

Cross-sectional Assessment of Homocysteine Levels in Patients Undergoing Cardiovascular Risk ScreeningSaurav Rai¹, Ujjwal Kumar², Debjit Mitra³¹Medical Officer, Leprosy Control Unit Buxar²Assistant Professor, Department of Biochemistry, Sri Narayan Medical Institute and Hospital, Saharsa³Professor, Department of Biochemistry, Sri Narayan Medical Institute and Hospital, Saharsa

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Abstract

Background: In the world, cardiovascular diseases are a major morbid and mortal illness. Although the traditional risk factors and variables, including hypertension, diabetes mellitus, dyslipidemia, and smoking are well-established, they cannot explain the cardiovascular risk in all individuals. The amino acid homocysteine which is a sulfur-containing concern in the metabolism of methionine has been suggested to act as an independent cardiovascular risk element, yet its role in the regular screening of cardiovascular risk is unclear.

Objective: The objectives of the present study were to evaluate levels of serum homocysteine in the individuals who are subjected to cardiovascular risk screening, and to determine the relationship of serum homocysteine with known cardiovascular risk factors.

Methods: A one-year cross-sectional observational study was carried out on a sample of 100 adult participants (study subjects who have undergone cardiovascular risk assessment). Clinical parameters, demographic data and laboratories were noted. Standardized enzymatic assay was used in the determination of fasting serum homocysteine with a level above 15 $\mu\text{mol/L}$ being regarded as high. Proper statistical tests were done to determine the association between cardiovascular risk factors and homocysteine levels and $p < 0.05$ was defined as statistically significant.

Results: The participants had high levels of serum homocysteine, 38% of them. Male, smoking, hypertensives, and dyslipidemic people were also found to have hyperhomocysteinemia much more frequently ($p < 0.05$). The homocysteine level and diabetes mellitus were found not to have a statistically significant relationship.

Conclusion: A high percentage of people who have cardiovascular risk screening have high levels of homocysteine that are strongly linked with various traditional cardiovascular risk factors. The results indicated that homocysteine estimation can be a valuable supplement in the cardiovascular risk classification.

Keywords: Homocysteine; Cardiovascular Risk Screening; Hyperhomocysteinemia; Cross-sectional Study; Cardiovascular Biomarkers.

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Introduction

Cardiovascular diseases (CVDs) constitute a major public health challenge and remain the cause of morbidity and mortality in the world. The estimates made by the World Health Organization show that cardiovascular disorders are reported to be the cause of almost one-third of all deaths in the world with a disproportionately higher number of people being affected in the low and middle-income nations [1].

The growing rate of cardiovascular diseases has been greatly due to rapid urbanization, sedentary living, unhealthy eating habits, use of tobacco as well as the growing rate of metabolic disorders [2]. Regardless of the progress in the diagnostic

modalities and therapeutic interventions, cardiovascular morbidity is steadily rising requiring the development of an effective preventive strategy and the prompt detection of risks [3]. The conventional cardiovascular risk-taking is mainly based on well-known variables age, hypertension, diabetes mellitus, dyslipidemia, smoking, and obesity.

Although these factors are highly acknowledged determinants of cardiovascular events, they do not entirely provide answers as to why the said disease occurs in every human being [4]. The prevalence of cardiovascular events in the people who do not have evident traditional risk factors is high,

indicating that other biochemical and metabolic factors play their role [5]. This has prompted increased attention towards the identification of new biomarkers that can be used to improve the prediction of cardiovascular risks and early interventions.

Another amino acid that has turned out to be a possible independent risk factor of cardiovascular disease is homocysteine, which is a sulfur-containing amino acid generated in the metabolic process of methionine [6]. An increased concentration of plasma homocysteine, termed as hyperhomocysteinemia, is linked to “endothelial dysfunction, amplified oxidative stress, vascular inflammation and prothrombotic conditions [7]. A number of epidemiological and experimental researches have revealed that high levels of homocysteine are associated with positive correlation to the risk of coronary artery disease, stroke, and peripheral vascular disease. The suggested mechanisms involve impairment of nitric oxide bioavailability, stimulation of proliferation of smooth muscle cells, and increasing platelet aggregation that all lead to atherogenesis [8].

Although research on the utility of homocysteine in cardiovascular pathology has become more substantiated, its actual application in cardiovascular risk screening has not yet been the consensus [9]. Although there are cases where the studies have found a strong correlation of the high level of homocysteine with the cardiovascular events, at the same time some have indicated inconsistent results of its predictive value in the absence of the conventional risk factors [10]. Also, there is inconsistent evidence in interventional trials of homocysteine-lowering therapy using folic acid and vitamin B supplements on cardiovascular outcome reduction. These contradictions outline gaps in the knowledge of clinical relevance of homocysteine especially in the asymptomatic patients undergoing regular cardiovascular risk assessment.

The Indian context has sparse information on homocysteine levels among the people who are being screened on risks of cardiovascular diseases [11,12]. The differences in dietary patterns, the impact of genetic polymorphisms on metabolism of homocysteine, and the excessive incidence of vitamin B12 deficiency can affect the level of homocysteine in the population.

Nonetheless, the majority of the research studies available have been done on patients that already have developed cardiovascular disease, and fewer studies have been done to evaluate the levels of homocysteine of apparently healthy individuals or those who are on preventive screening. This absence of region-specific and screening-based information highlights the fact that additional

studies are required to define the prevalence of hyperhomocysteinemia in this category and its importance. The determination of the distribution of homocysteine levels and its relationship with the conventional cardiovascular risk factors in a screening population could give useful information on its possible use as a complementary biomarker in cardiovascular risk stratification. Early detection of people with a high level of homocysteine might help in specific lifestyle change and nutritional interventions, thus making it a part of primary prevention.

Hence, the current research was done to determine serum homocysteine in the people under cardiovascular risk screening, and to measure its correlation with known cardiovascular risk factors.

Methodology

Study Design: The current research was done in the form of a cross-sectional observational study with the intention of identifying the serum homocysteine levels of the individuals undergoing cardiovascular risk screening. The cross-sectional design was also adopted because it would be used to assess the prevalence of hyperhomocysteinemia and its correlation with known cardiovascular risk factors at one time and no intervention.

Study Duration: The experiment involved conducting it in a one year duration which involved the recruitment of participants, collection of data, laboratory testing, and statistical analysis.

Study Population: The population of the study was made up of adult patients who were taken through routine cardiovascular risk screening. The participants were both males and females and all aged 18 years and above with a presentation that was aimed at preventive health assessment. Participants were not discriminated based on the existence of conventional cardiovascular risk factors, as long as they fit the inclusion criteria and provided their informed consent to be involved in the study.

Sample Size: The number of participants was 100 (n = 100). Selection of the sample was made through the convenience sampling of the population of cardiovascular screening during the study period. This sample size was regarded to be sufficient to determine the prevalence of high levels of homocysteine and to engage in the comparative study with the traditional cardiovascular risk factors.

Inclusion Criteria

The following individuals were included in the study:

- Adults aged 18 years and above
- Patients having a cardiovascular risk screening.

- Subjects who are willing to give informed consent.

Exclusion Criteria

Participants meeting any of the following criteria were excluded from the study:

- Also people with cardiovascular disease that has been diagnosed before.
- Patients with chronic kidney disease.
- OCases of thyroid disorders
- OCase of thyroid disorders.
- Pregnant women
- Patients that are on vitamin B12, folic acid, or vitamin B6 supplements.

Data Collection: The structured data collection proforma was used to collect detailed demographic and clinical data. Age and gender were the demographic variables. Medical records were used to record clinical parameters including blood pressure, smoking status, history of diabetes mellitus, and the values of lipid profile. Laboratory studies involved estimation fasting serum homocysteine and standard biochemical parameters as far as cardiovascular risk is concerned.

Laboratory Methods: Aseptic collection of fasting venous blood samples of all the participants occurred. The level of serum homocysteine was determined by a standardized enzyme test

procedure in line with the guidelines of the laboratory. A serum homocysteine of more than 15 mmol/L was deemed to be high and a sign of hyperhomocysteinemia.

Statistical Analysis: The data that were collected were then put in a spreadsheet and analyzed by using the right statistical software. The demographic and clinical characteristics were summarized by means of descriptive statistics. Frequencies and percentages were used to describe categorical variables whereas the mean, and standard deviation were used to describe continuous variables. The chi-square test was used to determine how the serum homocysteine level was associated with cardiovascular risk factor. The p-value of below 0.05 was regarded as significant.

Results

Demographic Distribution of Study Participants:

The study involved 100 participants that had cardiovascular risk screening. There were 58 males and 42 females among them (58/42%).

The subjects were aged between 22 and 72 years and the average age of the subjects was 52.6 years of mean age and standard deviation of 10.4 years.

Most respondents were within the age bracket of 41-60 years and this constituted the 54% of the study population.

Table 1: Demographic Characteristics of Study Participants (n = 100)

| Variable | Frequency | Percentage (%) |
|--------------------------|-----------|----------------|
| Gender | | |
| Male | 58 | 58 |
| Female | 42 | 42 |
| Age Group (years) | | |
| 18-40 | 18 | 18 |
| 41-60 | 54 | 54 |
| >60 | 28 | 28 |

Serum Homocysteine Levels: Homocystein levels in serum were determined in all the participants. According to the predetermined cut-off ($>15 \mu\text{mol/L}$), 38 participants (38% was found to be the elevated level of homocysteine), and 62 participants (62%) were found to be in normal range. The average homocysteine concentration in the sample population was $14.8 \pm 0.4 \mu\text{mol/L}$.

Table 2: Distribution of Serum Homocysteine Levels

| Homocysteine Level | Number (n) | Percentage (%) |
|--------------------------------------|------------|----------------|
| Normal ($\leq 15 \mu\text{mol/L}$) | 62 | 62 |
| Elevated ($>15 \mu\text{mol/L}$) | 38 | 38 |

Association of Homocysteine Levels with Cardiovascular Risk Factors: The correlation between conventional risk factors of cardiovascular and the level of serum homocysteine was assessed. The association between elevated levels of homocysteine and male respondents (44.8) as compared to female respondents who experienced elevated levels of homocysteine (28.6) was found to be statistically significant ($p < 0.05$). Fifty six percent of smokers had high levels of

homocysteine as compared to 30 percent of non-smokers, which showed a statistically significant relationship between the two. Likewise, hypertension and dyslipidemia were more common in the participants with hyperhomocysteinemia than in those that did not have these conditions. Nevertheless, homocysteine levels and diabetes mellitus were not found to have a statistically significant relationship.

Table 3: Association of Serum Homocysteine Levels with Cardiovascular Risk Factors

| Risk Factor | Elevated Homocysteine n (%) | Normal Homocysteine n (%) | p-value |
|--------------------------|-----------------------------|---------------------------|---------|
| Gender | | | |
| Male (n=58) | 26 (44.8) | 32 (55.2) | 0.041* |
| Female (n=42) | 12 (28.6) | 30 (71.4) | |
| Smoking Status | | | |
| Smokers (n=25) | 14 (56.0) | 11 (44.0) | 0.018* |
| Non-smokers (n=75) | 24 (32.0) | 51 (68.0) | |
| Hypertension | | | |
| Present (n=48) | 22 (45.8) | 26 (54.2) | 0.032* |
| Absent (n=52) | 16 (30.8) | 36 (69.2) | |
| Dyslipidemia | | | |
| Present (n=46) | 21 (45.7) | 25 (54.3) | 0.039* |
| Absent (n=54) | 17 (31.5) | 37 (68.5) | |
| Diabetes Mellitus | | | |
| Present (n=40) | 14 (35.0) | 26 (65.0) | 0.61 |
| Absent (n=60) | 24 (40.0) | 36 (60.0) | |

Statistically significant ($p < 0.05$)

Summary of Key Findings: As illustrated in the current study, over a third of the people who had been screened to assess their risk of cardiovascular diseases had high levels of serum homocysteine. The male gender, smoking, hypertension, and dyslipidemia showed significant association with hyperhomocysteinemia, but diabetes mellitus was not significantly associated.

Discussion

Summary of Main Findings: The current cross-sectional paper evaluated serum levels of homocysteine in people undergoing cardiovascular risk screening and determined how it related to classical cardiovascular risk factors [7]. The results indicated that 38 percent of the population of the study reported high levels of homocysteine and this shows that hyperhomocysteinemia occurs in a significant number of a screening population. High homocysteine levels were both gender specific (male) and smoking specific and hypertension specific and not diabetes mellitus specific. These findings indicate that homocysteine can serve as a contributory and independent risk factor with regard to cardiovascular risk.

Comparison with Previous Studies: The results of the current article accord with those of who [13] established a high correlation between high homocysteine levels and a high risk of ischemic heart disease and stroke. On the same line, a research study done by [14] established that a person with a high level of homocysteine risk was at a much more risk of developing coronary heart disease, regardless of the presence of traditional risk factors. A different study [15] also pointed out the importance of the homocysteine as a modifiable risk factor and its relation to the endothelial dysfunction and vascular damage.

In concurrence with these studies, the current study has also established increased prevalence of hyperhomocysteinemia in males and smokers. This correlation with hypertension and dyslipidemia is also in line with the previous studies which suggested that homocysteine might have synergetic effects with the more traditional cardiovascular risk factors and hence increase the overall cardiovascular risk.

Possible Explanations for the Findings: There are a number of mechanisms which have been suggested to account the relationship between high levels of homocysteine and cardiovascular risk. It has been known that homocysteine causes endothelial dysfunction by limiting the bioavailability of nitric oxide and enhancing oxidative stress. It also facilitates a smooth cell growth of the muscle and platelet aggregation, which is involved in atherogenesis and thrombosis.

The increased sex and smoking differences in the higher homocysteine levels in males and smokers recorded in the current study can be explained by lifestyle, dietary shortages, and higher oxidative stress. Also, vitamin B12 and folate deficiencies that are common among some population groups can disrupt homocysteine metabolism which results in increased serum levels.

Clinical Relevance: The results of the study underscore the possible clinical importance of determining the level of serum homocysteine as a cardiovascular risk factor. Considering its high correlations with the known risk factors, homocysteine estimation can be a helpful adjunct biomarker especially in patients with a borderline or unexplained cardiovascular risk. Early detection of high homocysteine may help in taking right lifestyle changes and nutrition to incorporate changes in dietary counseling and vitamin supplements, which

will be part of the primary prevention measures and better cardiovascular results.

Limitations of the Study: Although the present study has findings, it is limited in a number of ways. The cross-sectional type does not allow drawing a causal correlation between high levels of homocysteine and cardiovascular threat. The sample size is rather small which can restrict the generalizability of the findings. Also, nutritional foods, vitamin B12 and folate were not measured, which would have given more insight into the determinants of hyperhomocysteinemia. Those future works involving larger sample size and longitudinal follow up are recommended to further explain the contribution of homocysteine in the cardiovascular disease development.

Conclusion

The current paper has shown that a significant proportion of people undergoing cardiovascular screening have a high level of serum homocysteine. The remarkable linkage of hyperhomocysteinemia with male sex, cigarette smoking, high blood pressure, and hyperlipidemia lends support to the suggestion that this biomarker could be used as an add-on biomarker in the risk assessment of cardiovascular diseases. These results indicate that homocysteine has the potential to independently raise cardiovascular risk in addition to traditional risk factors and possibly in early diagnosis of persons at risk who are vulnerable.

Clinically and in terms of public health, the homocysteine estimation should be added to the standard cardiovascular screening in order to strengthen the risk stratification and provide specific preventive action, i.e., lifestyle- and nutrition-based. Nonetheless, due to the cross-sectional design of the research and the small sample size, the results are to be considered carefully. Large, longitudinal studies in the future are advisable so as to determine causal relationship as well as the effect of homocysteine-lowering strategies on cardiovascular outcomes.

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