vascular intervention in our country is depicted in the above table with smoking being the most common cause in our study.

Table 9: Distribution of severity of PVD in study subjects.

Severity of PVD	N	%
Normal	14	18.7
Mild PVD	12	16.0
Moderate PVD	25	33.3
Severe PVD	24	32.0

Our study revealed majority of the patients to have moderate and severe levels of PVD.
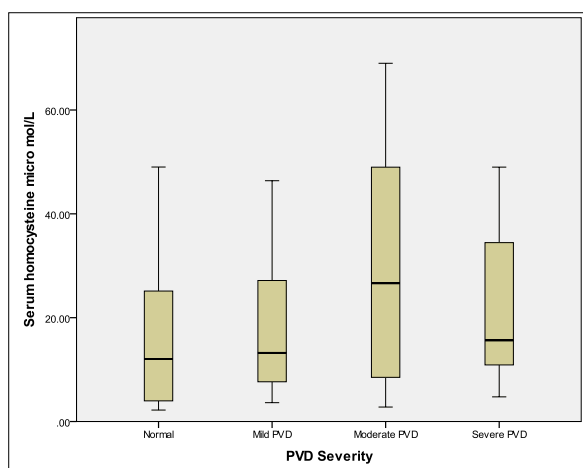
Figure 1: Association of serum Hcy with severity of PVD.

P=0.3, Kruskal wallis test.

Statistical analysis with binary logistic regression does shows a significant association with moderate severity of PVD and serum Hcy levels with 66.7% predictability.

This study showed a significant association of Serum homocyteine levels with moderate severity of PVD but Hcy levels did not correlate with mild and severe cases of PVD probably due to rampant use of multivitamins and necessitates further clinical trials on a larger population and preferably in different regions.

DISCUSSION

Raised Hcy levels is a well-established risk factor for coronary and peripheral vascular disease and it should be suspected especially in young patients in whom other risk factors are absent and with a gene frequency between one in 70 and one in 200 this condition may be more common than previously thought.[8]

According to a population-based study done in Department of Surgery, University of Hong Kong, Queen Mary Hospital, Hong Kong, patients with hyper-homocysteinemia had a four-fold increase in risk of PVD relative to patients with a normal Hcy level. These observations were in spite of no significant difference between the two groups with respect to various other factors such as patient demographics, biochemical risk factors, and disease pattern and severity etc.

However its role as marker of severity of PVD is yet to be tested and this study aimed to assess and establish Hcy as a reliable predictor of severity in PVD so that lowering homocysteine levels with multivitamin therapy becomes an additional established protocol in the treatment of PAD and may also be used as an effective screening tool in suspected subsets of population.

People with PAD have a risk of heart attack or stroke four to five times higher than that of the age-matched

population and hence treatment and control of all modifiable risk factors such as homocystinemia is of paramount importance.[6]

In this study of 75 patients, 50.6% of the patients was in the age group between 40-60 years, with equal distribution between 40-50 and 50-60 years with 78.7% predilection in males. The age and sex distribution is in accordance with the general demographics of PAD, the incidence of which increases with age, from approximately 0.3% per year for men aged 40 to 55 years to approximately 1% per year for men older than 75 years.

Normal limits of serum homocysteine values were reported in majority of the cases, i.e. in 34 out of 75 with prevalence of 45.3%. Mild and moderate homocystenemia have 26.7% and 28% prevalence in this study while severe levels were not recorded at all.

Doppler investigations revealed predominance of moderate PVD; 33.3%. The comparison of homocysteine levels with Doppler in assessing the grade of severity according to Kruskal Wallis test shows corresponding significant increase in serum Hcy levels only in cases of moderate PVD. Severe cases did not show an associated elevation in Hcy.

However statistical analysis with binary logistic regression does shows a significant association with moderate severity of PVD and serum Hcy levels with 66.7% predictability.

Homocysteine is prothrombotic, atherogenic agent, it promotes endothelial cell damage, platelet hyperactivity, and the production of abnormal clotting factors leading to the development of thromboembolic plaques in the coronary, carotid, and peripheral vascular systems and thereby confers an independent risk of vascular disease similar to that of smoking or hyperlipidaemia.[9]

Homocysteine levels were not raised in smokers or in those with co-morbid status of diabetes or hypertension.

Hyperhomocysteinaemia can be shown in up to 30% of patients with premature PVD as demonstrated by this population-based survey on the prevalence of diabetes and associated diseases conducted in Japanese-Brazilians which reported men in particular with PAD had higher prevalence rates of hyper-homocysteinemia as compared to women (22.7% vs 7.6%).[10]

Hence the need to evaluate Hcy as an effective marker and screening tool in mild to moderate cases of PAD in order to diminish the long-term complications in lower extremity PVD as a result of atherosclerotic complications in the coronary and cerebrovascular beds.

Primary therapy should be directed at treating the generalised atherosclerotic process i.e. the management

of lipids, blood sugar, and blood pressure and monitoring of homocysteine levels in such patients7 and primary treatment to lower the raised titres along with early referrals aid in improving the prognosis of those diagnosed with PVD and in those in whom clinical presentations are yet to manifest.

Folic acid and multivitamin drugs are the recommended treatment for raised homocysteine levels and can be included in the standard treatment regimen of PAD once high levels are detected.[1] Compared with nonusers of vitamin supplements, the small number of subjects taking such vitamins appeared to have a substantially lower risk of vascular disease, a proportion of which was attributable to lower plasma homocysteine levels as per Case-control study published as The European Concerted Action Project,1995 where a total of 750 cases of atherosclerotic vascular disease (cardiac, cerebral, and peripheral) and 800 controls of both sexes younger than 60 years were studied across nineteen centres in 9 European countries.

The rampant and judicious use of multivitamins even at rural areas can be considered one of the reasons for detecting normal to moderate levels of Hcy even in moderate to severe cases of arterial disease.

Highly prevalent in the Indian Subcontinent, arterial occlusive disease is a leading contributor to overall cause of death as a result of myocardial infarction or stroke, significant disability and loss of function which result in an enormous cost in impaired quality of life for our aging population and hence implementation of newer management; diagnostic and treatment protocols will aid is better prevention of PAD related morbidity and mortality.

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