Cardiovascular Diseases Risk Prediction by Homocysteine in Comparison to other Markers: A Study from Madhya Pradesh

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Abstract

Introduction: Atherosclerosis is associated with increased inflammatory activity and risk of vascular disease. However, the factors that promote inflammation are not apparently clear. In the present study we aimed to evaluate levels of homocysteine in comparison with other markers including inflammatory risk predictor of cardiovascular disease.

Methods: A total of 200 patients were included in the study, of these 100 were angiographically proven cardiovascular disease patients and 100 were not suffering from any cardiovascular disease. Levels of homocysteine, hs-CRP, lipoprotein(a), HbA1c and blood lipid were measured along with anthropometric and demographic parameters.

Results: Levels of homocysteine and other markers were significantly increased in diseased group in comparisons to control.

Conclusion: There is a very high sensitivity, specificity, and accuracy with 89% positive predictive value and 39.48 odds ratio for homocysteine in cardiovascular patients when compared to other risk factors.

Introduction

WHO has projected that cardiovascular disease (CVD) will become the greatest cause of morbidity and mortality in the world by the year 2015; and it is expected that Indians would be the most affected amongst all ethnic population.1 CVD rates among Asian Indians worldwide 50% to 400% higher than people of other ethnic origin. Asian Indians, compared with other subpopulation are at more risk for developing CVD and diabetes at a younger age.2 The prevalence of standard risk factor in Indian seems to be negated by a higher prevalence of several emerging risk factors.3 Therefore a more aggressive approach to prevention and treatment of both conventional and emerging risk factors is warranted in Asian Indians.2

Focus on newer factor is warranted as they may further improve our ability to predict future risk and to determine treatment when they are included along with conventional one. These newer risk factors are called “novel risk factors” which includes homocysteine (Hcy), hs-CRP, Lp(a) and HbA1c.

Several studies have demonstrated elevated levels of Hcy is an important cause for atherosclerosis.4,5 The adverse effects of Hcy, involves alteration of endothelial function by induction of oxidative stress, impaired generation of nitric oxide,6 decreased vasodilatation and increased proliferation of smooth muscle cells.7 Elevated plasma Hcy is also associated with lipid peroxidation.8 According to Chamber et al elevated plasma Hcy level is an independent risk factor for coronary artery disease (CAD) in Asian Indians compared to Europeans.9

A recent study by Nair et al reported that (MTHFR) gene mutation causing hyperhomocysteinemia is a risk factor for increased risk of CAD in Indians.10 However the results on...
hyperhomocysteinemia in CAD has been conflicting as several other studies failed to demonstrate an association between Hcy and CVD in Indians.\textsuperscript{11} Thus the evidence of increased plasma Hcy, as an independent risk factor in Indian community is not clearly understood. Therefore the aim of present study was to evaluate the relationship between the homocysteine levels and CVD in Central Indian population. We compared conventional and inflammatory risk factors with homocysteine in CVD patients to find the prediction rate of coronary heart disease (CHD).

**Material and Methods**

The study was case-controlled in design. We selected the patients as they presented. Patients included were all admitted to the ICCU of Chirayu Hospital Bhopal or attending the outpatient department of medicine at M.Y. Hospital attached to M.G.M. Medical College, Indore.

Consecutive 100 patients undergoing coronary angiography at our hospital over a period of 1 year, were included in the study. The diagnosis of CVD was made on the basis of clinical history and 12-lead standard electrocardiogram (ECG) before subjecting them to coronary angiography. The presence of any diameter stenosis ≥30% according to coronary angiography by visual assessment of coronary artery was included in the study.

Age and sex matched 100 subjects along with BMI were selected as control from Medicine OPD and Blood Bank with no history of CVD or had normal electrocardiogram (ECG) therefore not subjected to angiography. The name, age, sex, occupation and clinical history were taken on proforma. Previous histories of diabetes, smoking, hypertension were noted, subjects with proven nephropathy has been excluded. Fully informed consent was obtained from patients and controls of both groups.

All blood specimens were drawn at 8:00 am after 12 hour fast. Sample were centrifuged within 1 hour and frozen immediately at -20°C.

In our study smoking was defined as a regular smoking of cigarettes/beedies. Body mass index was calculated (kg/m\(^2\)) and blood pressure was measured with the person in the sitting position after 5 min rest. A participant was defined as having hypertension if systolic blood pressure was 160 mmHg or more, if the diastolic blood pressure was 95 mmHg or more or if the participant receiving drug treatment for hypertension. Diabetes mellitus was considered present in patients with a known history of diabetes and in patients with fasting glucose ≥126 mg/dl (7.0 mmol/L) according to the American Diabetic Association Criteria. A positive family history of CVD was defined as first-degree relative that had documented CVD <55 years in males or <65 years in females. For lipid analysis, samples were obtained after an overnight fast. Those patients whose body mass index is ≥25 kg/m\(^2\) were considered as obese. Patients who had serum concentration of total cholesterol (TC) ≥240 mg/dl, or triglyceride (TG) ≥300 mg/dl, or low-density lipoprotein cholesterol (LDL-C) ≥160 mg/dl or high-density lipoprotein cholesterol (HDL-C) ≤40.0 mg/dl or very low-density lipoprotein cholesterol (VLDL-C) ≥40.0 are considered as hyperlipidemics. Homocysteine, hs-CRP, Lp(a) levels ≥15.0 µmol/L, ≥30.0 mg/dl, ≥30 mg/dl were considered as higher or increased risk respectively.

Exclusion criteria included the use of aspirin, S-adenosylmethionine, vitamin supplementation, alcohol, anticonvulsant, estrogens, lipid lowering therapy, other infectious disease and medication that might affect hs-CRP, Lp(a) and homocysteine metabolism.

For assaying each parameter we used commercially available analytical system.

Lipid profile done on fully automatic analyzer using a) total cholesterol estimated by enzymatic, CHOD/PAP method, supplied by Roche Diagnostic Ltd., b) triglyceride estimated by enzymatic, GPO/PAP method, supplied by Roche Diagnostic Ltd., c) high density lipoprotein estimated by enzymatic, CHOD/PAP method, supplied by Roche Diagnostic Ltd., d) low density lipoprotein estimated by enzymatic, CHOD/PAP method supplied by Roche Diagnostic Ltd. and e) very low density lipoprotein estimated by enzymatic, CHOD/PAP method-supplied by Roche Diagnostic Ltd.

Fasting blood sugar estimation done on fully automatic analyzer by using enzymatic assay kit.

Homocysteine, hs-CRP and Lp(a) done by ELISA.

To ascertain the potential clinical usefulness of each putative risk marker we followed a priori analysis plan. Results are represented as mean ±SD. Student ‘t’ test and Pearson’s correlation analysis were performed in statistical evaluation. The diagnostic statistics namely, sensitivity, specificity, positive predictive value, accuracy, odds ratio were calculated for finding the diagnostic value of CHD risk factors. P values were computed using chi square distribution.

**Results**

The basic characteristics of the CVD patients and controls were given in Table 1. There were 100 controls with 69 (69%) males and 31 (31%) females. The control group had 29 diabetic, 26 obese and 12 with habit of smoking. The mean homocysteine is 6.53±1.59 µmol/L. In CVD patients 49 were diabetic, 51 were obese and 42 were smokers. The mean homocysteine was 16.06±3.34 µmol/L. For better analysis CVD patients were divided in to smokers and non smokers.
The mean ± SD value for Hcy in smokers and non-smokers was 16.07±1.31 and 16.06±1.38 µmol/L, there is no significant difference found between. Statistically significant increase in common risk factors are seen in CVD patients. Homocysteine was also increased significantly (p<0.001) in plasma of CVD patients when compared to control.

The diagnostic values for CVD patients for each of these conventional risk factors along with homocysteine were calculated statistically (Table 2).

Homocysteine showed the highest sensitivity (83%), specificity (89%), positive predictive value (88.29%), negative predictive value (84%), and accuracy (86%) when compared to other risk factors. The odds ratio for homocysteine was 39.48 which was highest when compared to others.

**Discussion**

Homocysteine is sulphur-containing amino acid derivative of dietary methionine, it has been associated with CVD. More than 75 clinical and epidemiological studies has shown a relation between elevated Hcy levels and coronary vascular disease, peripheral artery disease, stroke or venous thrombosis. The association of raised Hcy and thrombosis was reported by Mc Cully and Study of Alfinan G et al found a strong relation between increased Hcy levels and increased frequency of vascular disease. Several recent studies investigated the contribution of homocysteine to CVD risk both among immigrant Indians and those living in India. Chambers et al reported that elevated plasma homocysteine were independently associated with CVD in UK Indians. A population based study from Canada reported that South Asians had higher plasma homocysteine than European counterparts. Boushy et al showed homocysteine as an independent graded risk predictor for atherosclerosis disease in coronary, cerebral and peripheral vessels.

Recently Nair et al reported that methylenetetrahydrofolate reductase (MTHFR) gene mutation causing hyperhomocysteinemia as a risk factor for CVD. There are other studies from South India showing the association of homocysteine with CAD risk. In contrast to these studies there are some other studies showing negative relation. The limitations in these studies could be the numbers, to detect the significant differences between the study populations.

In our study the main modifiable risk factors considered were diabetes, hypertension, obesity and smoking other constitutional risk factors like age, family history of CVD were also compared between diseased and control group along with lipid profile and inflammatory markers.

Our data show a statistically significant increased Hcy levels in CVD patients compared to controls (Table 1), patients with diabetes, hypertension, smokers are significantly higher in diseased group; it could be the confounding factor for elevated Hcy levels but there is no significant difference found between CVD smokers and nonsmokers Hcy levels. Both have increased concentration of Hcy but some previous study had shown increased Hcy levels in smokers therefore it could be a potential factor. Diseased group is having significantly different lipid and inflammatory parameters in comparisons to control.

### Table 1: Risk factor distribution

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Group I controls n = 100</th>
<th>Group II CVD n = 100</th>
<th>P value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>45.29 ± 10.21</td>
<td>60.84 ± 10.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>23.2 ± 1.9</td>
<td>23.6 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>BPS (mmHg)</td>
<td>133.74 ± 10.48</td>
<td>130.82 ± 8.41</td>
<td>&lt;0.01</td>
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<td>BFD (mmHg)</td>
<td>79.58 ± 6.53</td>
<td>84.99 ± 6.35</td>
<td>&lt;0.20</td>
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<td>FBG (mg/dl)</td>
<td>110.29 ± 41.74</td>
<td>129.81 ± 49.00</td>
<td>&lt;0.01</td>
</tr>
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<td>TC (mg/dl)</td>
<td>156.95 ± 27.68</td>
<td>271.41 ± 31.14</td>
<td>&lt;0.01</td>
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<td>TG (mg/dl)</td>
<td>175.19 ± 66.91</td>
<td>226.16 ± 78.59</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>42.24 ± 8.54</td>
<td>28.96 ± 9.69</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>101.08 ± 27.95</td>
<td>158.68 ± 30.19</td>
<td>&lt;0.20</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>33.32 ± 11.39</td>
<td>44.31 ± 16.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hcy (µmol/L)</td>
<td>6.53 ± 1.59</td>
<td>16.06 ± 1.34</td>
<td>&lt;0.01</td>
</tr>
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<td>HBA1c (%)</td>
<td>6.14 ± 1.33</td>
<td>7.03 ± 2.30</td>
<td>&lt;0.02</td>
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<td>Lp(a) (mg/dl)</td>
<td>17.25 ± 8.03</td>
<td>50.11 ± 36.13</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>hs-CRP (mg/dl)</td>
<td>0.438 ± 0.317</td>
<td>1.11 ± 1.41</td>
<td>&lt;0.02</td>
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</table>

### Table 2: Comparison of diagnostic values

<table>
<thead>
<tr>
<th>Diagnostic values for CVD</th>
<th>FBS Sensitivity (%)</th>
<th>BMI Specificity (%)</th>
<th>Smoking PPV (%)</th>
<th>Hcy Accuracy (%)</th>
<th>HBA1c NPV (%)</th>
<th>Lp(a) - OR</th>
<th>hs-CRP - OR</th>
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<td>49</td>
<td>71</td>
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<td>84.00</td>
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<td></td>
<td>28</td>
<td>95</td>
<td>84.84</td>
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<td>61.50</td>
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</table>

sensitivity, specificity, positive predictive value, negative predictive value, accuracy and odds ratio were compared (Table 2) between these risk factors in CVD patients. Hcy was found to be with highest sensitivity, specificity, positive predictive value, negative predictive value, accuracy and odds ratio therefore it emerged as a best predictor of CVD risk.

Result of our study shows a significant raise in levels of hs-CRP, Lp(a) and HbA1c in diseased group as compared other, of them hs-CRP and Lp(a) were secondary for diagnostic value and prediction of CVD risk. But the most important outcome of this study is hs-CRP, Lp(a) and Hcy are marker of inflammation in CVD and this is supported by several studies. Thus results suggest that there is inflammatory activity and it could be a cause of CVD risk.

Good diagnostic value of HbA1c with an odds ratio of 14.1 suggests that there is a positive association of HbA1c with increased CVD risk.

In conclusion, our data suggests that plasma homocysteine levels were increased significantly in CVD patients when compared to controls and also homocysteine is the best predictor of CHD risk amongst other conventional risk factor in CVD patients.

References