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Original Article

Association Between Hyperhomocysteinemia and Early-Onset Atherosclerotic Peripheral Arterial Occlusive Disease

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ABSTRACT

Introduction: *Homocysteine is increasingly recognized as an independent risk factor for vascular disease especially peripheral arterial occlusive diseases. In the present study, an attempt was made to find out the association between the raised fasting plasma total homocysteine level and early onset atherosclerotic peripheral arterial occlusive diseases (PAOD).* ***Methods & Materials:*** *In this prospective observational study, a total of 50 study subjects were included. All patients were clinically and angiographically documented for atherosclerotic PAOD. All patients underwent surgical intervention as well as estimation of serum total homocysteine level. Arterial segment was sent for histopathological examination to find out whether atherosclerosis was present or not. Patients were divided into two groups- Group-1 included patients of 20-40 years of age and group-II included those of 41-60 years of age. The groups were compared to see association between elevated level of plasma homocysteine and atherosclerotic peripheral arterial occlusive diseases in elderly as well as in early age.* ***Results:*** *41 (82%) patients were male and 9 (18%) patients were female. Serum homocysteine level was higher in group I than group II (71.4% vs. 40.9%). Besides, the level of mean serum homocysteine level was significantly (p=0.02) higher in group I than group II (21.18±9.53 vs. 17.24±8.92 µmol/L).* ***Conclusion:*** *In conclusion, this study suggests that serum homocysteine has an association with early onset atherosclerotic PAOD. Therefore, a raised serum homocysteine level can be used as an independent biochemical predictor of early onset atherosclerotic PAOD.*

Key words: *Homocysteine, Atherosclerosis, Peripheral arterial occlusive diseases.*

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INTRODUCTION

Homocysteine and its derivatives cause endothelial dysfunction, intimal-medial thickening, oxidation of Low-Density Lipoprotein (LDL), and a pro-coagulant state, eventually leading to premature atherosclerosis[1]. Normal fasting levels of homocysteine range between 5 and 15 µmol/L[2]. Hyperhomocysteinemia can be classified as mild to moderate (16 to 30 µmol/L), intermediate (31 to 100 µmol/L), and severe (more than 100 µmol/L)[3].

Hyperhomocysteinemia is now recognized as an independent risk factor for atherosclerosis. Homocysteine is an unstable amino acid that undergoes autodigestion, producing free oxygen radicals[4]. Thus, hyperhomocysteinemia leads to increased production of free oxygen radicals and oxidative stress. Homocysteine-induced vascular pathologies include fragmentation of the intimal elastic lamina, disruption of elastic fibers, smooth muscle hyperplasia, and arterial and venous thrombosis[5].

Homocysteinemia results from deficiencies in vitamin B6, B12, or folate, or a combination of these vitamins. These vitamins are essential cofactors for key enzymes related to homocysteine metabolism; therefore, a deficiency would impair the activity of these enzymes and lead to an accumulation of homocysteine[6].

A series of cross-sectional and case-control studies, along with a meta-analysis of 27 observational studies, strongly supports the idea that increased plasma homocysteine levels are an independent risk factor for Coronary Artery Disease (CAD), cerebrovascular disease (CVD), and peripheral vascular disease[7]. Although the process of atherosclerosis begins early in life, its clinical manifestations usually occur in the elderly population. Thus, like CAD, atherosclerotic Peripheral Arterial Occlusive Disease (PAOD) is primarily a disease of the elderly[8].

Recent clinical experiences with PAOD patients at NICVD suggest a steady rise in the number of young patients suffering from atherosclerotic PAOD[9]. Even patients in their 20s and 30s have been diagnosed with documented atherosclerotic PAOD. The etiopathological characteristics of this disease are unclear. Although the association between atherosclerosis and homocysteine is well described in the literature, few studies address atherosclerotic PAOD in patients as young as their 20s and 30s and its possible

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association with homocysteine, particularly in the Bangladeshi population.

The importance of such a study is amplified by the fact that hyperhomocysteinemia is a potentially correctable metabolic disorder. If its association with early-onset atherosclerotic PAOD is established, a therapeutic target becomes readily available.

Therefore, we believe that the present study can enhance our understanding of the pathophysiological basis of early-onset atherosclerotic PAOD. No previous study has specifically investigated the role of elevated homocysteine in young patients with atherosclerotic PAOD in the Bangladeshi population. This study may provide important insights into the etiopathology of atherosclerosis in this patient population. Consequently, the study was undertaken to evaluate the possible association between higher serum levels of homocysteine and early-onset atherosclerotic PAOD.

METHODS & MATERIALS

Study design and site

This was a prospective observational study conducted in the vascular surgery department of the National Institute of Cardiovascular Diseases (NICVD), Dhaka, Bangladesh, over the period of January 2014 to December 2014. Patients aged between 20 and 60 years with angiographically documented peripheral arterial occlusive disease (PAOD) and fulfilling the inclusion and exclusion criteria were enrolled. A total of 50 patients were included in the final analysis.

Sampling Method and Data Collection

A purposive sampling technique was employed to recruit patients who fulfilled the inclusion and exclusion criteria. Ethical clearance for the study was obtained from the Ethical Committee of NICVD, and informed written consent was obtained from all participants prior to their enrollment in the study.

Data collection involved both preoperative and postoperative variables. Preoperative variables included demographic data such as age, sex, and occupation. Clinical findings recorded during the preoperative period included pulse, blood pressure, the presence of intermittent claudication, rest pain, ulcer, and smoking history. Laboratory investigations were conducted to measure fasting plasma total homocysteine (tHcy) levels, fasting blood sugar, fasting serum lipid profile, and serum creatinine.

Postoperative data included histopathological findings of the harvested arterial segments. These segments were analyzed using specialized staining techniques, including Hematoxylin and Eosin stain for cellularity and plaque structure, silver stain for elastic fibers, and Masson's trichrome stain for collagen content.

Assessment and Investigations

Each patient underwent a thorough medical history review, clinical examination, and relevant preoperative investigations. Risk factors for PAOD were evaluated, including smoking, hypertension, diabetes, dyslipidemia, and a family history of PAOD. Peripheral angiography was performed to assess the site, extent, severity, and pattern of arterial stenosis or occlusion.

Medications were reviewed, and all prescribed drugs were continued except for aspirin and clopidogrel, which were discontinued five days before surgery. During the operation, arterial segments containing atherosclerotic plaques were biopsied. Histopathological analysis of the specimens included:

- Hematoxylin and Eosin (H&E) stain for cellularity and plaque morphology.
- Silver stain for elastic fiber assessment.
- Masson's trichrome stain for collagen content evaluation.

Statistical Analysis

Data were analyzed using SPSS® statistical software (version 26). Numerical data were expressed as mean ± standard deviation, and categorical data were presented as frequencies and percentages. Group comparisons were performed using the Chi-square test for categorical variables and the student's t-test for continuous variables. Multivariate regression analysis was conducted to identify independent associations between variables.

Patients were divided into two groups based on age for subgroup analysis:

- **Group A**: Patients aged 20–40 years.
- **Group B**: Patients aged 41–60 years.

Ethical Considerations

Ethical approval for the study was obtained from the Ethical Committee of NICVD. Written informed consent was secured from all patients prior to participation, ensuring adherence to ethical standards for research.

RESULTS

A total of 50 patients were included in the study, divided into two age groups: Group I (20–40 years) comprising 28 patients and Group II (41–60 years) comprising 22 patients. The baseline characteristics and outcome variables were compared between these groups. The distribution of patients was nearly equal, with 56% in Group I and 44% in Group II. The study population included 41 males (82%) and 9 females (18%), with a mean age of 39.4 ± 9.1 years. An unpaired t-test revealed no statistically significant difference in mean age between male and female patients (39.8 ± 9.2 vs. 37 ± 8.5 years, $p = 0.39$). Systolic blood pressure (115.93 ± 10.83 mmHg vs. 114.57 ± 13.89 mmHg) and diastolic blood pressure (76.67 ± 8.98 mmHg vs. 73.91 ± 10.76 mmHg) were comparable between the groups, with no statistically significant differences ($p > 0.05$). Similarly, pulse rates were 78.0 ± 8.62 bpm in Group I and 73.22 ± 4.64 bpm in Group II, showing no significant difference ($p > 0.05$) (Table I).

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Table – I: Baseline Characteristics of the Study Population (*n* = 50)

Characteristic	Group I (20–40 years, n = 28)	Group II (41–60 years, n = 22)	p-value
Age (years)	32.4 ± 5.1	48.2 ± 5.3	-
Gender, n (%)			
Male	23 (82.1%)	18 (81.8%)	-
Female	5 (17.9%)	4 (18.2%)	-
Systolic BP (mmHg)	115.93 ± 10.83	114.57 ± 13.89	0.67
Diastolic BP (mmHg)	76.67 ± 8.98	73.91 ± 10.76	0.50
Pulse Rate (bpm)	78.0 ± 8.62	73.22 ± 4.64	0.08

*5% level of significance

Baseline characteristics, including blood pressure and pulse rate, were comparable between the groups.

Regarding clinical presentation in the Table II, rest pain was reported by 92.9% of patients in Group I and 81.8% in Group II, while ulcers were observed in 10.7% and 9.1% of patients in Group I and Group II, respectively.

Smoking was a notable risk factor, with 46.1% of Group I patients and 50.8% of Group II patients identified as smokers; however, the difference was not statistically significant (p = 0.81). Vasoactive drug use was significantly higher in Group I compared to Group II (92.9% vs. 59.1%, p = 0.01), while the use of antiplatelet therapy was more common in Group I (89.3% vs. 68.2%), though the difference was not statistically significant (p = 0.06).

Biochemical investigations revealed that fasting blood sugar, total cholesterol, triglycerides, LDL cholesterol, and serum creatinine levels were higher in Group II than in Group I, but these differences were statistically insignificant (p > 0.05). HDL cholesterol levels were higher in Group I than in Group II, though this difference was also not statistically significant (p = 0.80).

Table – II: Clinical Presentation and Risk Factors between Groups

Variable	Group I (20–40 years, n = 28)	Group II (41–60 years, n = 22)	p-value
Rest Pain, n (%)	26 (92.9%)	18 (81.8%)	0.27
Ulcers, n (%)	3 (10.7%)	2 (9.1%)	0.85
Smoking, n (%)	13 (46.1%)	11 (50.8%)	0.81
Vasoactive Drug Use, n (%)	26 (92.9%)	13 (59.1%)	0.01*
Antiplatelet Therapy, n (%)	25 (89.3%)	15 (68.2%)	0.06
Fasting Blood Sugar (mg/dL)	112.3 ± 10.4	116.2 ± 12.1	0.34
Total Cholesterol (mg/dL)	202.4 ± 19.6	210.6 ± 21.7	0.15
Triglycerides (mg/dL)	132.5 ± 14.8	138.3 ± 16.2	0.21
LDL Cholesterol (mg/dL)	130.4 ± 10.5	135.7 ± 12.3	0.19
HDL Cholesterol (mg/dL)	45.6 ± 5.3	44.9 ± 5.5	0.80
Serum Creatinine (mg/dL) Mean ± SD	0.93 ± 0.12	0.95 ± 0.14	0.56

*5% level of significance

Vasoactive drug use was significantly higher in Group I (p = 0.01). Other clinical presentations and biochemical parameters were comparable between the groups.

Homocysteine Levels

Table III illustrates the comparison of serum homocysteine levels between the two groups. Elevated serum homocysteine levels (>15 µmol/L) were observed in 71.4% of Group I patients compared to 40.9% of Group II patients. The mean serum homocysteine level was significantly higher in Group I than in Group II (21.18 ± 9.53 µmol/L vs. 17.24 ± 8.92 µmol/L, p = 0.02). These findings suggest that elevated plasma homocysteine levels are associated with the development of early-onset atherosclerotic peripheral arterial occlusive disease (PAOD).

Table – III: Comparison of Serum Homocysteine Levels Between Groups (*n* = 50)

Serum Homocysteine (µmol/L)	Group I (20–40 years, n = 28)	Group II (41–60 years, n = 22)	p-value
Elevated (>15) , n (%)	20 (71.4%)	9 (40.9%)	
Normal (5–15) , n (%)	8 (28.6%)	13 (59.1%)	
Mean ± SD	21.18 ± 9.53	17.24 ± 8.92	0.02*

*5% level of significance

Group I had significantly higher serum homocysteine levels compared to Group II, suggesting a stronger association between elevated homocysteine and early-onset atherosclerotic PAOD.

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Table – IV: Risk Factors for Early-Onset Atherosclerotic PAOD (Binary Logistic Regression Analysis)

Variables of interest	Univariate analysis			Multivariate analysis		
	OR	95% CI of OR	p-value	OR	95% CI of OR	p value
Smoking	1.89	0.797-3.538	0.17[ns]	1.59	0.699-3.443	0.16
Dyslipidemia	1.02	0.989-1.431	0.36 [ns]	1.00	0.987-1.132	0.42
Creatinine (mg/dl)	1.07	0.989-1.931	0.36 [ns]	1.00	0.987-1.032	0.42
Hyperhomocysteinemia > 15 µmol/L	2.01	1.142-3.144	0.02[s]	1.77	1.26-3.261	0.03*

*5% level of significance

Hyperhomocysteinemia (>15 µmol/L) was identified as a significant independent predictor of early-onset atherosclerotic PAOD in both univariate and multivariate analyses.

Risk Factors for Early-Onset Atherosclerotic PAOD

Table IV presents the results of univariate and multivariate analyses evaluating risk factors for early-onset atherosclerotic PAOD. In the univariate analysis, hyperhomocysteinemia (>15 µmol/L) emerged as a significant predictor of early-onset PAOD (OR: 2.01, 95% CI: 1.142–3.144, p = 0.02). Multivariate analysis confirmed hyperhomocysteinemia as an independent predictor (OR: 1.77, 95% CI: 1.26–3.261, p = 0.03). Other factors such as smoking, dyslipidemia, and serum creatinine levels were not found to be significant predictors (p > 0.05).

DISCUSSION

This prospective observational study was carried out at the vascular surgery department of the National Institute of Cardiovascular Diseases (NICVD), Dhaka, during the period from January 2014 to December 2014. Patients having histologically documented atherosclerotic PAOD were divided into two groups on the basis of their age; Group I (age 20-40 years) and Group II (age 41-60 years). No previous study could be found looking into the association between hyperhomocysteinemia and early onset atherosclerotic PAOD in Bangladesh. Reports on this subject are also few in published literatures worldwide. A few studies were found in India on hyperhomocysteinemia and peripheral vascular diseases. In these studies, age specification was limited and no study was found dealing specifically with early age.

The mean age of male patients in both groups were 39.8 ± 9.2 and of female patient were 37 ± 8; in total it was 39.4±9.1 years. Analysis revealed statistically insignificant mean age difference between male and female patients. Overall, 41 (82%) patients were male and 9 (18%) patients were female with a male- female ratio of 4.5: 1 In a similar study conducted on atherosclerotic coronary artery diseases by Rahman Md Arifur (2011), the majority of patients were male and male-female ratio was 3.6: 1, which was consistent with this study[10]. In group I, 23 (82.1%) patients were male and 5 (17.9 %) patients were female. In group II, male and female patients were 18 (81.8 %) and 4 (18.2 %) respectively. Hence, the sex distribution of the study patients was almost identical in both groups.

Differences between the two groups in terms of rest pain, ulcer, systolic blood pressure, diastolic blood pressure and pulse were not statistically significant. Comparison of risk factor between the two groups revealed that13 (46.1%) patients were smoker in group I and 11 (50.8%) patients in group II with difference between the groups being statistically insignificant. A study conducted by Mahdieh Momayyezi et al, 2024, showed negative association of smoking and serum lipid levels, which was consistent with this study[11]. Analysis of medications revealed that the use of vasoactive drugs was significantly higher (p=0.01) in group I than in group II (92.9% vs. 59.1%). The study also found that the use of antiplatelet was more in group I than in group II (89.3% vs. 68.2%) but the difference was not statistically significant (p=0.06). The rest of the drugs were used more or less similarly in both groups. Analyses of biochemical parameters demonstrate that fasting blood sugar, total cholesterol, triglyceride, LDL cholesterol and creatinine level were higher in group II than in group I with statistically insignificant difference (p>0.05). HDL cholesterol level was higher in group I than in group II patients with statistically insignificant difference (p=0.80). Even though, existing literature that identifies hyperhomocysteinemia as an independent risk factor for peripheral arterial disease (PAD)[7]. Further research is warranted to explore the potential benefits of homocysteine-lowering interventions in this patient population.

The number of patients with elevated serum homocysteine level was more in group I than in group II (71.4% vs. 40.9%). Likewise, the mean level of serum homocysteine level was also significantly (p=0.02) higher in group I than group II (21.18±9.53 vs. 17.24 ± 8.92 µmol/L). This data suggests a positive association between raised plasma homocysteine and early onset atherosclerotic peripheral arterial occlusive diseases (PAOD). Therefore, the aim of the study which was to evaluate the association between hyperhomocysteinemia and early onset atherosclerotic PAOD was statistically validated. A study previous study showed an elevated plasma tHcy level is now established as a strong and independent factor associated with all categories of atherosclerotic disease in both men and women, which is consistent with this study[12]. This study ascertained smoking, dyslipidemia and serum creatinine as dependent variables of early onset atherosclerotic peripheral arterial occlusive disease (PAOD) was while hyperhomocysteinemia (tHcy>15 µmol/L) was taken as independent variable. The binary logistic regression analysis of Odds Ratio for characteristics of the subjects likely to develop early onset atherosclerotic PAOD. The variables revealed to be significantly associated with early onset atherosclerotic PAOD by univariate analysis were all entered into the model directly. In univariate analysis,

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hyperhomocysteinemia was observed as significant predictor for developing early onset atherosclerotic PAOD with OR being 1.78. It was also observed in multivariate analysis, hyperhomocysteinemia was found to be the independent predictor for developing early on-set atherosclerotic PAOD with OR being 1.22. Smoking, dyslipidemia and serum creatinine were not observed as independent predictors for developing early on-set atherosclerotic PAOD ($p>0.05$). Each individual risk factor (high cholesterol, high triglycerides, low HDL, high LDL, smoking, and diabetes) separately using univariate regression, the study found a statistically significant association between each of these factors and the overall risk of developing the condition being studied, but none of these factors showed a significant association with Peripheral Arterial Occlusive Disease (PAOD), which aligns with the findings of the current research[13,14].

CONCLUSION

In conclusion, this study suggests that serum homocysteine has an association with early onset atherosclerotic PAOD. Therefore, a raised serum homocysteine level can be used as an independent biochemical predictor of early onset atherosclerotic PAOD. This study provided evidence that elevated level of fasting plasma total homocysteine level is not only significantly associated with early onset PAOD but also directly with the severity of PAOD.

Conflict of interest: None.

Source of Fund: Nil.

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