INTRODUCTION

Atherosclerosis is the leading cause of death in the developed world. In Pakistan; the disease seems to follow an accelerated course with ischaemic events occurring a decade earlier than those reported worldwide. While many risk factors of cardiovascular diseases are well established, many are still under evaluation; like homocysteine, which is now being considered an independent risk factor for cardiovascular diseases.

Homocysteine is a sulfur containing amino acid which is not our dietary constituent but is formed by metabolism of methionine, another amino acid present in our daily protein diet. In case of excess formation of homocysteine as compared to its consumption, it appears in urine if levels are too high. Normal plasma homocysteine concentration ranges from 5 to 15µmol/litre. Of this, almost 75% is bound to proteins, especially albumin, through disulfide bond. Classification of hyperhomocysteinemia described by Kang SS is followed widely.

Moderate hyperhomocysteinemia (15 to 30 µmol/litre)
Intermediate hyperhomocysteinemia (30 to 100µmol/litre)
Severe hyperhomocysteinemia (>100 µmol/litre)

Homocysteine is thought to promote atherosclerosis by affecting the coagulation system and thromboreistance of endothelium. It may also influence the vasodilator and antithrombotic effects of nitric oxide.

Yet some researchers have shown that elevation of total homocysteine is equivalent to acute phase protein rise and is not a true risk marker. Acute phase reactant proteins like C – Reactive proteins, however, are known to settle within 3–4 days of the acute event. This debate is ongoing, and two schools of thoughts - one, that homocysteine is a culprit; and the other, that it is an innocent bystander - are present in the scientific community. Similarly we are also facing differing opinions regarding need to treat hyperhomocysteinemia.

If hyperhomocysteinemia is proved to be an independent risk factor and not an innocent bystander, it can be lowered pharmacologically by vitamin B6 or vitamin B12 and folic acid administration. Therefore we conducted a study to discover a causal relationship between homocysteine and coronary artery disease and also see whether total homocysteine level is an acute phase reactant marker or a true representative risk factor.
OBJECTIVES

The main objectives of this study were to:

1. Compare the total homocysteine levels of patients and of controls to define a causal association of homocysteine to coronary artery disease.

2. Assess whether total homocysteine level is an acute phase reactant or a true representative risk factor.

MATERIALS AND METHODS

It was an experimental case controlled study carried out in the medical departments of two tertiary care hospitals in Lahore from September 2009 to August 2010. All the patients with acute myocardial infarction (MI) (between 18-80 years) were included in this study. Acute MI diagnosed on 2 out of 3 criteria of (I) typical ischemic chest pain of more than half an hour, not relieved by sublingual nitrates, (II) ECG suggestive of acute MI, or (III) serially elevated cardiac enzymes.

All patients were given detailed information about the aim of the study, and all gave written informed consent for participation. No restrictions were made as to the age, sex and severity of disease when patients were chosen. Asymptomatic unrelated individuals from community, of the same age and sex, preferably of same socioeconomic status, were selected as controls.

Exclusion criteria were: Patients on drugs like methotrexate, carbamazepine, phenytoin, septran, levodopa, vitamin B6, B12, folic acid or metformins; patients with known malignancy like Acute Lymphoblastic Leukemia, carcinoma - breast, pancreas or ovary; known chronic liver disease patients; patients with GFR less than 50% and known hypothyroid patients. Same exclusion criteria were used for the selection of control subjects.

Sixty participants in total; thirty patients of acute MI with same number of healthy controls were included in this study. Sampling for measurement of serum homocysteine levels was done in sitting position and in a fasting condition. Non-haemolysed clotted samples were properly centrifuged at 1000 x g for 10 minutes within 6 hours of collection and were sent for analysis on ice pack.

Apart from complete history and physical examination, all baseline investigations like haemoglobin, Total leukocyte Count (TLC), Differential Leukocyte Count (DLC), Serum (S)/Na+, S/K+, Prothrombin time (PT), Activated partial thromboplastin time (APTT), S/albumin, Liver function test (LFTs), Blood (B)/urea, serum creatinine, blood sugar, along with 12 lead ECG and cardiac enzymes (CK-MB, Troponin T, serum LDH were carried out in all subjects.

Statistical analysis

All the data obtained was entered on the spread sheet of SPSS version 17. Day one and day seven homocysteine levels of all the cases were compared by paired t-test. Comparison of numerical variables (age, blood urea, serum creatinine and blood pressure) between cases and controls was done by independent sample t-test. A p value of < 0.05 was taken as significant.

RESULTS

Sixty participants in total; thirty patients of acute MI with same number of healthy controls were included in this study. Out of these, 39 were males and 21 were females. Baseline clinical characteristics including age, sex, diabetic and hypertensive status, smoking and s/creatinine levels of the patients and control group showed no statistically significant difference (Table 1). Among the comparisons of total homocysteine levels with other variables like hypertension, diabetes mellitus, smoking and post MI angina, only smoking turned out to have significant association with elevated total homocysteine levels in healthy asymptomatic controls only (Table 2).

Comparison of patients’ day 1 total homocysteine levels (Mean ± SD 12.99 ± 3.51 µmol/liter) with that of controls (Mean ± SD 13.47 ± 4.44 µmol/litre) showed no statistically significant difference (Table 3). Frequency of hyperhomocysteinemia was higher in patients group only on 7th day (43.3%) as compared to controls (33.3%).

There was an average of 14.8% rise in total homocysteine on 7th day of the coronary event in patients of acute MI. This rise in total homocysteine was statistically significant when patients’ day 1 (Mean ± SD: 12.99 ± 3.51 µmol/litre) values were compared to their day 7 levels (Mean ± SD 14.92 ± 4.54 µmol/litre) (Table 4).

Table 1: Baseline characteristics of patients and controls at study entry

<table>
<thead>
<tr>
<th>Age (Mean+/− Std. Dev.)</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>51.4 ± 7.7</td>
<td>51.1 ± 6.7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Diabetic</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Smokers</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Creatanin Clearance (Mean)</td>
<td>109*</td>
<td>113*</td>
</tr>
</tbody>
</table>

*P value for the difference = 0.276
We analysed our data to testify the association of homocysteine with coronary artery disease in our population. We compared controls’ homocysteine values with patients’ homocysteine values on day 1 and day 7 separately. None of the comparisons reached the statistical significance to prove an association. So we report a lack of association of homocysteine with coronary artery disease in population. These results in this regard are in agreement with some international studies\textsuperscript{12, 13} and two studies from Pakistan\textsuperscript{14, 15}. However, smaller sample size and case control design of the study warrants further evaluation of this hypothesis in a larger scale prospective study.

This study revealed a statistically significant variation in homocysteine levels in patients of acute MI between day 1 and day 7 of the disease event i.e. mean homocysteine levels increased by 14.8% from day 1 to day 7 (12.99 ± 3.51 to 14.92 ± 4.54 µmol/litre). These results coincide with the available pool of data in international literature. Egerton et al. in their study on Australian population reported 25% difference in homocysteine levels between day 1 and day 7 in patients of acute MI. They found this elevation to be persistent till 180 days of follow up\textsuperscript{16}. Al-Obaidi et al. noted a small but statistically significant difference in homocysteine values between day 2 and day 7 in patients of acute MI. Those with unstable angina showed no significant variation in homocysteine levels over time\textsuperscript{17}.

### Table 2: Hyper-homocysteinemia (>15 µmol/litre) according to diabetic, hypertension and smoking status

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;15 µmol/litre</td>
<td>&lt;15 µmol/litre</td>
<td>&gt;15 µmol/litre</td>
<td>&lt;15 µmol/litre</td>
</tr>
<tr>
<td>Smokers</td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Non Smokers</td>
<td>2</td>
<td>13</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>3</td>
<td>14</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Non Hypertensive</td>
<td>2</td>
<td>11</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Diabetic</td>
<td>2</td>
<td>10</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Non-Diabetic</td>
<td>3</td>
<td>15</td>
<td>5</td>
<td>13</td>
</tr>
</tbody>
</table>

### Table 3: Comparison of hyper-homocysteinemia between patients of acute MI and controls at day 1

<table>
<thead>
<tr>
<th>Serum tHcy levels</th>
<th>&gt;15 µmol/litre</th>
<th>≤15 µmol/litre</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>05 (16.7 %)</td>
<td>25 (83.3 %)</td>
<td>12.991</td>
<td>3.507</td>
<td>0.664</td>
</tr>
<tr>
<td>Controls</td>
<td>10 (33.3 %)</td>
<td>20 (66.7 %)</td>
<td>13.471</td>
<td>4.444</td>
<td>0.664</td>
</tr>
</tbody>
</table>

### Table 4: Comparison of hyper-homocysteinemia on day 1 and day 7 in patients of acute MI

<table>
<thead>
<tr>
<th>Serum tHcy levels</th>
<th>&gt;15 µmol/litre</th>
<th>≤15 µmol/litre</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients at day 1</td>
<td>05 (16.7%)</td>
<td>25 (83.3%)</td>
<td>12.991</td>
<td>3.507</td>
<td>0.001</td>
</tr>
<tr>
<td>Patients at day 7</td>
<td>13 (43.3%)</td>
<td>17 (56.7%)</td>
<td>14.918</td>
<td>4.542</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Succu et al. noted a statistically significant difference between homocysteine levels on 24th hour of admission and day 7. In all of these studies, however, variation was not reaching 25% figure to make homocysteine an acute phase reactant protein by definition. Same is the situation in this study which favours a rise on 7th day homocysteine levels but not beyond 25%. Al-Obaidi et al. and Succu et al. followed their patients till six months and three months, respectively, and both found that homocysteine levels on admission were comparable with that in convalescent phase. Our study, however, lacked these follow up observations that could be of help in elaborating these serial changes in our patients.

In Pakistan, various studies have given different results about its association with coronary artery disease. However, none of the studies has addressed the issue of acute phase variation of homocysteine in patients of acute MI, which could disturb the interpretation of results in a diseased population. Results of our study differ in homocysteine values of patients from those in other studies reported from Pakistan in previous years. Interestingly none of previous studies has mentioned the number of days passed after the acute event when blood samples were taken from patients for homocysteine analysis. This study shows lower homocysteine levels in patients group than in all studies in Pakistan.

Much higher values in patients in other studies may be explained if the samples might have taken after few days of acute event when levels are said to be higher than the actual. However, as the difference between patients’ homocysteine values in this study and others’ is more than 15%, i.e. the difference we observed between day 1 and day 7 values in our population, this may only be a partly explaining fact for this observation. Nevertheless, 7th day mean homocysteine level in patients (14.92±4.54µmol/litre) in this study coincide well with that of one previous study by Salahuddin et al. in which they reported mean homocysteine level in patients as 14.97 ± 1.13 µmol/litre.

Regarding homocysteine value in control subjects, mean homocysteine value (13.47 ± 4.44 µmol/litre) in this study is lower than those reported in two local studies; one from Rawalpindi by Akhtar et al. (19.09 ± 7.68 µmol/litre) and the second from Karachi by Iqbal et al. (16.4 ± 4.9 µmol/litre). Contrarily, value in this study in control group is higher than those reported by two other studies; one from Karachi by Salahuddin et al. (11.87 ± 1.55 µmol/litre) and other from Rawalpindi by Aamir et al. (10.8 ± 4.1 µmol/litre). The lack of accord in homocysteine values in asymptomatic populations in studies reported within each city is surprising. These differences, including this study’s variations from others’ results, may be explained by difference in vitamin and folate status of the populations studied. Only one study in Pakistan by Iqbal et al. has concomitantly measured serum folate and vitamin B12 levels in patients and control groups.

They have documented an insignificantly lower folate and vitamin B12 levels in patients as compared to control group. Taking their data as reference for the time being, even Pakistani healthy populations appears to be deficient in folate (4.93 ± 2.93nmol/litre, control group as measured by Iqbal et al.) when we compare folate levels to values in the Hordaland Homocysteine follow up study by Nurk et al. 21 (6.7 ± 0.07 to 7.8 ± 0.10 nmol/litre in different age groups). Corresponding homocysteine levels were 8.8 to 11.9µmol/litre in different age groups in that study, whereas Iqbal et al. reported mean homocysteine in controls as 16.4 ± 4.9 µmol/litre.

Our study lacked these measurements which might explain this possible underlying reason for within country variability of observations. But considering socioeconomic status of our patients, we suspect it to be a stronger factor and we suggest that further studies on homocysteine be carried out along with folate and vitamin B12 levels in Pakistani population. This issue will be more important in countries where most of the population is vegetarian.

We also extend this thought to the phenomenon of acute phase variation of homocysteine in patients of acute MI. During hospital stay, restricted dietary intake may provide less vitamins and folate to patients thus raising their homocysteine levels on seventh day. We suggest a further study with folic acid supplementation on day 1 in these patients to see its effect on homocysteine rise on seventh day.

Association of homocysteine with other factors was also analysed in both patients and control groups. As documented by Al-Obaidi et al. and Succu et al., that homocysteine levels on admission are better representative of true homocysteine status of the patient. We used patients’ day 1 values for these comparisons. We analysed the association of homocysteine with smoking, hypertension, diabetes mellitus in both patients and control groups, and with post MI angina in patients group.

Smoking showed a statistically significant association with elevated homocysteine in our population in asymptomatic controls only. This is in agreement with Aamir et al. and Iqbal et al. in Pakistan, and with Bazzano et al. and O’Callaghan et al. who reported the same observations. This study however showed no association of elevated homocysteine levels with smoking in patients of acute MI contrary to the above mentioned studies. This observation however needs further evaluation owing to the small sample size of this study.

This study underscores the importance of consistency in taking blood sample from patients on a particular day after an acute event and it should be mentioned while reporting the study so that comparison between studies may not be biased by this important effect of homocysteine concentration. We emphasise the need for a further large, multicentre study about homocysteine in patients with acute MI, with a longer follow-up and special emphasis on folate and vitamin status along with other major risk factors, and also noting ethnic and racial background of patients and controls to know whether these elevated levels settle in our population in the same way as reported in international literature.

An alternative outcome may imply an abnormal vascular response to homocysteine in our population, irrespective of homocysteine being a cause or consequence of the disease. This will help establish or refute the role of homocysteine as a risk factor in this multi-racial nation already burdened with many major risk factors for cardiovascular diseases.

Until unequivocal evidence on the benefit of vitamin supplementation is proved, balanced diet will be the best advice for reducing CVD risk associated with hyperhomocysteinemia. As various studies are ongoing and as this is a topic currently in debate worldwide, we expect some conclusive evidence in the near future.
REFERENCES


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