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Role of Homocysteine and Status of Lipid Profile in Myocardial Infarction.

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ABSTRACT

Homocysteine is an intermediate in methionine metabolism and receiving a lot of attention these days as a new risk factor for a variety of diseases including coronary heart diseases, cerebrovascular diseases and peripheral vascular diseases. The aim of our study is to evaluate a role of homocysteine in myocardial infarction (MI). Serum homocysteine and lipid profile were estimated in myocardial infarction and compared with control subjects. Thirty MI patients admitted to medicine ward of Krishna hospital, Karad (Maharashtra), were included and compared with same age and sex matched healthy controls. Homocysteine (P<0.001, 248.13%), Triglycerides (P<0.01, 27.08 %), Very Low Density Lipoprotein (P<0.01, 25.64%), ratio of Total Cholesterol/High Density Lipoprotein (P<0.001, 27.38%) and ratio of Low Density Lipoprotein / High Density Lipoprotein (P<0.01, 29.64%) were significantly increased in MI patients as compared to controls. High density lipoprotein (P<0.001, -16.8%) is significantly decreased in MI as compared to controls. Total cholesterol, Low density lipoprotein and Non High density lipoprotein were non-significant in MI as compare to controls. It conclude that the increased serum homocysteine level in this study might be possible cause for the myocardial infarction and now days it is one of the most important biomarker apart from the lipid profile parameters in risk stratification for myocardial infarction.

Keywords: Homocysteine, Myocardial Infarction, Lipid Profile.

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INTRODUCTION

All living organism require sulphur in some form and in mammals this need is met by the sulphur containing amino acids. Homocysteine is a sulphur containing amino acid formed during the metabolism of methionine, the average intake of which is more than 1.8g/day [1]. In diet homocysteine is present in only trace amount; dietary intake does not affect the plasma homocysteine levels [2]. Homocysteine present in plasma in different forms about 1% circulates as the free thiol, 70-80 % is disulphide bound to plasma chiefly albumin and remaining 20-30% combines with itself to form the dimmer homocysteine or with the other thiols including cysteine, with which it forms the homocysteine-cysteine mixed disulphide [3]. Normal range of homocysteine is 5-15µmol/l [4].

Now days homocysteine is consider a new risk factor for a variety of diseases. During the past 10 years, the vast majority of over 100 cases–control retrospective studies have shown that homocysteine is strong independent risk factor for coronary artery diseases, cerebrovascular diseases and peripheral vascular diseases [5]. An elevated plasma level of homocysteine is found in inherited genetic defects of cystathionine B-synthase [CBS] and methylene tetrahydro folate reductase [MTHFR] or acquired cause such as deficiencies of folic acid, Vitamin B6, Vitamin B_{12} [6].

Despite steady progress in treatment of cardiovascular diseases, people are still dying of these diseases, although at later ages [7]. In 1987, Israelson et-al found abnormally high plasma homocysteine levels in patients of myocardial infarction when investigated with 7 years after their myocardial infarction. In patient with acute myocardial infarction elevated homocysteine levels are associated with higher risk of recurrent coronary events and death [8]. Homocysteine increases activation of procoagulant endothelial cells factor & inactivates anticoagulants like protein C & thrombomoduline promoting formation of thrombin, homocysteine stimulates generation of thromboxia. Homocysteine stimulates generation of thromboxane A_2 which is vasoconstrictor & cause aggregation of platelets & binding of lipoprotein (a) to fibrin thus by preventing fibrinolysis [9].

Alteration of lipoprotein levels in MI are well documented in literature, Increased serum triglycerides, very low density lipoproteins, low density lipoproteins and decreased high density lipoproteins leads to myocardial infarction are well know.

Therefore, we have planned this study to see the effect of serum homocysteine level and lipid profile status in myocardial infarction patients of our region.

MATERIAL AND METHODS

Study Design:

The study was carried out in the department of Biochemistry and Medicine, Krishna hospital, Karad (Maharashtra), India. In this study 30 Myocardial Infarction (MI) admitted to medicine ward were taken and compared with 30 same age and sex matched healthy controls.The diagnosis of acute MI was based on the presence of ischemic chest pain of at least 30 minutes duration and a typical increase in cardiac enzymes.
Several diseases such as renal and thyroid dysfunction, cancer, psoriasis, and diabetes as well as various drugs, alcohol, tobacco, coffee, and menopause, are believed to be associated with moderately elevated homocysteine concentrations are not included in our study.

Biochemical parameters

Overnight fasting blood samples were collected for estimation of homocysteine and lipid profile.

Homocysteine

Serum homocysteine was measured by Autopure homocysteine enzymatic kit method by Accurex biomedical Pvt. Ltd, using the principle of conversion of oxidized Homocysteine to reduced form and then converting into S-adenosyl-L-Homocysteine [10].

Cholesterol

Serum cholesterol was estimated by using Cholesterol Oxidase Peroxidase (CHOD-PAP) method. Cholesterol esters are enzymatically hydrolysed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.The hydrogen peroxide combines with 4-aminoantipyrine to form a chromophore (quinoneimine dye) which may be quantitated at 505 nm. [11]

Triglycerides

Serum triglycerides estimated by (GPO / PAP Method) using the principal lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol 3 phosphate, which is oxidised by the enzyme glycerol phosphate oxidase to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound and 4 aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of triglycerides present in the sample [12]

HDL

Serum HDL was estimated by using modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method [13] LDL, VLDL and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER).

The enzymes selectively react with HDL to produce H2O2 which is detected through a Trinder reaction.

LDL ,VLDL and Non- HDL

Serum LDL and VLDL was calculated by using Friedwald's Equation.

[LDL- Cholesterol] = [Total Cholesterol] − [HDL-Cholesterol] − [Triacylglycerol] / 5.
VLDL = Triglycerides / 5
Serum Non-HDL Cholesterol concentrations were calculated by sub tracting HDL-C concentrations from total cholesterol concentrations. [14].

Statistical analysis

Statistical analysis was done by Student 't' test.

RESULTS

Table 1: Mean and SD of Homocysteine and Lipid Profile of MI and Healthy Control Subjects

Sr. No	Parameters	Control Group (n=30)	Myocardial Infarction (n=30)
1	Homocysteine (µmol/lit)	5.63 ± 4.1	19.6 ± 4.7***
2	Total Cholesterol (mg/dl)	157.8 ± 22.3	162 ± 33.6
3	Triglyceride (mg/dl)	104 ± 31.5	132 ± 49.7**
4	HDL (mg/dl)	46.6 ± 9.2	38.7 ± 7.7***

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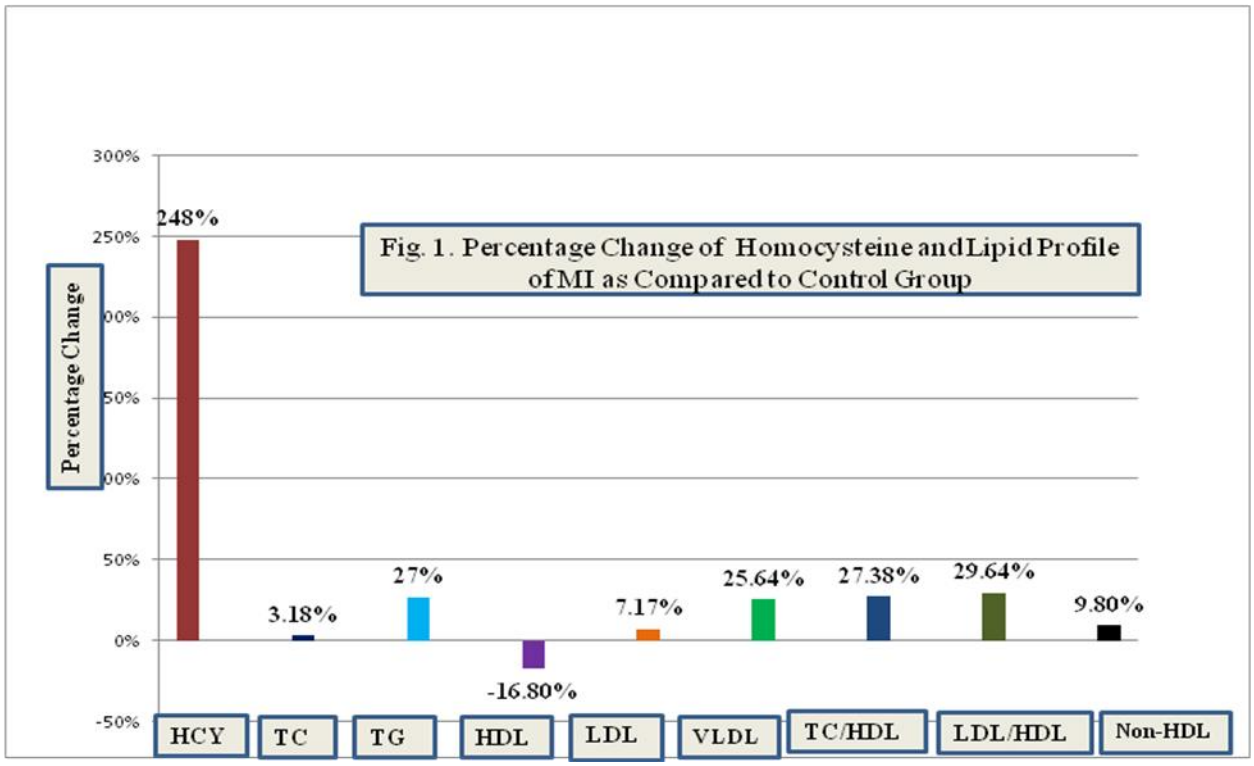
5	LDL (mg/dl)	90 ± 20.7	97 ± 28 .
6	VLDL (mg/dl)	20 ± 6.5	25 ± 9.5 **
7	TC / HDL	3.36 ± 0.63	4.28 ± 1.13 ***
8	LDL / HDL	1.99 ± 0.64	2.58 ± 0.94 **
9	Non HDL(mg/dl)	112 ± 22.7	123 ± 32.9 .

Figures in table indicate mean and SD.

**p<0.01, ***p< 0.001, . Non Significant as compared to control group



DISCUSSION

Serum homocysteine level was significantly increased in Myocardial Infarction (p< 0.001, 248.13%) as compared to control may be due to inherited genetic defects of Cystathionine β-Synthase [CBS] and N^5 N^{10} Methyleinetetrahydrofolate Reductase [MTHFR] and methionine synthase or deficiencies of folic acid, Vitamin B_6, Vitamin B_{12}. The most common one that is detected worldwide and has a high incidence in different populations, is single nucleotide polymorphisms of N^5, N^{10}-methylenetetrahydrofolate reductase which has been associated with mild (13–24 µM) and moderate (25–60 µM) hyperhomocysteinemia [15]. We are unable to rule out the exact cause of hyperhomocysteinemia in MI, since we have not measured these enzymes and vitamins levels due to fund constraint.

There has been an indication towards a significant correlation between hyperhomocysteinemia and cardiovascular disease and its complications such as heart attacks and strokes [16]. It is believed that hyperhomocysteinemia leads to endothelial cell damage, reduction in the flexibility of vessels, and alters the process of haemostasis [16]. Hyperhomocysteinemia may lead to an enhancement of the adverse effects of risk factors like hypertension, smoking, alteration in lipid and lipoprotein metabolism, as well as promotion of the development of inflammation [16]. Increasing age, male sex, smoking, coffee consumption, high blood pressure, altered lipid profile, high creatinine and faulty diet are some of the factors associated with increased homocysteine levels [17].

On the other hand, physical activity, moderate alcohol consumption, good folate and vitamin B_{12} status are associated with lower homocysteine levels. Vegetarians may be at a higher risk of hyperhomocysteinemia due to low plasma B_{12} levels but the difference is likely to be insignificant [17].

If homocysteine is not converted to the methionine then it get accumulated and builds up plaque in the endothelial cells lining of the arteries through various mechanisms, example. The highly reactive by products of homocysteine oxidation combines with LDL to form aggregates. Macrophage then take up these particle to become foam cells in plaque that later become atheromatous plaque [18].

Therefore, from past reports and present results, it is tempting to speculate that the increased serum homocysteine level might be cause of myocardial infarction in our study.

We have estimated the lipid profile in myocardial infarction patients and found significantly increased levels of serum triglycerides ($p<0.01$, 27.08%), VLDL ($p<0.01$, 25.64%), and ratio of TC/HDL ($p<0.001$, 27.38%), LDL/HDL ($p<0.01$, 29.64%) as compared to control subjects. Serum HDL level ($p<0.001$, -16.8%), is significantly decreased and serum total cholesterol, LDL and Non- HDL levels were not altered in MI as compared to control group.

Several studies reported that very high triglycerides has been linked to a higher chance of developing heart disease and having a heart attack or stroke. While research is still underway to uncover the exact relationship between triglycerides and cardiovascular disease, we know that very high levels tend to cluster with other risk factors including being obese, high blood pressure, and high cholesterol [19]. Also in other studies it is found that very high triglycerides often occur along with: Lower levels of “good” or HDL cholesterol that help clear cholesterol from the bloodstream, smaller particles of the “bad” cholesterol that can speed up atherosclerosis, changes to the body’s ability to break down blood clots, which can increase the risk of stroke. In our result we also observed that the HDL level is significantly decreased ($p<0.001$, -16.8%), However, the LDL level was slightly increased (7%) but not statistical significant.

The ratio of TC/HDL and ratio of LDL/HDL is significantly increased in MI patients as compared to controls. This may be due to significant decrease in HDL and increased in total cholesterol and low density lipoproteins. However, in our study we found slight increase of total cholesterol and LDL in MI as compared control subjects, but these results are statistically non significant. Several studies have advocated the value of ratios of LDL/HDL and TC/HDL as a correlate of the severity and extent of coronary artery stenosis [20].

According to the study done by Asutosh P Chauhan, Piyush B Tailor et al the low levels of LDL-C and high levels of HDL-C did not protect the patients against the homocysteine induced coronary artery disease. They also show that in patients who did not have high levels of total cholesterol, but the higher levels of serum homocysteine triggered the coronary artery disease [21].

Therefore, from past reports and present results, it conclude that the increased serum homocysteine level in this study might be possible cause for the myocardial infarction and now days it is one of the most important biomarker apart from the lipid profile parameters in risk stratification for myocardial infarction.

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