INTRODUCTION

Coronary artery disease (CAD) defines a disease spectrum of diverse aetiology and atherosclerotic plaque is its most common cause.1,2 Although more than 200 coronary risk factors have been reported including systemic hypertension, Diabetes mellitus and smoking as risk factors in patients with acute myocardial infarction (AMI) and changes in the former levels with vitamins supplementation.

ABSTRACT

Objective: To assess the relationship of serum total homocysteine (tHcy) and lipoprotein (a) [Lp(a)] levels with systemic hypertension, Diabetes mellitus and smoking as risk factors in patients with acute myocardial infarction (AMI) and changes in the former levels with vitamins supplementation.

Study Design: An interventional study.

Place and Duration of Study: Medical College for Women and Hospital (MCW&H), Dhaka, Bangladesh, from July 2008 to December 2009.

Methodology: Consecutive AMI patients were recruited from the Coronary Care Unit (CCU) at MCW&H, Dhaka. Blood samples were collected at inclusion (Patient-I0). They were given conventional treatments and prescribed vitamins (vitamins B6=25 mg, B12=2 mg and folic acid=2.5 mg) daily for 2 months. After follow-up, blood samples were taken again (Patient-II0). A group of 25 normal subjects were also included as controls. Serum tHcy and Lp(a) were measured by kinetic method and nephelometric method respectively.

Results: Serum tHcy (µmol/L) and Lp(a) (mg/dl) levels were elevated in Patient-I0 that reduced in Patient-II0 after vitamins supplementation, but not to the normal control level. tHcy of Patient-I0 was 25.1 ± 4.7 µmol/L, of Patient-II0 was 20.1 ± 4.5 µmol/L and of controls 12.1 ± 3.3, p < 0.001. Lp(a) of Patient-I0 was 43.1 ± 15.2 mg/dL, of Patient-II0 was 35.6 ± 10.2 mg/dL, Control: 22.3 ± 5.2 mg/dL, p < 0.001. Elevated tHcy and Lp(a) levels were independent of the traditional risk factors (p > 0.1). However, in a significant proportion of patients tHcy and Lp(a) levels were reduced to control levels (tHcy: p < 0.001, Lp(a): p < 0.01).

Conclusion: These results indicated that tHcy and Lp(a) levels were possibly atherogenic risk factors independent of conventional risk factors. Since both tHcy and Lp(a) levels responded in a similar fashion, a common point of the metabolic and pathogenetic pathways of tHcy and Lp(a) may be influenced by the vitamins supplementation.

Key words: Homocysteine. Lipoprotein (a). Acute myocardial infarction. Vitamin B. Folic acid.
pathophysiology of CAD are highly complex and not fully settled.12

Some studies were performed to determine lowering of tHcy level by B vitamins, e.g. folic acid, cobalamine and pyridoxine.7,13 Their results suggested that B-vitamins supplementation might have beneficial effects on clotting activation and improved the coronary endothelial function by lowering tHcy levels in blood. These delicate metabolic interactions and the efficacy of specific drug therapy need to be elucidated and evaluated in different populations. Only limited published studies on Bangladeshi patients along this line were available as indicated by the literature survey.14 The present study was, therefore, undertaken with the objectives to: (i) assess the serum levels of tHcy and Lp(a) as non-traditional risk factors and the relationship between non-traditional [tHcy, Lp(a)] and traditional [systemic hypertension, Diabetes mellitus and smoking] risk factors and (ii) investigate in Bangladeshi patients that tHcy and Lp(a) levels are reduced through vitamins supplementation.

METHODOLOGY

The diagnosis of AMI in patients was made according to standard clinical and laboratory criteria.1,15,16 After obtaining institutional approval and patients’ consent, clinical evaluation was made including electrocardiogram (ECG), the patient’s proforma was completed and about 10.0 ml blood sample (first specimen) was drawn from each patient by venepuncture. The serum separated was aliquoted and stored frozen at -30°C or below until analysed for the special biochemical parameters, i.e. tHcy and Lp(a). Cases were the admitted patients in the Coronary Care Unit (CCU) at Medical College for Women and Hospital (MCW&H), Dhaka with central chest pain and suspected AMI. Patients with history of central retrosternal chest pain with radiation and with or without sweating and supporting evidence of AMI, i.e. (a) ECG showing ST elevation with or without Q waves and (b) Troponin-I positive were included. Patients having chest pain without ECG change specific for AMI and Troponin I negative results were excluded.

A total of 45 patients were selected with positive evidence for AMI with ECG and positive cardiac enzymes, with or without Diabetes mellitus, hypertension and dislipidaemia. Those patients were explained about the role of vitamins B6, B12 and folic acid in reducing homocysteine level, reducing further risk of recurrence attack of ischaemic heart disease and smooth recovery from CAD. Written or verbal consent was taken from each patient, cardiac vitamins were prescribed and asked them to take the vitamins daily (Folic acid-2.5 mg, Pyridoxine-25 mg and Cyanocobalamine-2.0 mg) for 2 months. They were asked for 1st follow-up after 1 month and 2nd follow-up after 2 months (Patient-I0).

At the first follow-up ECG was taken and advise was given. At the second follow-up after 2 months, blood samples were collected again at 2 months for tHcy and Lp(a) levels estimation, ECG were taken and advised (Patient-II0). These were cases of AMI who received folic acid, vitamin B6 and B12 regularly and attended the CCU after 1 month and again after 2 months. A total of 20 patients attended for follow-up in Patient-I0. Others were either lost to follow-up or went to other centres for coronary intervention, etc. The patients were given the necessary conventional treatments as per requirement of the patients. A group of 25 healthy subjects, matched for age and gender were included in this study as normal controls.

The serum total homocycteine (tHcy) level was measured by kinetic method following the conversion (decrease) of NADH to NAD+ at 340 nm which was proportional to the tHcy concentration in the sample using diagnostic reagent “Homocysteine FS” (Cat No. 134099910930; Cat No. 134009910041), from Diasys Diagnostic Systems GmbH, Germany. The intra-assay and inter-assay coefficients of variation were 2.1% and 4.5% respectively with the lower detection limit of 1 µmol/L.

The serum total lipoprotein (a) [Lp(a)] level was determined nephelometrically at 700 nm from a standard curve using antigen-antibody reaction between antibodies against Lp(a) bound to latex particles and Lp(a) present in the sample. The diagnostic reagents used were Lp(a) 21FS (Cat No. 171399910930; Cat No. 171399910931; Cat No. 171409910059) from Diasys Diagnostic Systems GmbH, Germany. The intra-assay and inter-assay co-efficient of variation were 1.6% and 3.1% respectively with the lower detection limit of 3.9 mg/dl.17

The significance at 5% level of the results were evaluated by Student’s t-test, Paired t-test, Chi-squared (χ²) test and analysis of variance (ANOVA) using standard statistical test equations and procedures.18

RESULTS

The results of serum tHcy and Lp(a) levels presented in Table I showed that serum tHcy and Lp(a) levels were elevated in Patient-I0. Serum tHcy and Lp(a) levels were decreased in Patient-II0 after vitamins supplementation, but remain elevated above the normal levels (p < 0.05).

In significant proportion of patients, however, serum tHcy and Lp(a) levels were reduced to normal levels (Table II). The elevated levels of serum tHcy and Lp(a) levels were independent of conventional risk factors, i.e. hypertension, Diabetes mellitus and smoking (Table III: p > 0.1).
Discovery of new markers associated with an increased risk of CAD may provide a better insight into the pathology of coronary atherosclerosis and facilitate the development of preventive and therapeutic measures. The present study showed that serum tHcy level was significantly higher in both patient groups (Patient-I0, Patient-II0). Our results particularly in the patient-I0 were consistent with some other studies.14,19 This indicated that tHcy may be an atherogenic risk factor which responded to vitamins supplementation, but not to the full extent as indicated by the tHcy level in patient-II0. Two months supplementation period probably was short to obtain complete response. The results suggested that elevated tHcy level as an atherogenic risk factor was independent of traditional risk factors, i.e. systemic hypertension, Diabetes mellitus, smoking. However, hyperhomocysteinemia may promote atherosclerosis and thrombosis and hence CAD (AMI) through a number of mechanisms.3,20

The present study also showed that serum Lp(a) level was elevated in Patient-I0 which responded to vitamins supplementation, but not to the full extent as it remained elevated in Patient-II0. However, elevated Lp(a) level was also independent of traditional risk factors, i.e. hypertension, Diabetes mellitus and smoking. Thus Lp(a) may be an independent atherogenic factor and accelerate advanced atherogenic lesion formation and hence may play an important role in CAD (AMI) as reported in other studies also.3,17,21

Interestingly, both tHcy and Lp(a) responded to vitamins supplementation in a similar fashion and both the parameters did not return to the control levels. These results mean that the metabolic and pathogenetic pathway of tHcy and Lp(a) may have a common point which was influenced by the vitamins supplementation.

### Table I: Serum homocysteine (µmol/L) and lipoprotein (a) (mg/dl) levels in cases (Patient-I0 and Patient-II0) and controls and their statistical analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum homocysteine level (µmol/L)</th>
<th>Serum lipoprotein (a) level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient-I0</td>
<td>Patient-II0</td>
</tr>
<tr>
<td>Number</td>
<td>46</td>
<td>20</td>
</tr>
<tr>
<td>Observed range</td>
<td>18.1-39.7</td>
<td>14.5-25.3</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>25.1±4.7</td>
<td>20.1±4.5</td>
</tr>
<tr>
<td>95% Range</td>
<td>15.8-34.6</td>
<td>11.1-29.1</td>
</tr>
<tr>
<td>ANOVA (Patient-I0, Patient-II0, control):</td>
<td>F-ratio=19.49, (df=2, 88), p &lt; 0.001</td>
<td>F-ratio=17.68, (df=2, 88), p &lt; 0.001</td>
</tr>
<tr>
<td>Student’s t-test:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient-I0 vs. control:</td>
<td>t=3.95, df=69, p &lt; 0.001</td>
<td>t=2.743, df=69, p &lt; 0.01</td>
</tr>
<tr>
<td>Patient-II0 vs. control:</td>
<td>t=2.46, df=43, p &lt; 0.01</td>
<td>t=2.121, df=43, p &lt; 0.05</td>
</tr>
<tr>
<td>Paired t-test:</td>
<td>(Patient-I0 vs. Patient-II0):</td>
<td>t=1.75, df=64, p &lt; 0.05</td>
</tr>
</tbody>
</table>

**p < 0.05: Significant;   p > 0.05: Not significant.**

### Table II: Distribution of subjects according to serum homocysteine levels within normal range (≤ 18.7 µmol/L) or above the normal range (> 18.7 µmol/L) and serum lipoprotein (a) levels within the normal range (≤ 32.7 mg/dl) or above the normal range (> 32.7 mg/dl) and their chi-square (χ²) tests.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Serum homocysteine level (µmol/L)</th>
<th>Serum lipoprotein (a) level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 18.7</td>
<td>&gt; 18.7</td>
</tr>
<tr>
<td></td>
<td>≤ 32.7</td>
<td>&gt; 32.7</td>
</tr>
<tr>
<td>NC</td>
<td>24 (92%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Patient-I0</td>
<td>5 (11%)</td>
<td>41 (89%)</td>
</tr>
<tr>
<td>Patient-II0</td>
<td>10 (50%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>53</td>
</tr>
<tr>
<td>Chi-squared (χ²) test</td>
<td>χ² = 26.7, df=2, p &lt; 0.001</td>
<td>χ² = 9.89, df=2, p &lt; 0.01</td>
</tr>
</tbody>
</table>

**p < 0.05: Significant;   p > 0.05: Not significant.**

### Table III: Serum homocysteine (µmol/L) and lipoprotein (a) (mg/dl) levels in patients according to complications and their statistical analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Homocysteine levels (µmol/L) according to complications in Patient-I0</th>
<th>Lipoprotein (a) levels (mg/dl) according to complications in Patients-I0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Diabetes mellitus</td>
<td>Smoking</td>
</tr>
<tr>
<td>Number</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Observed range</td>
<td>18.4-39.7</td>
<td>18.4-28.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>25.6±5.1</td>
<td>24.4±3.2</td>
</tr>
<tr>
<td>ANOVA</td>
<td>F-ratio = 0.142, (df = 2, 56), p &gt; 0.1</td>
<td>F-ratio = 0.106, (df = 2, 56), p &gt; 0.1</td>
</tr>
</tbody>
</table>

**p < 0.05: Significant;   p > 0.05: Not significant.**
Serum total homocysteine and lipoprotein (a) levels in acute myocardial infarction

(folic acid, B12, B6)? This is possible as oxidation stress affects both tHcy and Lp(a), although they are entirely different unrelated molecules.3,14

Regarding mechanisms, vitamins supplementation reduced the serum level of Hcy probably by optimisation of its catabolism via different metabolic pathways. Hcy remethylation to methionine is increased by B12-dependent methyl synthetase in the presence of S-methyltetrahydrofolate via reduction by methylenetetrahydrofolate reductase. Also, Hcy entry into the catabolic transsulfuration pathways are augmented through B6-dependent enzymes such as cystathione β-synthetase and cystathionase producing inorganic sulphate which is excreted in the urine. Lp(a) is essentially an LDL-particle in which apo(a) is linked to apoB through a disulphide bridge. Higher levels of Hcy can lead to its binding with Lp(a) via apoB resulting in aggregation of Lp(a) particles. Vitamins supplementation probably reduces Lp(a) aggregation due to reduction in serum tHcy level. Hcy also contributes to oxidation stress which is possibly reduced due to lowering of Hcy level by vitamins supplementation. Through these probable mechanisms more Lp(a) are made available for its catabolism through normal metabolic pathways with other lipoproteins, particularly LDL, and thus reduces the serum Lp(a) level. However, much about the biochemistry, physiology and pathophysiology of Lp(a) are yet to be understood fully.3,4,14,22

Eating more fruits and vegetables, specially leafy green vegetables can help lower tHcy and Lp(a) levels by increasing folate and additional vitamin B5 and vitamin B12 supplements may also help the body process tHcy and Lp(a). Here, one must consider the role of vitamin E, other antioxidants and lipid lowering drugs which also reduce oxidation stress.14,17,23,24,25

In recent overviews on the management of primary hyperlipidaemia by statins, plasma/serum baseline Lp(a) level and its reduction were not mentioned and considered in the discussion. Even the updated National Cholesterol Education Program (NCEP) report, USA discussed and debated LDL-C only and no consideration for Lp(a) level was suggested in the NCEP report.24,25 More recent studies have indicated that Lp(a) measurement may have a significant role to play in the prediction and management of patients relevant to atherosclerosis including CAD (AMI), stroke and severity of long-term complications such as retinopathy, neuropathy, CAD and CVD in DM.17,25 Considering these facts and findings, Lp(a) has started to get its rightful place in the management, diagnosis, treatment and follow-up of patients with hyperlipidaemia particularly CAD(AMI), DM and others.24,25

The limitations of this study include a small sample size; moreover, status of folic acid, vitamin B5, B12, index of renal function, other lipoproteins status and methyltetrahydrofolate refucact activity were not known in these patients. tHcy and Lp(a) were measured after MI infarction in cases (Patient-I) at presentation. Thus, question remains whether elevated tHcy and Lp(a) levels were precursor or a consequence of MI. Metabolically, however, it may not be possible for such a high levels to occur in such a short post MI period of 8-12 hours at presentation.

CONCLUSION

Elevated serum tHcy and Lp(a) levels are associated with CAD (AMI) independent of traditional risk factors such as hypertension, Diabetes mellitus and smoking and common metabolic or pathogenetic pathway may be influenced by the vitamins supplementation. Further studies with larger sample size, a longer follow-up and supplementation period and study of other lipid parameters and antioxidants are required to substantiate the present findings.

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