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Original Article

Title of the manuscript: Serum uric acid, homocysteine and lipid peroxidation status in patients of coronary artery disease

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ABSTRACT

Coronary artery disease (CAD) is one of the major causes of mortality and morbidity in India. Over the last two decades, it has been seen that along with other risk factors like diabetes, hypertension, smoking, alcohol etc, the free radicals (FR) and reactive oxygen species (ROS) play an important role in the development and progression of atherosclerosis. Oxidative stress enhances the possibility of low density lipoprotein (LDL) oxidation and development of atherosclerotic plaque formation. Hence the present study was planned to assess the levels of various oxidative stress markers like malondialdehye (MDA), uric acid and homocysteine in patients of CAD and explore their role in the causation and progression of coronary artery disease.The other parameters included in the study were cardiac biomarkers like creatine kinase (CK-MB), Aspartate transaminase (AST), Troponin-I and lipid profile. The studied population consists of fifty three CAD patients and fifty normal healthy controls. The levels of MDA and homocysteine were extremely raised in the CAD patients than controls (<0.0001). Serum uric acid level was found to be significantly high in patients of CAD when compared with healthy controls. The levels of CK-MB, AST, total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were significantly raised in CAD patients. High density lipoprotein cholesterol (HDL-C) levels were significantly lower in patients of CAD.This study concluded that increased level of MDA could prove the presence of oxidative stress in the patients of CAD. Increased levels of homocysteine, uric acid and lipid profile might contribute to additional risk factors in the development of atherosclerosis

1. Introduction

Coronary Artery Disease [CAD] is the major cause of morbidity and mortality worldwide [1]. It causes more deaths, disability and economic loss than any other non-communicable disease. According to WHO reports of 2005, 53% deaths reported in India, were due to chronic diseases and out of these 29% were due to cardiovascular diseases alone [2]. With the explosive rise in the incidence of CAD, it would be the principal cause of disability and deaths in India, by the year 2020 [3]).

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CAD is a multi-factorial disease, till date more than 200 risk factors were identified. Major risk factors that initiate or accelerate the process of atherosclerosis are dyslipidemia, hypertension, diabetes mellitus, obesity, alcohol consumption, smoking, stress and oxidized low density lipoprotein [Ox-LDL [4]. Dyslipidemia is a well established risk factor for CAD. Elevated low density lipoprotein cholesterol [LDL-C] and decreased high density lipoprotein cholesterol [HDL-C] was commonly observed in patients of coronary artery disease [5]. Atherosclerosis is the most common cause of CAD.

It has been seen that various oxidants like reactive oxygen species [ROS] and free radicals [FR] of endogenous and environmental origin play an important role in the pathogenesis and development of various disease like Alzheimer,

atherosclerosis and CAD [6]. Normally, the oxidants are kept in check by antioxidant scavenger system of the body, which includes various enzymes (superoxide dismutase, catalase and glutathione peroxidase) and antioxidant vitamins C, A and E and other carotenoids [7]. They scavenge, dispose, control and oppose the actions of oxidants. The body maintains the balance between pro-oxidants and anti-oxidants, but under various patho-physiological conditions this delicate balance can be altered in favors of the former, thus leading to a condition known as oxidative stress [OS] [8].

Free radicals are highly reactive molecules. They are mostly derived from oxygen and are continuously produced in low concentration from the cells of aerobic organisms. Under normal conditions they are produced by every layer of the vessel wall like endothelium, smooth muscle and adventitia. They act as the mediators of cell signaling pathway which regulate the contraction and relaxation of vascular wall. In diseased conditions like CAD, the production of pro-oxidants become increased and causes cellular dysfunctions and sometimes cell death. The major sources of ROS are xanthine oxidase, cyclooxygenase, cytochrome P450, lipooxygenase, uncoupled nitric oxidase Synthase and NAD(P)H and mitochondria [9].

In free radical hypothesis, the LDL is considered as 'bad cholesterol'. Free radicals can oxidatively modify LDL particle (Ox-LDL) and this is considered to be an early and critical step in the formation of atherosclerosis. Ox-LDL is taken up by sub-endothelial macrophages through scavenger receptor pathway. By this pathway monocyte/macrophages engulf unlimited amounts of Ox-LDL and convert into 'foam cells'. Accumulation of lipid laden foam cells under endothelium will produce fatty streaks. Fatty streaks are the earliest histopathological evidence of the development of atherosclerotic plaque. Ox- LDL also stimulates the release of monocyte-derived TNF- α and IL-1β which leads to smooth muscle cell proliferation and ultimately formation of plaque and fibrosis [10].

Oxidative stress can damage a wide range of macromolecules like lipids, proteins and nucleic acid. Auto-oxidation of polyunsaturated fatty acids of cell membrane epithelium produce, a diene product known as malondialdehyde [MDA], which is considered to be an important biomarker of oxidative stress [11]. Homocysteine increases oxidative stress in the body and increase levels act as an independent risk factor of CAD. The redox reaction which converts sulfhydral group of homocysteine to disulfide produces number of pro-oxidants. These oxidants initiate lipid peroxidation and are responsible for endothelial injury [12]. Various risk factors like smoking, alcohol consumption, tobacco, pollution and various metabolic abnormalities increases the formation of ROS and leads to oxidative damage [13].

Creatine kinase [(CK] and particularly its co-enzyme CK-MB are normally present in the cell. They leak into the plasma due to dissolution of contractile elements and sarcoplasmic reticulum during myocardial infaction. Elevated levels of CK-MB and Troponin-I have been considered as biochemical markers of myocytes necrosis [14]

Uric acid is an end product of purine metabolism. During its synthesis, the enzyme xanthine oxidase is convert hypoxanthine into xanthine, which further oxidized into uric acid. This reaction produces ROS as byproduct, which plays a significant role in the increased vascular oxidative stress [15]. Hyperuricemia has been correlated with endothelial dysfunction, insulin resistance and cardiovascular disease. The risk factors for CAD like hypertension would cause local tissue hypoxia, which increase the production of lactate. Serum lactate decreases the excretion and ultimately increases the level of uric acid in the body. There is also a controversial opinion about the pro-oxidant and antioxidant properties of uric acid. It is recognized as a marker of oxidative stress, but also as a protective factor acting as an antioxidant [16].

There is evidence that anti-oxidants can protect against free radical production and therefore inhibit their damaging effects. Population based study conducted in 2004 gave an inverse relationship between coronary artery disease and anti-oxidants [17]. Therefore, the present study was planned to study the role of uric acid, homocysteine and MDA, if any, in causation of disease in CAD patients and whether they are considered as risk factor or act in conjunction with other parameters leading to endothelial injury.

2. Material And Method

2.1. Study Design

The study group consisted of fifty-three patients with Coronary artery disease. Patients attending medicine OPD and admitted in the Cardiac care unit [CCU and CTVS of Himalayan institute of hospital trust University, Dehradun, were included in the study. Fifty normal healthy volunteers from general population were also included as controls. The patients and healthy controls of age group between 30-60 years were included in the study. The subjects (patients and controls) who were taking antioxidants or vitamin supplements, patients of renal failure and gout were excluded from the study.

The mean age of all the subjects was 44.9 ±10years.The diagnosis of CAD was based on history of prolonged ischemic chest pain, positive troponin-I test, elevated creatine kinase isoenzyme MB (CK-MB), characteristic electrocardiogram [ECG] changes and was confirmed by angiography. Diabetes mellitus was diagnosed if fasting plasma glucose was >126 mg/dl. Patients were considered to be hypertensive if systolic blood pressure was >140mm Hg and diastolic blood pressure was >90mm Hg or self reported use of anti-hypertensive drugs [9]. The patients who had total cholesterol [TC] level of >220mg/dl or triglyceride [TG] >200 mg/dl, high density lipoprotein [HDL] levels <45mg/dl in males and < 50mg/dl in females were considered to be dyslipidemic.

CAD patients were grouped on the basis of age, gender and risk factors involved in the causation of CAD. On the basis of positive troponin-I test, patients presented within 4 hours of onset of chest pain were classified as group I and diagnosed as acute myocardial infarction [AMI] and those presented after 4 hours were classified as group II and diagnosed as chronic coronary artery disease [ch.CAD].The factors of oxidative stress factors were compared between these two groups. Written consent was taken

from the patient or patient's relatives and normal subjects, prior to the study. Study was started after approval from institutional ethical committee.

2.2. Sample collection:

All subjects were instructed to observe an overnight fast for 12 hours prior to the venepuncture. 10ml of blood sample was drawn under aseptic conditions in plain [yellow top] vaccutainer for the estimation of lipid profile, uric acid and homocysteine. The blood was allowed to stand for an hour, after clot formation, the supernatant was centrifuged and tests were performed subsequently. For the estimation of MDA, 3-4ml of blood sample was collected in EDTA vaccutainer [purple top] and plasma was separated by centrifugation.

2.3. .Lipid profile assay:

Serum total cholesterol [TC], serum triglyceride [TG] and serum high density lipoprotein cholesterol [HDL-C] were estimated by commercially available enzymatic reagents on auto-analyzer [DxC 900Beckman culter]. Serum low density lipoprotein cholesterol [LDL-C] and serum very low density lipoprotein cholesterol [VLDL-C] was calculated using Friedwald's formula for samples with TG value less than 350 mg/dl [18].

2.4. Uric acid and homocysteine assay

Serum uric acid and serum homocysteine were estimated by using commercial kits from Weldon Biotec on auto-analyzer [Beckman coulter DxC 900].

2.5. Lipid peroxidation assay:

MDA is a secondary product of lipid peroxidation. It was estimated by measurement of thiobarbituric acid [TBA] in plasma. Proteins were precipitated from plasma with 40% trichloroacetic acid. Precipitated proteins were incubated with TBA reagent in boiling water bath for 60 minutes. The pink chromogen formed was estimated against blank at 535 nm. Results were expressed as µmol/l [19].

2.6. CK-MB and qualitative Troponin-I assay:

CK-MB was estimated by immuno-inhibition, IFCC, UV-Kinetic method, by the kit provided by SIEMENS. Qualitative Troponin-I was determined by One-step rapid assay kit from QDx TROP I.

2.7. Statistical analysis:

The data analysis was carried out by using Statistical Package for the Social Sciences [SPSS] Version 17.0. Results were expressed as Mean ± Standard Deviation. The statistical significance of difference between the various groups was determined by using the student's t-test; $p > 0.05$ not significant, $p < 0.05$ was significant, $p < 0.001$was highly significant and $p < 0.0001$ was extremely significant.

3. RESULTS

Serum homocysteine levels of CAD patients were highly significant [<0.0001] as compared to normal healthy controls. The levels of serum uric acid were found to significantly high in patient when compared with normal individuals [p<0.05]. Statistical analysis showed, MDA was also extremely significant in patients of CAD than controls [p<0.0001; Table I].

Table-I Comparison of serum homocysteine, MDA and serum uric acid between conrols and CAD patients.

Parameters	Controls (Mean±SD)	Patients (Mean±SD)	p-value
Homocysteine (µmol/l)	6.9±3.5	23.4± 4.4	<0.0001**
MDA (µmol/l)	4.9±1.0	19.9±1.7	<0.0001**
Uric acid (mg/dl)	4.9±1.6	6.1±2.4	<0.05*

*Significant **Extremely significant

Figure 1

The CAD patients and controls were grouped on the basis of gender. Male patients had significantly high levels of serum homocysteine, MDA and serum uric acid as compared to normal healthy male controls. It was depicted in Figure 1.

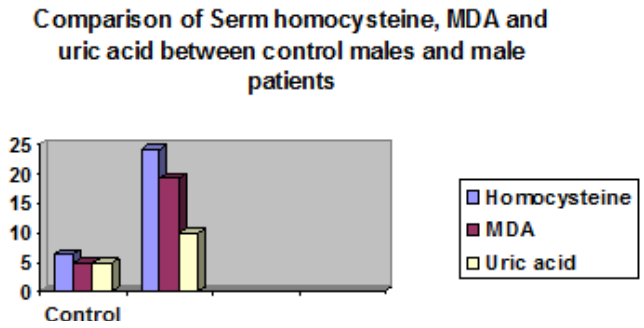
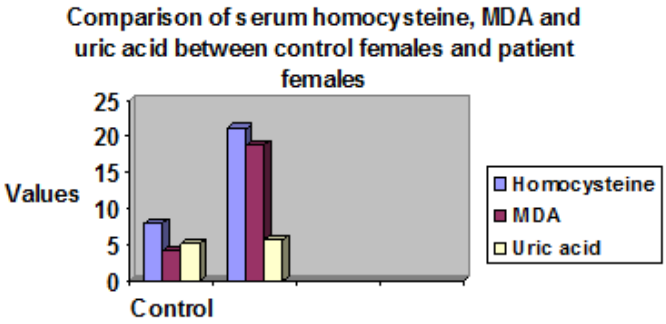


Figure 2

Figure 2 showed that female CAD patients had significantly high levels of serum homocysteine and MDA when compared with female controls. No significant variation was observed when the levels of serum uric acid were compared between female patients and healthy control females.



The comparison of Troponin-I, CPK-MB, Aspartate transaminase [AST] and lipid profile in controls and CAD patients were described in Table II. Study showed significantly increased levels of CK-MB and AST in CAD patients than controls. CAD patients presented within 4 hours of onset of chest pain, had qualitative troponin-I test positive (n=27) and the test was found to be negative in controls. Significantly high levels of TC, TG, LDL-C and VLDL-C were observed in patients of CAD as compared to normal healthy controls. HDL-C was found to be significantly lower in patients than controls.

CAD patients were divided into two groups on the basis of duration of the disease. Those who presented early and had positive [within four hours] qualitative troponin-I test were considered to be suffered from AMI than those with negative troponin-I had chronic coronary disease. Statistically there have been no significant difference in serum homocysteine, MDA and serum uric acid in two groups [Table III].

The patients of CAD when reviewed by their case notes, it has been observed that the patients with very high levels of lipid peroxidation i.e MDA and homocysteine had multiple risk factors. In the present study from the total 53 CAD patients, eight were having hypertension, five were diabetic mellitus, seven were smokers, eleven were taking tobacco with alcoholic and smoking and rest with more than one risk factors.

Table-II Comparison of Troponin-I, CK-MB, AST, Lipids and lipoproteins in patients of CAD to that of normal individuals

Parameters	Controls (Mean±SD)	Patients (Mean±SD)	p-value
Troponin-I	Negative	Positive	
CK-MB (U/L)	12± 2.9	89± 5.8	<0.001*
AST (U/L)	25±5.2	54±6.1	<0.0001**
TC (mg/dl)	156.1±28.5	198±21.0	<0.001*
TG (mg/dl)	125.9±34.0	167±29.6	<0.001*
HDL-C(mg/dl)	44.8±8.6	39±6.7	<0.0001**
LDL-C(mg/dl)	84.2±29.3	121±14.6	<0.0001**
VLDL-C(mg/dl)	25.4±6.9	60±18.9	<0.0001**

*** Highly Significant** **** Extremely significant**

Table-III Variation of serum homocysteine, MDA and serum uric acid between AMI and Chronic CAD.

Parameters	AMI (Mean±SD) (n=27)	Ch CAD(Mean±SD) (n=26)	p-value
Homocysteine (µmol/l)	21.9±3.3	23.9±4.1	0.06 NS
MDA(µmol/l)	19.0±1.5	19.7±0.8	0.07 NS
Uric acid (mg/dl)	6.2±2.6	6.2±2.7	1.0 NS

NS-not significant

4. DISCUSSION

CAD is the most common, serious, chronic and life threatening disease. Atherosclerosis is the most common causes of CAD. Recent studies have demonstrated that cardio-vascular risk factors and oxidative stress play an important role in abnormal vasomotor responses. These responses are considered to be an important cause of ischemia in the formation of atherosclerosis [20].

The role of uric acid represents a marker for oxidative stress associated with increase xanthine oxidase activity (21). The present study showed significant increased levels of uric acid in patients of CAD than the normal healthy controls. This is in accordance with study who demonstated that hyperuricemia reflects increased xanthine oxidase enzyme which produces more oxygen free radicals. This increase oxidative stress may induce endothelial dysfunctions of the blood vessels and leads to the formation of plaque [22]. Studies done recently demonstrated that when high dose of allopurinol is given to CAD patient, it lower the risk of cardio-vascular events, mortality, oxidative stress and improves vascular or endothelial dysfunctions [23]. In one of the previous study, hyperuricemia was considered as a predictor of mortality for both men and women. A 1mg/dl increase in serum uric acid levels was associated with 26% increase in mortality [24]. Uric acid might consider playing an important role in diagnosis, prognosis and therapy monitoring of CAD patients. In the present study, uric acid levels were found to be significantly high in male patients as compared to female patients while no significant variation has been observed in case of male and female control groups.

Homocysteine is considered to be an independent risk factor of CAD. The current study observed highly significant increased levels of serum homocysteine in coronary artery disease patients. The findings of the present study were similar to other clinical studies which have shown that hyper-homocysteinaemia exhibit dysfunctions of the cells of endothelium and increase the oxidative stress. This process was completely prevented by administration of anti-oxidants [25]. Homocysteine inhibit the action of thrombomodulin which is a powerful anti-thrombotic of the endothelium thus induce thrombosis and ischemia [26]. Similar observations also noted by other studies, which showed elevated levels of homocysteine in CAD [27]. When patients and controls were grouped on the basis of gender, no statistically significant difference has been observed.

Oxidative stress generated by ROS may play a causative role in the pathogenesis of coronary artery disease. MDA is a product of auto-oxidation of polyunsaturated fatty acids and is used as an index of oxidative damage (28). The present study observed a significantly increased in the levels of MDA in CAD patients as compared to the healthy controls. The raised MDA indicates increased membrane lipid peroxidation [(29]. The findings of this study are in consistence with previous reports which observed that decreased levels of antioxidants increase the oxidative stress and inflammation in CAD patients [30]. Similarly, the risk factors for coronary artery disease could be reversed by administration of agents which are capable of scavenging ROS/FR like vitamin C [31]. Various other previous studies reported elevated levels of MDA in patients of CAD [32]. There was no significant difference in serum MDA levels of male patients as compared to female patients as well as between male and female healthy controls.

CK-MB and qualitative Troponin-I are highly sensitive and specific markers of myocardial damage. Our study showed significant increased level of CK-MB CAD patients as compared to controls. HDL-C is considered to be a good cholesterol. A negative correlation exists between HDL-C and atherosclerosis because of its active participation in reverse transport of cholesterol [33].Similar findings have been shown by present study wherein HDL levels were found to significantly decrease in CAD patients than controls. The present study reported a significant increased in total cholesterol, triglycerides, LDL-C and VLDL-C in patients of CAD. These findings are in agreement with previous studies which considered increased levels of LDL-C are associated with risk factors of atherosclerosis [34]. Ox-LDL inhibit the synthesis prostacyclin, has atherogenic and pro-inflammatory properties [35].

In the present study we observe increased levels of lipid peroxidation or the oxidative stress in patients of CAD that were associated with an additional risk factors. These findings were similar to the study which showed in the patients of CAD, the risk factors increase the oxidative stress by increasing the production of free radicals and these ROS can stimulate the oxidation of LDL which can lead to foam cell formation and atherosclerotic plaque [36].

5. CONCLUSION

The study has shown high oxygen free radical production, suggested by increased levels of MDA, showed the incidence of oxidative stress in coronary artery disease. Increased levels of uric acid and homocysteine may be an additional risk factor in the initiation and progression of CAD. Other additional risk factors like dyslipidemia, smoking, hypertension, tobacco, diabetes mellitus and alcohol etc might increase the oxidative stress in patients of CAD. The study concluded with the message that the assessment of oxidative stress markers are use-full if recommended and accordingly anti-oxidants may be used as secondary therapy to prevent oxidative damage in the management of coronary artery disease.

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