Assessment of lipid per-oxidation and endothelial dysfunction in patients of coronary artery disease

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Abstract

Aim: To find the difference in the levels of homocysteine and lipid-peroxidation in male and female patients of coronary artery disease.
Materials and Methods: 71 subjects were included in this study over a period of 12 months out of which 12 female patient of CAD, 29 male patient of CAD and 30 were normal healthy subjects as controls. Estimation of plasma malondialdehyde (MDA) was done by colorimetric technique on RA 50 semi-automated chemistry analyzer. The homocysteine level in the plasma was estimated using the Hcy enzymatic assay on SYNCHRON CX5, Automated Chemistry Analyzer of Beckman Coulter Ltd.
Results: We found significant increased levels of Hcy and MDA in male CAD patients than in female CAD patients.
Conclusion: In female CAD patients, decreased levels of Hcy and MDA areindication of low oxidative stress that may be due to female sex hormones.

Keywords: Coronary artery disease (CAD), Homocysteine (Hcy), Lipid-peroxidation, S-adenosylhomocysteine (SAH).

Introduction

Atherosclerotic disease that involves the cerebro-vascular, coronary and peripheral system is a major health problem in the adult problem worldwide in the developed and developing nations.[1] And of these, the CAD is the leading disease in the industrialized nations and has emerged as a dominant chronic condition in many parts of the world. It is predicted to become the leading cause of disability and death worldwide in the 21st century, and is expected to claim almost 25 million lives annually, thus surpassing infectious disease as the world number one cause of death and disability.[2] A few emerging risk factors have also been identified such as lipoprotein'a', homocysteine, prothrombotic factor and pro- inflammatory factor along with the classical risk factors for the development of CAD.[3]

The combination of homocysteine, homocystine, homocysteine thiolactone, and homocysteine mixed disulfides in the serum is described by the term Homocysteine (tHcy or H (e). Elevation in the plasma tHcy is multifactorial, i.e. increased dietary intake, heritable enzyme deficiencies and vitamin cofactor deficiencies all play a part (4).An elevation of plasma tHcy>15 µmol/ L is usually defined as Hyperhomocysteinemia, and may be caused by genetic defects, nutritional deficiency of folate, vitamin B_6 or Vitamin $B_{12,}$ renal insufficiency, certain drugs, or the phyiological factors such as the advancing age, males, menopause.[5]

The exact mechanisms leading to tHcy toxicity are unknown, and it is believed that they are due to its metabolite that adversely affects vascular endothelium and inducing atherosclerosis by several mechanisms such as

1. Auto oxidation of homocysteine
2. By causing endothelial injury
3. Effect on haemostasis
4. Effect on smooth muscle proliferation
5. Oxidative modification of low density lipoprotein
6. Formation of Hcy-thiolactone.

Hcy auto-oxidation and thiolactone formation promote the production of free radicals which are well known initiators of lipid peroxidation in cells. Aldehydes such as thiobarbituric acid reacting substances (TBARS) are widely accepted general marker for free radical production, of which, the one measured most commonly is malondialdehyde (MDA).[6] The objective of the present study was to find the difference in the levels of Hcy and MDA in male and female patients of coronary artery disease.

Materials and Methods

The present study was conducted on 71 subjects over a period of 12 months in the Department of Biochemistry and Cardiology of Himalayan Institute of Medical Sciences, Swami Rama Nagar, Doiwala, Dehradun after getting approval from ethical committee of Himalayan Institute of Medical Sciences and informed consent were taken from all the subjects prior to the study. The patients were from the intensive care unit of Cardiology Department at HIMS. The study included 12 female cases and 29 male cases of CAD. The study also included 30 normal age matched healthy adults (20 males and 10 females), who served as controls.

Study Group: The study included 12 female cases and 29 male cases of CAD according to the standard diagnostic criteria.[7]

Control Group: Included 30 normal age matched healthy adults (20 males and 10 females), without clinical evidence of coronary artery disease and with normal ECG constituted the control group.

Exclusion Criteria: Subjects with renal failure, hepatic dysfunction, pregnancy, hypothyroidism, and those taking Methotrexate, Carbamazepine or phenytoin were excluded from the study.

Collection of Samples: All samples were collected in the morning after 12hrs of overnight fasting. 2 ml of blood was drawn by venepuncture from the antecubital vein in an EDTA vacutainer for estimation of plasma MDA and homocysteine. Estimation of plasma malondialdehyde was done by colorimetric technique on RA 50 semi-automated chemistry analyzer. MDA was measured by method of Ceconi, Cargoni, Pasini et al. 100µl of serum was diluted with 500µl of distilled water. The samples were kept in boiling water bath for 15 minutes. To the diluted sample 1 ml of trichloric acid and thiobarbituric acid reagent was added. The reaction mixture was cooled and centrifuged. The optical density of pink coloured supernatant was taken on 535 nm on colorimeter. The concentration of MDA is directly proportional to the optical density. Estimation of plasma homocysteine was done on SYNCHRON CX5, Automated Chemistry Analyzer of Beckman Coulter Ltd. Homocysteine level in the plasma was estimated using the Hcy enzymatic assay as marketed by Diazyme. In Diazyme enzyme assay oxidized Hcy is first reduced to free Hcy, which then react with S- adenosylmethionine to form free methionine and S- adenosyl homocysteine (SAH). SAH is further assessed by coupled enzyme reaction including SAH hydrolase, adenosine deaminase and glutamate dehydrogenase. The result of all the parameters undertaken were tabulated and statistically analysed by students "t test" from which p value were obtained.

Results

Table 1 shows the mean and standard error in control subjects. The mean level of Hcy were significantly increased in male control subjects as compared to female control subjects but there is no significant difference between the mean level of MDA in female and male control subjects. Table 2 represents the mean level of MDA and Hcy in CAD patients. Higher levels of plasma Hcy and MDA were obtained in male than female cases of coronary artery disease; this difference was statistically insignificant for both the parameters (MDA & Hcy).

Table 1: Hcy and MDA in males and females subject of control group

Parameters	Status	N	Mean± SD	p-value
Hcy µmol/L	Control Female	10	8.66 ±0.98	p<0.001
	Control Male	20	15.84 ±2.02	
MDA µmol/L	Control Female	10	0.60 ±0.04	p=0.20
	Control Male	20	0.53 ± 0.03	

P<0.05 considered as statistically significant

Table 2: **The mean levels of Hcy and MDA in female and male subjects with MI**

Parameters	Status	N	Mean± SD	p-value
Hcy µmol/L	Control Female	12	28.89 ±1.27	p<0.001
	Control Male	29	34.64 ±2.33	
MDA µmol/L	Control Female	12	1.43 ±0.09	p=0.03
	Control Male	29	1.51 ± 0.10	

P<0.05 considered as statistically significant

Discussion

In the present study, we found significant increased levels of Hcy in male as compared to female in both control and CAD patients. Our study findings for Hcy level is in agreement with Falloni et al.[8] Modi et al[9] and Zogte et al[10] in their study observed insignificant increase in Hcy in male subjects as compared to female subjects. The present study indicates that hyperhomocysteinemia is present to a greater extent in male gender and it is possible that this may be related to sex hormones.[11] Low levels of homocysteine in females may be due to their exercise habit and day to day physical activity.[12]

In addition, it was also observed in our studies that plasma MDA level in male CAD patients (1.51±1.97 µmol / L) was higher than female CAD patients (1.433±0.224 µmol / L), none of whom had previous history of MI. This finding is found to be in agreement with the observations of Nielsen, Mikkelsen, Andersen et al who also found higher values of MDA in male subjects having CAD as compared to similar female subjects.[13] Estrogen has protective effect on lipoprotein metabolism, vascular functions and

endothelial cell lining. It can protect atherosclerosis by reducing oxidative stress and lowering total plasma cholesterol.[14] In addition to vascular and myocardial effects, estrogen also posses antioxidant property. All estrogen has phenolic group in their structure that causes scavenging of oxygen free radicals. Estrogen can induce antioxygen enzyme expression by sitmulating antioxidant defence system.[15] High protein contents in diet, alcohol intake and smoking habits in males may be the cause of high levels of Hcy and MDA in male subjects.

Oxidative stress may play role in several disease including atherosclerosis, diabetes mellitus and hypertension and any gender differences associated with oxidative stress could have implications in the mechanisms for these cardiovascular diseases. Pre-menopausal females have lower level of oxidative stress as compared to men this could be due to the anti-oxidant properties of estrogen.[16] Generally, females were more concerned about body shape, eating and body shape and BMI may also play a role in developing oxidative stress and estrogen may not be the only reason for the differences between males and females.

Conclusion

Higher levels of Hcy and MDA were obtained in males cases than in female's cases, indicating protective effect of female sex hormones for hyperhomocysteinemia and oxidative stress in coronary artery disease.

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