

## Study to Evaluate Serum Homocysteine Levels as Marker of Peripheral Arterial Disease in a Tertiary Care Hospital in India

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### ABSTRACT

**Background:** The study is aimed to evaluate the association between raised serum Homocysteine levels and severity of Peripheral Vascular Disease (PVD) and its role as a prognostic marker in PVD. Thereby, Vitamin K therapy can be instituted for lowering Homocysteine levels as prophylaxis against cerebral and coronary events in PVD patients.

**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 abovevalues with Doppler ultrasonography (USG) in the duration between 2020-2022(18 months)

**Results:** The comparison of homocysteine levels with Doppler shows corresponding significant increase in Serum homocysteine 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 80% 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 randomized trials are required to elucidate its clinical relevance in PAD as a prognostic marker of Severity.

**KEYWORDS:** Homocysteine, Peripheral arterial disease, Peripheral vascular disease

### ARTICLE DETAILS

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### INTRODUCTION

Peripheral arterial disease (PAD) is the most common condition affecting the arteries of lower extremity. Compromise of arterial flow due to stenosis and occlusion can result in limb ischemia, which may manifest as claudication, rest pain, local tissue loss (ulceration) and even loss of limb due to amputation. It adversely affects the functional status of the limb and is associated with poor quality of life. Patients with PAD may also be asymptomatic. These patients have an increased risk of myocardial infarction and stroke.

The most common cause in the lower limb arterial occlusive

disease is atherosclerosis.<sup>1</sup>Atherosclerosis is a systemic disease affecting the medium and large arteries. The pathophysiology of this disease involves the accumulation of fibrin and lipid between the tunica intima and media of the blood vessel leading to narrowing of the vessel lumen. This results in decreased blood flow which may lead to ischemia. It is called peripheral arterial disease when it occurs in a non-cardiac or cerebral vessel. Ankle Brachial Pressure Index (ABI) is used to diagnose PAD. ABI of less than 0.90 is considered diagnostic of peripheral arterial disease<sup>2</sup> while an ABI of more than 1.30 is suggestive of non-compressible vessels indicative of arterial

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disease<sup>3</sup>, especially of the systemic type.

Atherosclerosis, inflammation and thrombosis are the most common causes of decreased peripheral blood flow.<sup>4</sup> 20% - 50% Peripheral Arterial Disease (PAD) cases are asymptomatic while 10% – 35% present with claudication pain and another 20% present with nonspecific local symptoms<sup>5</sup>. Diagnosis of PAD is critical because people with PAD have 4-5 times the risk of a heart attack or a stroke than the age-matched population.<sup>6</sup> These asymptomatic patients realize their predicament too late, even for secondary prevention. They continue to smoke and are non-compliant with medications for diabetes mellitus as they are asymptomatic and lead a comfortable life, therefore not needing to change their current lifestyle. It is a known fact and proven by studies that prevalence of PAD is associated with diabetes<sup>7,8,9</sup>, male gender, smoking, hypertension, dyslipidaemia, race, hyperhomocysteinemia and renal disease.<sup>6</sup> Less common causes include thromboembolism, acute thrombotic occlusion, micro embolism, trauma and vasculitis including vasospastic disorders and Beurger's disease.<sup>10</sup>

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 hyperlipidemia.<sup>11,12</sup> The association is independent of other factors, is 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 randomized trials, that lowering serum homocysteine is followed by a significant reduction in athero thrombotic vascular disease.<sup>13</sup> Hyperhomocysteinemia can be shown in 30% of patients with premature peripheral vascular disease (PVD).<sup>12</sup> 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.<sup>14,15</sup>

## MATERIALS AND METHODS

### Study Design

Hospital based; single center prospective observational study.

### Study population

All the cases PVD admitting in hospital in the one year period meeting the inclusion and exclusion criteria.

### Study area

Department of Surgery in a tertiary care government hospital in West India with attached Medical College.

### Sample size

Sample size was established as 84 patients was calculated at 95% confidence at 80% power to predict the severity of PAD patients at 67.75 predictability.

### Inclusion Criteria

- All diagnosis of peripheral vascular disease of upper and/or lower limbs in which Doppler ultrasound was done.
- Age >18 years
- Given written consent

### Exclusion Criteria

- Ongoing or previously treated for hyperhomocysteinemia.

### Procedure

The patients were identified with symptoms and/or signs of peripheral arterial disease visiting OPD and Emergency Department were included in study. Serum homocysteine was sent in these patients along with preliminary Doppler USG.

Classification of homocysteine levels give in points-

- Mild: 15-30  $\mu\text{mol/l}$
- Moderate: 30-100  $\mu\text{mol/l}$
- Severe: Above 100  $\mu\text{mol/l}$

## STATISTICAL ANALYSIS

Descriptive statistics was done measuring mean and proportions. Kruskal-Wallis test were done for logistic regression analysis. All statistical calculations documented in this report were done using SPSS (Online free version). For statistical calculations a p value of <0.5 considered as significant.

## RESULTS

The study included 84 patients. The prevalence of peripheral arteries disease increases with age affecting up to 65% of patients over the age of 41 years.

The age and sex distribution are in accordance with the general demographics of peripheral arteries disease, the incidence of which increases with age, from approximately 0.3%/year for

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men aged 40 to 55 years to approximately 1%/year for men older than 72 years. (Table-1) Most of the 45.24% patients are between the age group of more than 51 years. Followed by 21.43%, 19.05% and 14.29% patients are between the age group of 41-50 yrs, 21-30 yrs and 31-40 yrs age group respectively.

Table-2 shows male to female distribution of cases. 65.5% patients were male & 34.5% patients were female.

The majority of 73.8% patients studied had unilateral involvement of atherosclerosis at presentation. While 26.2% patients studied had bilateral involvement. (Table-3)

Only 23.8% of patients had normal pulsations (premature PVD) with maximum 48.8% having feeble pulses. 27.4% of patients had no normal pulsation. (Table-4)

In the study group, 49 (58.3%) were non smokers while 35 (41.7%) were smokers. (Table-5) There were 26

(31%) of participants who had hypertension and 18 (21.4%) had diabetes mellitus. (Table-6-7)

Normal limits of serum homocysteine values were reported in majority of the cases, i.e. in 40 out of 84 with prevalence of 47.6%. Mild and moderate homocystenemia have 25% and 27.4% prevalence in this study while severe levels were not recorded at all. (Table- 8) Table -9 shows that 56 (66.7%) patients received surgical intervention for PAD while 28( 33.3%) didn't receive any surgical intervention. Amputation was done in 17 ( 20.2%) of patients. ( Table- 10)

Table- 11 shows that maximum Thrombectomy interventions was found in 15.4% patients. Followed by Aortofemoral Bypass, Femoropoplital Bypass and debridement interventions was found 11.9%, 10.7% and 8.3% respectively. Majority of the patients to have moderate and severe levels of PVD. (Table-12)

**Table 1: Age wise distribution in study subjects**

Age group	Frequency	Percentage
21-30	16	19.05%
31-40	12	14.29%
41-50	18	21.43%
51-60	19	22.62%
> 60	19	22.62%
<b>Total</b>	<b>84</b>	<b>100.0%</b>

**Table 2: Gender wise distribution in study subjects**

Gender	Frequency	Percentage
Male	55	65.5%
Female	29	34.5%
<b>Total</b>	<b>84</b>	<b>100.0%</b>

**Table 3: Limb wise distribution in study subjects**

Limb	Frequency	Percentage
Unilateral	62	73.8%
Bilateral	22	26.2%
<b>Total</b>	<b>84</b>	<b>100.0%</b>

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

Peripheral pulse	Frequency	Percentage
Present	20	23.8%
Absent	23	27.4%
Feeble	41	48.8%

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<b>Total</b>	<b>84</b>	<b>100.0%</b>
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**Table 5: Distribution of Smokers among study subjects**

Smokers	Frequency	Percentage
Yes	35	41.7
No	49	58.3
<b>Total</b>	<b>84</b>	<b>100.0%</b>

**Table 6: Distribution of Hypertension among study subjects**

Hypertension	Frequency	Percentage
Yes	26	31.0%
No	58	69.0%
<b>Total</b>	<b>84</b>	<b>100.0%</b>

**Table 7: Distribution of Diabetes Mellitus among study subjects**

Diabetes Mellitus	Frequency	Percentage
Yes	18	21.4%
No	66	78.6%
<b>Total</b>	<b>84</b>	<b>100.0%</b>

**Table 8: Distribution of Homocysteine Levels among study subjects**

Hcy levels	Frequency	Percentage
Normal (<15 umol/l)	40	47.6%
Mild (15-30 umol/l)	21	25.0%
Moderate (30-100 umol/l)	23	27.4%
Severe (>100 umol/l)	0	0.0%
<b>Total</b>	<b>84</b>	<b>100.0%</b>

**Table 9: Nil surgical interventions in study subjects**

Nil	Frequency	Percentage
Yes	28	33.3%
No	56	66.7%
<b>Total</b>	<b>84</b>	<b>100.0%</b>

**Table 10: Amputation interventions in study subjects**

Amputation	Frequency	Percentage
Yes	17	20.2%
No	67	79.8%
<b>Total</b>	<b>84</b>	<b>100.0%</b>

**Table 11: Other interventions in study subjects**

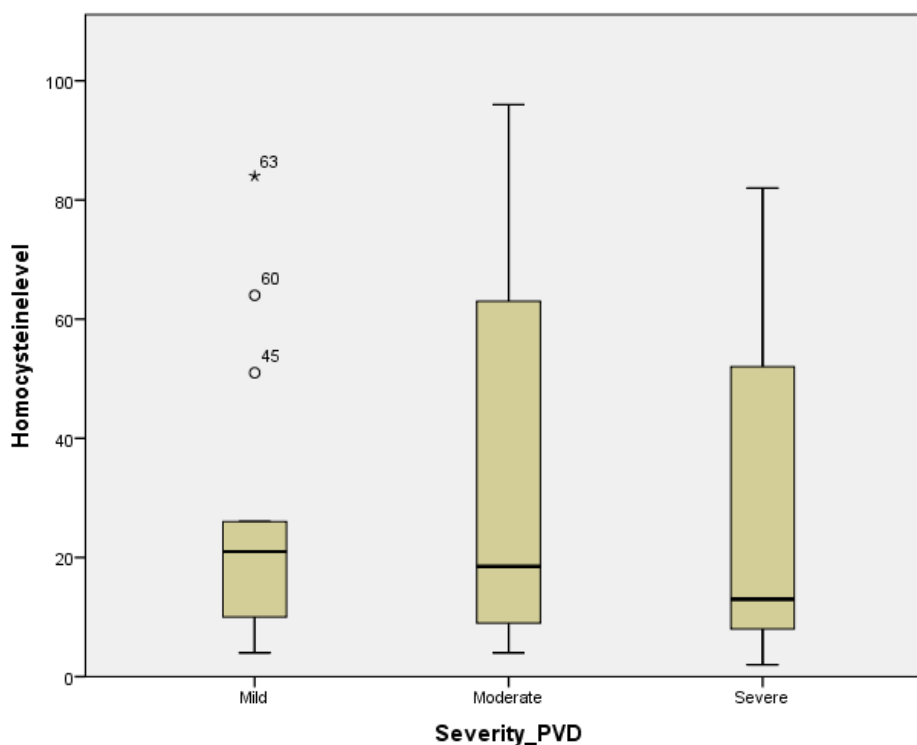
Other interventions	Frequency	Percentage
Aortofemoral Bypass	10	11.9%
Debridement	7	8.3%

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Femoropoplital Bypass	9	10.7%
Thrombectomy	13	15.4%
<b>Total</b>	<b>39</b>	<b>46.4</b>

**Table 12: Distribution of severity of PVD in study subjects**

Severity of PVD	Frequency	Percentage
Normal	15	17.9%
Mild	9	10.7%
Moderate	32	38.1%
Severe	28	33.3%
<b>Total</b>	<b>84</b>	<b>100.0%</b>



### Association of serum Hcy with severity of PVD

This study showed a significant association of Serum homocysteine 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 Homocysteine 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.<sup>16</sup>

A study was done in Department of General Surgery, tertiary care government hospital in western India showed that patients with hyper-homocysteinemia had a four-fold increase in risk of PVD relative to patients with a normal Homocysteine 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.

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Patients 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.<sup>14</sup>

In this study of 84 patients, 54.77% of the patients was in the age group between 21-50 years, with equal distribution between 51-60 and more than 60 years with 45.24%. Maximum 65.5% patients was found of male category. 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 72 years.

Normal limits of serum homocysteine values were reported in majority of the cases, i.e. in 40 out of 84 with prevalence of 47.6%. Mild and moderate homocystenemia have 25% and 27.4% prevalence in this study while severe levels were not recorded at all.

Doppler investigations revealed predominance of moderate PVD; 38.1%. 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 more than 80% 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.<sup>17</sup>

Homocysteine levels were not specifically 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%).<sup>18</sup>

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 generalized atherosclerotic process i.e. the management of lipids, blood sugar, and blood pressure and monitoring of homocysteine levels in such patients<sup>15</sup> and primary treatment to lower the raised titers 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.<sup>1</sup> 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.

Highly prevalent in the Indian Subcontinent, vascular 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 PVD related morbidity and mortality.

We recommend more studies with larger sample for greater insights into the subject for confirming the association between homocysteine levels and severity of PVD for effective management of the patient.

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