Plasma homocysteine level and risk of Gallstone Disease in North-Eastern Uttar Pradesh

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INTRODUCTION:

Homocysteine (Hcy) is an endogenous amino acid containing sulfur, a metabolite product of essential amino acid methionine [1]. Hcy works in different functions affecting several diseases [2]. High levels of total plasma homocystine (tHcy) were associated with premature atherosclerosis and cardiovascular disease risk, independent of traditional atherothrombotic risk factors [3, 4]. Moreover, serum tHcy (total homocysteine, free and protein-bound) is also reported to be high in patients with cancerous diseases [5]. Plasma concentration of Hcy depends on age, gender, life style factors (which includes consumption of coffee, alcohol, smoking, physical activity, etc), genetic mutation lead to a severe decrease in homocysteine catabolism enzyme activity, drugs and other diseases which interfere with its metabolism and most specifically intake of vitamins B. The plasma levels of vitamin B - group (folic acid, pyridoxine and cobalamin) found to be inversely related to homocysteine because of their involvement in the catabolism of homocysteine [6]. Therefore, hyperhomocysteinemia (HHcy), referring to fasting homocysteine plasma concentrations of up to 15 μM [6], is associated with reduced levels of folate. HHcy is a known risk factor for cardiovascular diseases in the general population [7].

Gallstone disease (GSD) and Coronary heart disease are showed an association with coronary heart disease [8, 9]. This may relatively be described by common risk factors between the two coronary risk factors which correlate with the presence of GSD: physical inactivity [10, 11], cigarette smoking [11-16], low vegetable intake [10, 17], obesity [11, 12], high serum triglyceride [11, 18], high total cholesterol [18, 19], low HDL cholesterol. It is not clear whether plasma levels of tHcy are associated with the GSD presence. In this cross-sectional study, we assessed the association between gallstone disease presence and total homocysteine concentration in the North-Eastern Uttar Pradesh.

MATERIALS AND METHODS

Study Design

The present cross-sectional study has investigated the Hcy plasma levels in patients with gallstone disease.

Settings

The study was carried out in gallstone patients who were admitted to surgical unit of hospital, Banaras Hindu University, Varanasi. Study was performed between the periods of May 2015 to September 2015. Informed consent was obtained from each subject. The study has been approved by an Ethics Committee of the Institute. The 5 ml blood sample from the patients was collected after an overnight fast. The collected blood samples were centrifuged within 15-25 min and stored at ~80 ° C until further use.

Participants

The study included 100 patients with gallstones. Only
ultrasonography were included in our study. Patients having coronary artery disease, malignancy, pregnancy, diabetes and hypothyroidism were excluded. The body mass index (BMI) was measured as the weight in kilogram divided by the height in squared meters. Each gallstone patient were sent to get routine analysis involving total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride measurements.

**Measurement of plasma Hcy levels**

Total plasma homocysteine was calculated using a fluorescence polarization immunoassay kit (Abbott IMx; homocysteine, Abbott, IL, USA).

**Bias**

The experiment was repeated thrice to validate the results. Each sample was quantified independently in three sets of experiment. We compared the mean value of the plasma homocysteine level in gallstone disease patients.

**Study Size**

The number of cases admitted due to cholelithiasis in our area during the study period decided the sample size.

**Quantitative variables**

Plasma homocysteine was divided in three groups i.e. <10, >10 <=12 and >12 mg/dl. Those were having more than 12 mg/dl considered at the high level of Hcy levels.

**Statistical analyses**

The statistical analyses were performed on SPSS version 16.0. The values of quantitative variable were presented by mean with standard deviation and one way ANOVA was applied for comparison between the means. The p value <0.05 was taken significant.

**RESULTS**

**Participants**

This study was carried out in 100 patients who underwent cholecystectomy. Out of 100, 34 were male and 66 female. Mean age was 42.7 years with age range from 16 to 65 years. Abdominal pain was the main symptom observed in 88 cases of gallstone. Vomiting and belching symptoms were present in 6 and 16 cases respectively, of patients.

**Homocysteine Level**

Table 1 represents the comparison of mean values of characteristics in gallstone disease patients. The plasma Hcy levels were significantly higher (p<0.001) in GSD patients. It was observed that elevated homocysteine level was associated with the presence of gallstone. Moreover, BMI, triglyceride and HDL also showed a statistical significant difference. All the patients were found to have less than 240 mg/dl of total cholesterol. LDL was also less than 160 mg/dl in all the patients of gallstone.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Gallstone Patients (n = 100)</th>
<th>F - value</th>
<th>p - value</th>
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</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
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<tr>
<td>&lt;=20</td>
<td>16</td>
<td>281.231</td>
<td>.000</td>
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<tr>
<td>&gt;20 &lt;=25</td>
<td>64</td>
<td></td>
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<tr>
<td>&gt;25 &lt;=30</td>
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<tr>
<td>&gt;30</td>
<td>10</td>
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<tr>
<td><strong>Total Cholesterol (mg/dl)</strong></td>
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<tr>
<td>&gt;240</td>
<td>0</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>&lt;=240</td>
<td>100</td>
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<tr>
<td><strong>Serum Triglyceride (mg/dl)</strong></td>
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<tr>
<td>&lt;=150</td>
<td>90</td>
<td>1.067</td>
<td>.000*</td>
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<tr>
<td>&gt;150</td>
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<td><strong>HDL (mg/dl)</strong></td>
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<tr>
<td>&lt;=40</td>
<td>57</td>
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<td>.000*</td>
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<td>&gt;60</td>
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<td><strong>LDL (mg/dl)</strong></td>
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<tr>
<td>&gt;160</td>
<td>0</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>&lt;=160</td>
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<tr>
<td><strong>Total homocysteine (mg/dl)</strong></td>
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<td></td>
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<tr>
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<td>18.692</td>
<td>.012*</td>
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<tr>
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<tr>
<td>&gt;12</td>
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**DISCUSSION**

Homocysteine plays an important role in disease production and progression, especially cardiovascular diseases [20, 3]. Hcy has been shown to be related to the inflammatory / immune system [2]. In addition, increasing levels of plasma Hcy were associated with increased risk of neural tube defect [21, 22]. Other studies also reported to establish an association with Dementia, Alzheimer’s disease and loss of cognitive function with the increased Hcy plasma levels [23-26]. In this study, we looked at the association between Hcy and gallstones. In our study, total cholesterol and LDL was less than 240 mg/dl and 160 mg/dl respectively, in all the patients. Serum Triglyceride was less than 150 mg/dl in 90 cases and more than 150 mg/dl in 10
cases of GSD. The possible mechanism underneath the tHcy-gallstone association isn’t evident. Antioxidants reduce the prevalence of gallstones so possibly tHcy-induced oxidative stress may be implicated in gallstones development in patients with hyperhomocysteinemia. The elevated level of Hcy in the blood is commonly believed to promote endothelial dysfunction, contributing to atherosclerosis and other cardiovascular diseases. Gallstone disease presence was associated with low HDL but had not been associated with triglyceride, total cholesterol and LDL. Majority of patients with gallstone disease have hyperhomocysteinemia hence it may be associated with gallstones disease. However in most of the patients having hyperhomocysteinemia, total cholesterol was normal.

Sun et al. (2002) showed that homocystine levels increased in different tumor cell lines (A549, human lung cancer; SK-N-AS, human metastatic neuroblastoma; MCF-7, human breast adenocarcinoma) compared with normal cell lines. A study by Sun et al. (2002) reported increased homocystine levels in various human cell lines (SK-N-AS, Human metastatic neuroblastoma; MCF-7, Human breast adenocarcinoma; A549, human lung cancer) in comparison to normal cell lines. The rise in levels of Hcy is related to depletion of folates and rapid proliferation of tumor [5]. However, higher homocystine levels were observed in lung cancer patients [27]. Increase tHcy were reported in both serum and cystic fluid of an ovarian cancer patient [28]. Other also reported that women with higher homocystine may be at an increased risk for breast cancer [29]. Similar to our results, a Japanese study also suggested that the plasma tHcy was associated with gallstone presence. However they find no association with triglyceride or total cholesterol in middle-aged Japanese men [30]. In a recent study, homocystine has also been observed to be higher in chronic renal failure patients [31]. A meta-analysis study found a correlation with increased serum tHcy level and cerebral infarction and also suggested it as biomarker [32].

This study demonstrated first time Hcy plasma levels in gallstone disease patients in North Eastern part of Uttar Pradesh, India. Gallstone disease prevalence is very high in this region. We reported that elevated levels of total homocystine were significantly associated with the risk of gallstone disease. The results of this study appear to suggest that the plasma homocystine levels might be a risk factor for gallstone disease. But further studies are needed.

COMPLIANCE WITH ETHICAL STANDARDS:

Competing Interest: All the authors declared there was no conflict of interest.

Ethical approval: All procedures conducted in the studies involving human subjects were in compliance with the ethical standards of their institutional research committee.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Availability of data and materials: All data generated or analysed during this study are included in this published article.

Funding: Departmental research grant

References:


