Original Article

Serum Homocysteine Levels in Patients with Ischemic Heart Disease and Normal Individuals: A Comparative Study

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Abstract

Objective: To compare the serum homocysteine levels of patients with ischemic heart disease (IHD) with those of normal individuals (those without ischemic heart disease) in our population.

Methods: This is a case-control study, carried out at CMH Rawalpindi during the course of 6 months from June 2024 to Dec 2024. This study included 200 individuals, among whom 130 were patients with ischemic heart disease (angiographically diagnosed coronary artery disease), within the last 3 months, and 70 normal individuals (those without ischemic heart disease), with the closest matching of baseline characteristics with the cases, which comprised the control group. Individuals having other diseases or medications that could raise serum homocysteine levels were excluded from the study. Fasting serum homocysteine levels were measured in both groups and compared.

Results: Mean serum homocysteine levels in patients with ischemic heart disease were higher (23.12 ± 7.16 µmol/L) as compared to normal individuals (9.60 ± 3.17 µmol/L). This difference was statistically significant, with a p-value less than 0.05.

Conclusion: This study demonstrates that patients with ischemic heart disease have higher serum homocysteine levels as compared to normal individuals, suggesting a potential role of homocysteine in the pathogenesis of ischemic heart disease. These findings highlight the importance of serum homocysteine measurement in the risk assessment and management of ischemic heart disease patients.

Keywords: Coronary artery disease, Homocysteine, Risk factor.

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Introduction

Throughout the world, cardiovascular disease (CVD) is one of the most common causes of premature death and disability.[1] The cardiovascular system encompasses the heart and its blood vessels. CVD consists of these four entities: coronary artery disease (CAD), aortic atherosclerosis, cerebrovascular disease and peripheral artery disease (PAD)[2]. CAD is caused by a reduction in myocardial perfusion, leading to angina due to ischemia and further leading to myocardial infarction and heart failure.[2]

CAD has been responsible for 16.2% of all cause deaths and 7.19% of disability-adjusted life years (DALY) throughout the world in 2019, as per Global Burden of Disease (GBD) data.[3] In Pakistan, CVD incidence has been reported to be 918.18 per 100,000 as per GBD 2019 estimates.[4] CVDs were self-reported by 18.9% of participants in the National Socioeconomic Registry Survey [5]

Different international studies continue to study the impact of various risk factors on cardiovascular disease. These studies classify the risk factors into modifiable and non-modifiable risk factors. Hypertension, hyperlipidemia, diabetes, obesity, poor diet, smoking, stress and sedentary lifestyle are classified as modifiable risk factors. While age, ethnicity, gender and family history of CAD come under non-modifiable risk factors.[6]

Atherosclerosis, which is an important risk factor for CAD, is linked with serum homocysteine levels. Serum homocysteine levels have been implicated as an early atherosclerotic promoter.[7] The different mechanisms by which serum homocysteine acts as a risk factor for CAD are through impaired nitric oxide production and coronary microvascular endothelial dysfunction, which is the earliest detectable form of coronary atherosclerosis [8]

Homocysteine is an amino acid which is biosynthesised from methionine through various steps, followed by two major metabolic pathways, remethylation and transsulfuration. Dysfunction in the enzymes involved in homocysteine biosynthesis leads to increased levels of serum homocysteine. Other than this, increased methionine consumption, some drugs and certain diseases also lead to hyperhomocysteinemia.[9]

Certain vitamins, like vitamin B12, vitamin B6 and folic acid, play a role in the chemical breakdown of homocysteine. Deficiency of these vitamins also causes elevated serum homocysteine levels.[10] MTHFR (Methylenetetrahydrofolate Reductase) is an enzyme involved in folate metabolism and thus maintaining methionine and homocysteine balance. Certain mutations in the MTHFR gene lead to hyperhomocysteinemia and homocystinuria, and therefore an increased risk for CVD.[11]

Various studies have studied this association between elevated serum homocysteine levels and increased risk for CAD and other cardiovascular diseases, establishing serum homocysteine to be an independent risk factor for these diseases. A study that used the SYNTAX score to assess the severity of CAD showed serum homocysteine levels to be significantly higher in patients with a high SYNTAX score.[12] A meta-analysis of 10 studies demonstrated that for every 5umol/L rise in plasma homocysteine levels, the risk of CAD increases by 22%.[13]

The normal serum homocysteine levels range from 5-15umol/L/L. Serum homocysteine levels of more than 15 umol/L are defined as hyperhomocysteinemia.[14] An 8-12-hour fast is preferred before sampling. Medications and vitamin supplements (especially folate and B vitamins) can affect test results.[15] Total homocysteine is quantified after chemical reduction of disulfide-bound forms. The primary accurate measurement method for serum homocysteine is Isotope dilution liquid chromatography-tandem mass spectrometry (ID-LC/MS/MS). .[16]
Our research aims to study the association between serum homocysteine levels and CAD by measuring serum homocysteine levels in patients with angiographically detected CAD and comparing these with serum homocysteine levels of patients without CAD. This will provide evidence of this association in our population so that clinicians can use serum homocysteine as a predictive marker of CAD, and preventive and precautionary measures can be taken in patients with elevated levels.

Materials And Methods

This was a case-control study that was conducted at CMH Rawalpindi after taking approval from the hospital's ethical review committee/institutional research board (ERC letter no.855 CMH Rawalpindi, dated 23/04/2024). The duration of our study was 6 months from June 2024 to December 2024. Sample size calculation was done using the WHO sample size calculator, at a 95% confidence interval and 8% margin of error.
Our sample size was 200 individuals. Out of these 200 individuals, Group 1 included 130 patients with already diagnosed CAD based on angiography (moderate to severe stenosis in one or more major coronary arteries) within the last 3 months, which comprised the case group. Our exclusion criteria were individuals with any other heart disease, chronic kidney disease, COPD, any malignancy or any endocrine disorder. Patients using any vitamin supplements, methotrexate, antidepressants or anti-convulsant drugs were also excluded from the study. Pregnant females or those using oral contraceptive pills (OCPs) were also excluded. Group 2 included 70 healthy individuals from the general population with the closest matching of basic characteristics (age, gender, BMI, smoking status, presence of DM and HTN) with the participants of Group 1, for comparison. This comprised the control group.
In our study, a case to control ratio of 1.85:1 (130 cases and 70 controls) was employed because 1) more patients with ischemic heart disease were available during the study period, 2) recruiting strictly screened healthy individuals posed considerable practical and ethical challenges, and 3) statistical principles affirm that this ratio still provides robust power and precision, while maximizing resource utilization.
Informed consent was taken from all the participants, and their demographic details were obtained. Using aseptic technique, fasting blood samples were collected from all the participants and sent to the Armed Forces Institute of Pathology (AFIP) laboratory for the measurement of serum homocysteine levels. Results were obtained by keeping a record of the lab IDs of all the participants. The reference range of serum homocysteine at the AFIP lab is 5-15 µmol/L.
The statistical analysis of our study was done using the Statistical Package of Social Sciences (SPSS) version 23. Frequency, along with percentage, was calculated for categorised data. Mean, along with standard deviation calculation, was done for continuous variables. In order to determine any statistically significant difference between the serum homocysteine levels of the two groups included in our study, a t-test at 5% level of significance was applied. A *p*-value of less than 0.05 was considered statistically significant for data analysis results.

Results

A total of 74 patients were enrolled in the study, which was divided into two groups. Group A patients underwent Submucosal diathermy, and Group B underwent Partial Inferior Turbinectomy. The mean age of the participants enrolled in the study for groups A and B was 28 and 29, respectively. Figure 1 indicates the distribution of participants based on gender among both groups. Postoperative complications such as haemorrhage and crusting were also recorded among the individuals. 13 participants out of 37 from group A, undergoing submucosal diathermy, were noted to have crusting post-surgery. In comparison, only 8 participants were identified with the same complication in group B. (Figure 2)

Table 1 indicates the preoperative assessment of the patients of both sides from each group—the majority of participants presented with no fogging in both groups, indicating complete obstruction.

Table 1: Basic Characteristics of Cases and Controls

Patient Characteristics	Cases (%age within cases)	Controls (%age within controls)	p-value
Age (in years)	57.03 ± 5.55	56.50 ± 5.76	0.52
Gender			
Male (N)	85 (65%)	42 (60%)	
Female (N)	45 (35%)	28 (40%)	0.53
Smoking (N)	46 (35%)	21 (30%)	0.53
BMI (kg/m2)	30.25 ± 3.82	29.81 ± 4.21	0.45
Hypertension (N)	75 (57%)	36 (51%)	0.45
Diabetes (N)	56 (43%)	28 (40%)	0.76

Serum homocysteine levels were measured in all the participants. The mean serum homocysteine levels in patients with CAD were 23.12±7.16 µmol/L, and in normal individuals were 9.60±3.17 µmol/L. This difference in the serum homocysteine levels of the two study groups was significant with a p-value less than 0.05.

Table 2: Serum homocysteine levels in cases and controls

Study group	Serum homocysteine (µmol/L)	p-value
Cases (Patients with CAD) 23.12 ± 7.16	23.12 ± 7.16	<0.001
Controls (Normal Individuals)	9.60 ± 3.17	

Discussion

Many different studies, both national and international, have studied the relationship of serum homocysteine with different cardiovascular conditions. Our study, which investigated the association between serum homocysteine and risk of CAD, showed a positive correlation between these two variables (i.e serum homocysteine and CAD). This was established by comparing the serum homocysteine levels of patients with CAD with those of the normal individuals, which came out to be 23.12 ± 7.16 µmol/L and 9.60 ± 3.17 µmol/L, respectively.

Our results are comparable to the results of previous studies. A Turkish study that used the SYNTAX score (which is a score to grade the severity of CAD) showed that patients with higher SYNTAX scores (more severe CAD) had higher serum homocysteine levels than patients with lower SYNTAX scores (less severe CAD).[12] In 2022, a meta-analysis of 10 case-control studies on the association between plasma homocysteine levels and coronary heart disease (CHD) was carried out. It showed that for every 5 µmol/L rise in serum homocysteine levels, the incidence of CHD increased by 22%.[13] Another meta-analysis and systematic review of 59 studies showed a significant association between plasma homocysteine levels and coronary artery disease, with greater strength of this association in Asian and African populations than their European and American counterparts [17]

Another prospective study that was carried out on patients with coronary heart disease who underwent stent implantation, in which the study participants were followed for 1 year, showed a greater incidence of major life-threatening cardiovascular and cerebrovascular events in patients with high serum homocysteine levels. Also, the stenosis degree in these patients was comparatively higher than the control group.[18] A Pakistani case control study showed significantly high plasma homocysteine levels, i.e 44.5±14.01 µmol/L in the cases (patients with angiographically diagnosed moderate to severe stenosis in one or more major coronary arteries), than in the control group (6.3±2.05 µmol/L).[19]

Homocysteine is an essential amino acid that contains sulphur. It is formed during the conversion of methionine to cysteine, as an intermediate product. It is remethylated into methionine through pathways in which vitamin B12, vitamin B6 and folic acid act as coenzymes. Homocysteine cannot be obtained from the diet, and the only pathway in humans for the production of homocysteine is from methionine.[20]

Serum homocysteine is a significant biomarker of overall health status, and elevated fasting serum homocysteine levels are associated with various pathological disorders, including renal dysfunction, bone health, neurodegenerative diseases, cognitive impairment, congenital defect development, and as an independent risk factor in cerebrovascular disease and coronary heart disease.[21] High levels of circulating serum homocysteine interfere with low-density lipoprotein (LDL) oxidation, have an impact on endothelial cells lining vessels and have prothrombotic properties, making serum homocysteine an independent and non-traditional risk factor for coronary artery disease.[22]

Given that elevated serum homocysteine is associated with coronary artery and other cardiovascular diseases, factors that aid in reducing serum homocysteine levels should be considered. These include daily supplementation of vitamins like vitamin B12, vitamin B6 and folic acid, the deficiency of which leads to increased serum homocysteine levels.[23]

Although the association between serum homocysteine levels and coronary artery disease has been explored internationally, our study offers distinct contributions. First, it provides recent region-specific data from Pakistan, where large-scale contemporary studies remain limited despite notable lifestyle and dietary changes in recent years. We also applied rigorous inclusion and exclusion criteria- enrolling only clinically confirmed coronary artery disease patients while excluding individuals with other cardiac disorders, CKD, COPD, malignancies, endocrine disorders, and those using vitamin supplements- to minimise confounding factors and strengthen internal validity. Furthermore, our investigation emphasises homocysteine as a modifiable cardiovascular risk factor in a setting where its screening is not routinely implemented. These findings have practical implications for improving risk stratification and guiding targeted nutritional interventions in the Pakistani population.

Conclusions

Serum homocysteine levels are significantly elevated in patients with coronary artery disease (CAD) in comparison to individuals without CAD, establishing the fact that serum homocysteine is a risk factor for CAD and can be used effectively in the monitoring of susceptible individuals. Our study used a small sample size. Studies being conducted subsequently on this topic should use a larger sample size in order to yield more reliable results. Also, the effect of other risk factors of CAD other than serum homocysteine could not be ruled out. Moreover, vitamin supplementation of at-risk individuals and following them with their serum homocysteine levels would also provide an insight into the effect of vitamin supplementation on serum homocysteine levels and also on the chance of further CAD events.

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