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Original Research Paper

Correlation between Serum Homocysteine & Lipid Profile in patient with metabolic syndrome

Authors:
Davina Hijam, Niketa Ashem, Wahengbam Diana Devi, Tina Das, Bidyarani Haobam, Rk Ranjit Singh
Associate Professor, Department of Biochemistry, Regional Institute of Medical Sciences, Imphal
Senior Resident, Department of Biochemistry, Regional Institute of Medical Sciences, Imphal
Ex-Post Graduate Trainee, Department of Biochemistry, Regional Institute of Medical Sciences, Imphal
Ex Senior Resident, Department of Biochemistry, Regional Institute of Medical Sciences, Imphal
Post Graduate Trainee, Department of Biochemistry, Regional Institute of Medical Sciences, Imphal
Research Scientist – II, MRU, Regional Institute of Medical Sciences, Imphal
Corresponding Author:
Niketa Ashem
Senior Resident, Department of Biochemistry, Regional Institute of Medical Sciences, Imphal

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ABSTRACT:
Background: Metabolic syndrome (MetS), a powerful determinant of diabetes and cardiovascular disease, varies from 9% to 27% depending on geographical location and age of the study population. Homocysteine (Hcy) is a biomarker for cardiovascular disease. There is limited number of data concerning relation between Hcy and lipid profiles in the North Eastern region of India. **Aim:** This study aims to determine association of homocysteine and lipid profile levels in patients of metabolic syndrome. **Materials and Methods:** This cross-sectional study was done in the Department of Biochemistry in collaboration with the department of Medicine, RIMS, Imphal from February 2020 to January 2022 which consisted of 100 patients aged 30 years and above with MetS identified using IDF (2006) criteria. The patients were divided into two groups based on the Hcy levels ((Hcy≥15 µmol/L and Hcy<15 µmol/L) for analysis. The correlation of Homocysteine with lipid indices was calculated using Pearson's coefficient analysis. **Results:** The values of BMI (P=0.023), SBP (P=0.008), DBP (p=0.009), TC p=0.024), TG (p=0.003), HDL-C (p=0.045) and LDL-C (p=0.016) were significantly higher in MetS patients whose Hcy >15 µmol/Dl than patients with Hcy ≤ 15 µmol/dL. Pearson's correlation was used to access the correlation of homocysteine and cardiovascular risk factors. The results demonstrated that age (r=0.235, p<0.05), BMI (r=0.212, p<0.05), TC (r=0.433, p<0.05) and LDL-C (r=0.423, p<0.05) were positively correlated with Hcy. **Conclusion**: The present study showed that hyperhomocysteinemia being an independent predictive biomarker for cardiovascular disease has a strong positive association with total cholesterol, triglyceride and LDL which provides evidence that homocysteine might effect lipid (TC, TG & LDL) metabolism.

Keywords: Metabolic syndrome, Homocysteine, Triglyceride, Cholesterol

INTRODUCTION:

Homocysteine (Hcy), a sulphur containing non-essential amino acid, is the byproduct in the conversion of methionine to cysteine. Cofactors of vitamin B12, vitamin B6, and folic acid and enzymes involved in methionine metabolism influenced blood Hcy level.[1] Methionine condenses with adenosine triphosphate (ATP) to form S-adenosylmethionine (SAM), by hydrolysing all the three phosphate bonds in ATP. The methyl group attached to the tertiary sulphur in SAM can be transferred to a variety of acceptor molecule and this leads to the production of S-adenosylhomocysteine from SAM. S-adenosylhomocysteine is hydrolysed to homocysteine and adenosine. Homocysteine has two fates. If there is deficiency of methionine, homocysteine may be remethylated to methionine. If methionine stores are adequate, homocysteine may enter the trans sulfuration pathway, where it is converted to cysteine.[2]
Hyperhomocysteinemia (HHcy) is considered as a risk factor for the development of arteriosclerotic vascular disease. According to a number of studies, methylenetetrahydrofolate reductase (MTHFR) polymorphism is another risk factor for hyperhomocysteinemia and its related consequences.[3,4] In the Indian population, the MTHFR C677T gene polymorphism is more mutated.[5] Prior studies in India

have documented the incidence of hyperhomocysteinemia among young Asian Indians, the teenage population, western Indians, poor socioeconomic strata of north India, and rural and urban areas of India.[3,6-9]One of the potential outcome of hyperhomocysteinemia is a decline in endothelial-dependent dilation via: (1) Endoplasmic reticulum stress, which induces endothelial cell apoptosis; (2) oxidative stress, which is the disruptive uncoupling of nitric oxide (NO) synthase activity, extinction of NO, and enzymatic inhibition; and (3) chronic inflammation/prothrombotic conditions.[10]

Metabolic syndrome (MetS), which significantly increases CVD mortality, is a cluster of conditions characterized by elevated blood pressure, high blood sugar, increased body fat around the waist, and abnormally high levels of triglycerides or cholesterol in the blood. Insulin resistance is considered to be the major underlying pathophysiological feature of the metabolic syndrome, as it interferes in many metabolic pathways.[11] It is not yet known whether the increased cardiovascular risk associated with metabolic syndrome can be explained by the individual components only, or other risk factors associated with both atherosclerosis and insulin resistance are involved. Homocysteine, is one such factor which is considered to be an indicator of risk for the development of cardiovascular disease.[12] Hence, this study was taken up to determine association of homocysteine and lipid profile levels in patients of metabolic syndrome.

MATERIALS AND METHODS:

Study populations: A total of 100 patients aged 30 years and above from the general population with MetS identified using IDF (2006) criteria, were included in the study. This cross-sectional study was carried out in the Department of Biochemistry in collaboration with the MRU, Regional Institute of Medical Sciences (RIMS), Imphal, for a period of two years from February 2020 to January 2022.

Exclusion criteria: Patients with serious illness, congenital heart diseases, Congestive Heart Failure, pericardial disease, pulmonary disease, severely impaired renal and or hepatic functions, history of acute infections, thyroid dysfunction, prolong supplementation of B-complex vitamin especially vitamin B_6, vitamin B_{12} and folic acid and individuals with pregnancy.

This study was approved by the Research Ethics Board, Institutional Ethics Committee (IEC), Regional Institute of Medical Sciences (RIMS), Imphal.

Blood sample collection and laboratory measurements: After an overnight fasting for 8 hours, 3 ml of blood samples were collected from the antecubital vein in the early morning and centrifuged for 10 minutes at 3000 rpm. Serum samples were separated within 30 minutes of collection and were stored at -80^{0}C. Serum lipids and total homocysteine were measured with Enzyme Linked Immunosorbent Assay (ELISA) method. As per the findings of earlier research of Yang B et al, hyperhomocysteinemia (HHcy) is commonly defined as Hcy ≥15 μmol/L.[1] Furthermore, in the current investigation, the upper quartiles of Hcy had a cut-off value of 15 μmol/L, thus we designated Hcy≥15 μmol/L as HHcy and split the participants into two groups (Hcy≥15 μmol/L and Hcy<15 μmol/L) for analysis.

Metabolic syndrome is defined according to IDF criteria, which require a waist circumference (WC) ≥ 90cm in men or 80 cm in women (for Asian population) plus any two or more of the following risk factors: serum triglycerides (TG) ≥150mg/dl, serum HDL < 40mg/dl in men, <50mg/dl in women, blood pressure ≥130/85 mmHg or treatment of previously diagnosed hypertension and fasting plasma glucose ≥ 100 mg/dl or previously diagnosed diabetes mellitus.[13]

Statistical analysis: Statistical analysis were carried out with IBM SPSS version 21 for windows. Continuous variables were given a mean ± SD (if normal distribution) and were compared using Student's t-test. The categorical variables were shown as numbers and percentages. The chi-square test was used to compare mean values of the categorical variables between the groups. The correlation of Homocysteine with lipid indices was calculated using Pearson's coefficient analysis. The results were evaluated within 95% confidence interval (CI) and at a significance level of two-sided p-value less than 0.05.

RESULTS:

The present study consists of 100 MetS patients consisting of 49 males and 51 females and the highest percentage of subjects belong to age group 56-65 years. **Table 1** shows the baseline characteristics and biochemical data of MetS patients.

Table 1: Baseline characteristics of study population.

Hcy (μmol/dL)	≤ 15 μmol/dL (N=52)	>15 μmol/dL (N=48)	p-value
Age (year-old), mean±SD	57.04 ± 10.82	61.20 ± 12.42	0.341
BMI (kg/m^2), mean±SD	23.53 ±1.82	28.24 ±3.20	0.023

Sex, N (%)			
Male	21 (40.7 %)	28 (60 %)	0.426
Female	31 (59.3%)	20 (40.7%)	
SBP (mmHg), mean ±SD	116.83 ± 9.19	138.65 ± 11.99	0.008
DBP (mmHg), mean ±SD	78.00 ± 5.04	86.63 ± 8.74	0.009
FBG (mg/dl), mean ±SD	120.54 ± 10.16	140.33 ± 12.43	0.234
TC (mg/dl), mean ±SD	156.6 ± 32.9	179.4 ± 32.2	0.024
TG (mg/dl), mean ±SD	135.75 ± 35.07	198.56 ± 40.09	0.003
HDL-C (mg/dl)	33.42 ±4.91	26.45 ±7.91	0.045
LDL-C (mg/dl)	133.2 ± 35.4	153.1 ± 35.0	0.016

Abbreviations: TC-Total cholesterol, TG-Triglycerides, HDL-C-High density lipoprotein cholesterol, LDL-C-Low density lipoprotein cholesterol, Hcy-Homocysteine, BMI-Body mass index, FBG-Fasting blood glucose, SBP-Systolic blood pressure, DBP-Diastolic blood pressure

The values of BMI (P=0.023), SBP (P=0.008), DBP (p=0.009), TC (p=0.024), TG (p=0.003), HDL-C (p=0.045) and LDL-C (p=0.016) were significantly higher in MetS patients whose Hcy >15 µmol/Dl than patients with Hcy ≤ 15 µmol/dL.

Table 2 shows the Pearson's correlation of homocysteine and cardiovascular risk factors.

Table 2: Correlation between homocysteine and cardiovascular risk factors

Variable	r-value	p-value
Age	0.235	0.009
BMI	0.212	0.042
Fasting blood sugar	0.167	0.104
Systolic BP	0.160	0.077
Diastolic BP	0.070	0.446
TC	0.433	0.001
TG	0.089	0.532
HDL-C	0.177	0.208
LDL-C	0.423	0.002

Abbreviations: TC-Total cholesterol, TG-Triglycerides, HDL-C-High density lipoprotein cholesterol, LDL-C-Low density lipoprotein cholesterol, Hcy-Homocysteine, BMI-Body mass index, FBG-Fasting blood glucose, SBP-Systolic blood pressure, DBP-Diastolic blood pressure

The results demonstrated that age (r=0.235, p<0.05), BMI (r=0.212, p<0.05), TC (r=0.433, p<0.05) and LDL-C (r=0.423, p<0.05) were positively correlated with Hcy.

DISCUSSION:

The results of this study shows a significant correlation of Hcy with age, which was similar to the findings of the study done by Framingham Offspring cohort and the Hordaland Homocysteine study.[14,15] Refsum H et al[16] reported that increasing age is one of the factor associated with increased Hcy levels. Saw SM et al[17] found that age was positively correlated with Hcy concentration. They reported that there was approximately 1µmol/L increase, on average, in homocysteine concentration with per decade increase in age between 45 and 75 years as well as approximately 2µmol/L higher serum Hcy concentration in men than women. In our study there is a significant positive correlation between Hcy and lipid profiles which is similar with the study done by Real JT et al,[18] and Obed R and Herrmann W.[19]

The result of this study showed a positive correlation of homocysteine levels in relation with impaired fasting blood sugar level though statistically not significant. In this study, it was found that there was a statistical significant association between BMI, Total cholesterol and LDL-C which was similar with studies done by Kang JY et al[20] and Shin KP et al[21]. One of the major impacts of obesity is that it is accompanied with metabolic syndrome and higher BMI has consistently been associated with higher risk of developing metabolic syndrome. The relation between BMI and Hcy concentrations suggest that person with greater BMI will have higher serum Hcy level. In a study done by Glueck CJ et al[22], 3.7% of patients with hyperlipidemia had high plasma Hcy. Results from in vitro studies suggest that Hcy may interact with cholesterol by increasing LDL oxidation, thus predisposing to atherosclerosis.

Studies have suggested that the mechanism of Hcy affecting lipid metabolism are mainly related to down regulation of key players in HDL production [Apo-A1, lecithin-cholesterol acyltransferase(LCAT)][23] and reducing of the liver Apo-A1 mRNA expression.[24] The inhibition of phosphatidylcholine(PC) conversion to phosphatidylethanolamine(PE) and the low ratio of PE/PC caused by hyperhomocysteinemia are key issues in the relationship between hyperhomocysteinemia and triglycerides accumulation.[25,26] Various other mechanisms which explained the harmful effect of Hcy are 1)It enhances the expression of sterol regulatory element binding proteins (SREBP's) to increase intracellular accumulation of total cholesterol and triglycerides.[27] It also causes protein misfolding in the endoplasmic reticulum and oxidative stress, which might affect lipoprotein particle production.[27,28] 2) It affects the vascular wall's elastic quality, raises the growth of vascular smooth cells, and lowers the amount of nitrogen oxide in the blood. The weakened vascular wall has an impact in the emergence of hypertension.[29] 3) In addition, hyperhomocysteinemia causes aneurysms, atherosclerosis, cardiac hypertrophy and other cardiovascular disorders.[30,31] Additionally, high homocysteine levels can harm the kidney's microcirculation, which could result in chronic renal disease.[32] The present study also has several limitations. Serum lipid profiles might relate to dietary habits that were not assessed in detail due to lack of such data. The study is only one center cross sectional study and multiple center large population is required to further confirm the results.

CONCLUSION:

This study has suggested that homocysteine being an independent predictive biomarker for cardiovascular disease has a strong positive association with total cholesterol, triglyceride and LDL which provides evidence that homocysteine might effect lipid (TC, TG & LDL) metabolism. Hence, Homocysteine along with lipid profile might be used as a predictor of cardiovascular disease.

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