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Clinical relevance of serum bilirubin and uric acid as antioxidants in coronary artery disease

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ABSTRACT

Currently known risk factors account for only about 50-70% of coronary artery disease (CAD) patients. Oxidative stress induced by reactive oxygen species has been implicated as a major new risk factor in the pathogenesis of variety of vascular diseases including atherosclerosis and CAD. Thus the present study has been designed to see the biochemical changes related to Malonyldiadehyde (MDA as a lipid peroxidation marker) and antioxidants including reduced glutathione (GSH), Serum Bilirubin and Uric acid in patients of CAD. A randomized, controlled, prospective study of 100 patients divided into two groups, Group 1 comprising of 50 patients of CAD and Group 2 including 50 age and sex matched people from general population was done. An increase was observed in the levels of MDA in patients of CAD as compared to controls which was statistically highly significant ($p<0.001$). There was a decrease in the levels of GSH, S. Bilirubin and Uric acid in patients of CAD as compared to controls depicting the decrease in antioxidant status in CAD patients as compared to controls which was statistically highly significant. The levels of MDA showed inverse correlation with the levels of GSH, S. Bilirubin and uric acid whereas GSH, S. Bilirubin and uric acid showed a positive correlation amongst them in patients of CAD.

KEYWORDS

Angina;
Bilirubin;
Uric acid;
Reduced Glutathione;
Malonyl dialdehyde;
Coronary artery disease.

INTRODUCTION

Although CAD is a vastly investigated topic, currently known risk factors account for only about 50 to 75% cases of CAD. Over the past decade, several new markers have been considered as probable known risk factors. These include factors such as Lipoprotein a [Lp(a)], LDL subclasses, oxidized LDL, Glutathione, Superoxide-dismutase (SOD) and Malonyldialdehyde (MDA) and other markers of oxidative stress; Metabolic factors such as insulin resistance and Homocystiene ; Haematological factors such as fibrinogen, factor VII, tissue Plasminogen Activator (tPA) and Platelet Activator Inhibitor (PAI); Inflammatory markers such as C- reactive protein and Infective markers such as Chlamydia pneumoniae. Role of Oxidative stress in

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Coronary Artery Disease is the biggest concern these days[1].

Free Radical induced Oxidative stress contributes to CAD. The ROS oxidize apo B component of LDL molecules. Due to the attack of oxygen free radicals on the lipid component of membrane, the lipid peroxide content is elevated. To evaluate this, estimation of levels of many intermediate lipid peroxides and their end-products has been used to indirectly evaluate the oxidative stress. The most reliable indicators are Malonyldialdehyde (MDA) or Thiobarbituric acid reducing substances (TBARS)[2].

Malonyldialdehyde (MDA) is an end-product of peroxidation of cell membrane lipids caused by oxygen-derived free radicals and is considered a reliable marker of myocardial cell damage[3]. Studies have shown increased levels of MDA in all patients of CAD that is acute MI, stable and unstable angina. Sanderson reported elevated levels of of lipid peroxides in patients with peripheral vascular disease. The raised levels of MDA signifies the increased susceptibility of LDL oxidation in CAD patients[4].

Glutathione (GSH) is an intracellular tripeptide that directly quenches the Reactive Oxygen Species (ROS) and protects against effects of free radicals. Reduced Glutathione (GSH) is important in the metabolism of RBC in part to counteract the action of potentially toxic peroxides. The RBC's can synthesize reduced glutathione (GSH) and require NADPH to return oxidized glutathione (GSSG) to reduced state (GSH)[1].

The modestly higher serum uric acid concentrations have been reported in coronary heart disease patients than in controls. It has been suggested that serum uric acid is an additional risk factor in prediction of coronary heart disease. This is further complicated by the correlation of serum uric acid concentrations with several established coronary risk factors (such as blood pressure), with use of diuretics and with chronic renal disease. So uric acid does not help to predict heart disease but may still be involved in triggering heart disease[5].

Bilirubin, the principle bile pigment, is the end product of heme catabolism. For many years, bilirubin was considered as toxic waste product formed during heme catabolism. However, more recent evidence suggests that bilirubin is a potent physiological antioxidant that may provide important protection against the atherosclerosis, coronary artery disease (CAD), and inflammation.

Several studies have found that different circulating forms of bilirubin are powerful antioxidants, viz. free bilirubin, albumin-bound bilirubin, conjugated bilirubin, and unconjugated bilirubin were all found to be effective scavengers of peroxyl radicals and able to protect human LDL (low density lipoprotein) against peroxidation. Heme oxygenase (HO) is the rate-limiting enzyme of bilirubin production. It is a microsomal enzyme, present in both central and peripheral tissues, that converts heme to biliverdin and CO[6]. Biliverdin is subsequently reduced to bilirubin by the cytosolic enzyme biliverdin reductase[7]. At least two isoforms of HO have been identified and found to be products of different genes and to differ intheir tissue expression, function, and ability to respond to stimuli[6-9]. HO-1 (*M*r > 32 000) is an inducible form that is expressed at a low concentration in vascular endothelial and smooth muscle cells and is markedly induced by heme, metals, oxidative stress, inflammatory mediators, oxidized LDL, and hypoxia. A variety of experiments have suggested that HO-1 is a stress-response protein that plays an important function in cell defense mechanisms against oxidative injury. HO-1 activity is responsible for increased CO and bilirubin formation as well as iron release in pathological conditions such as cardiovascular shock, hypoxia, ischemia-reperfusion, and hypertension[8-15]. The proposed mechanism for antioxidant activity of Bilirubin:

Bilirubin can scavenge the chain-carrying peroxyl radical by donating a hydrogen atom attached to the C-10 bridge of the tetrapyrrole molecule to form a carbon-centered radical.

LOO. + Bil →LOOH + Bil.

Bil. + LOO →Bil-OOL

Bil + O2 ↔Bil-OO.

LOO + BV. →LOO-BV.

At concentrations as low as 10nM, Bilirubin can protect against 10,000-fold greater concentrations of H_2O_2. Under physiologic conditions, bilirubin provides more potent protection against lipid peroxidation than α..-tocopherol, formerly known to be most effective in preventing lipidd peroxidation[16].

Inspite of many efforts to explain the role of oxida-

tive stress in Coronary Artery Disease, the predictive role of oxidative stress is still not very clear. In order to fill these lacunae and to establish the utility of antioxidant vitamins in delaying the progression of CAD, the present study was conducted.

MATERIAL AND METHODS

A total of 100 subjects were included in the present study. These 100 subjects were divided into two groups:

GROUP I (Patients) comprised of 50 clearly defined cases of coronary artery disease attending the OPD or admitted in the department of Medicine of the institution.

GROUP II (Controls) comprised of 50 age and sex matched healthy individuals from the general population, who volunteered for getting included in the present study.

INCLUSION CRITERIA

Individuals of either sex and belonging to age group of 40-60 years having history of or other evidence of CAD were included in the study.

EXCLUSION CRITERIA

The individuals either taking Diuretics and OCP' s were excluded from the study group.

Before starting the study approval of the institutional ethical committee was obtained.

The clinical examination of all patients was done and history was recorded as per the proforma attached. On the basis of clinical features as recorded in the proforma attached, ECG (Electrocardiography) reports and TMT (Tread Meal Test) in relevant patients, the CAD patients were confirmed for diagnosis.

All the individuals selected for study were examined and investigated for Lipid Profile, Reduced Glutathione, Malonyldialdehyde, Serum Bilirubin and uric acid.

METHODS

1. Blood glutathione estimation (GSH) - Beutler et al 1963[17].
2. Malonyldialdehyde (MDA): Beuge and Aust (1978)[18].
3. Serum Uric acid - By enzymatic uricase method of Fossati et al (1980)[19].
4. Serum Bilirubin : Total and direct bilirubin (Diazotised sulfanilic acid method)[20].

RESULTS

A total of 100 subjects were included in the present study. Group I (Patients) comprised of 50 clearly defined cases of coronary artery disease attending the OPD or admitted in the department of Medicine of the institution. Group II (Normal Healthy Individuals, NHI) comprised of 50 age and sex matched healthy individuals from the general population, who volunteered for getting included in the present study. TABLE I shows the variations in levels of lipids, lipid peroxidation marker MDA and various antioxidants GSH, Uric Acid and S.Total Bilirubin.

TABLE 1

No	Parameter	Patients of CAD	NHI	Significance
1.	TC (mg%)	229.87±40.17	184.14±33.08	P<0.001; HS
2.	TAG(mg%)	168.70±61.03	113.96±22.38	P<0.001; HS
3.	HDL(mg%)	40.38 ± 6.01	49.54 ± 4.72	P<0.001; HS
4.	LDL(mg%)	155.16±35.12	11.34±29.17	P<0.001; HS
5.	VLDL(mg%)	33.50±12.1	22.78+4.47	P<0.001; HS
6.	TC/HDL	5.71 ± 1.26	3.73 ± 0.65	P<0.001;HS
7.	TG/HDL	4.2 ± 1.48	2.3 + 0.47	P<0.001;HS
8.	GSH (mg%)	19.66 ± 10.47	45.96 ± 11.13	P<0.001; HS
9.	MDA(mMoles/L)	6.06 ± 1.61	1.93 + 0.66	P<0.001; HS
10.	Uric Acid	5.71 ± 1.40	6.68 + 1.37	P<0.001; HS
11.	S. Bilirubin μmoles/L)	7.2 ± 4.0	11.8 + 6.1	P<0.0001; HS

TABLE I shows the variations in Serum lipids, lipoproteins, lipid peroxidation product MDA and antioxidants Reduced Glutathione, Uric Acid and S. Bilirubin in patients of CAD and Control group. The values of TC, TG LDL and VLDL were statistically significantly higher in patients of CAD as compared to controls ($p<0.001$) whereas mean $\pm$ SD of HDL was significantly lower in patients of CAD as compared to controls ($p<0.001$). The Atherogenic Indices like the Total cholesterol/HDL and TG/HDL were calculated in patients of CAD and controls. TABLE 1 shows the difference in Lipoprotein Ratios in Cases and Controls. The mean value of TC/HDL was 5.76 ± 1.20 in pa-

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tients of CAD as compared to controls in whom the mean value of TC/HDL was 3.73 ± 0.65. These values were statistically highly significant depicting the importance of these ratios in CAD. Another atherogenic index TG/HDL predicted similar results with Mean value in patients of CAD 4.2±1.48 as compared to 2.3 ± 0.47 in healthy subjects. These values were statistically highly significant (p< 0.001).

TABLE 2 : Correlation of serum malonyldialdehyde (MDA) with reduced blood glutathione (GSH) in patients of CAD

Parameter	Mean± SD	r value
GSH	19.66±10.47	
MDA	6.06 ± 1.61	- 0.335

TABLE 2 shows a negative correlation between lipid peroxidaion marker MDA and antioxidant GSH

TABLE 3 : Correlation of serum uric acid with GSH and serum malonyldialdehyde (MDA) in patients of CAD

Parameter	Mean ± SD	r value
UA	5.71 ± 1.40	-
GSH	19.66±10.47	0.08
MDA	6.06 ± 1.61	-0.09

S. Uric Acid shows a positive correlation between GSH whereas a negative correlation was found between UA and MDA in patients of CAD

TABLE 4 : Correlation of serum bilirubin with GSH, Serum MDA and UA in patients of CAD

	Mean ± SD	r value
Bilirubin	7.1 ± 1.52	-
GSH	19.66±10.47	0.171
MDA	6.06 ± 1.61	-0.06
UA	5.71 ± 1.40	0.04

A positive correlation was observed between S. Bilirubin, GSH and UA whereas a negative correlation was found between S. Bilirubin and MDA

TABLE 5 : Correlation of serum low density lipoprotein with reduced blood glutathione (GSH) and malonyldialdehyde (MDA) in patients of CAD and normal healthy individuals

	Mean ± SD	r value
LDL	155.16 ± 35.1	-
GSH	19.66±10.47	- 0.021
MDA	6.06 ± 1.61	0.023
S.Bilirubin	7.1 ± 1.52	- 0.280

According to TABLE 4, there is a negative correlation between correlation between Serum LDL and GSH and S. Bilirubin whereas MDA and LDL show a positive correlation in patients of CAD

It was observed that mean GSH, S. Bilirubin and UA concentrations were lower in patients of CAD as compared to controls which was statistically highly significant (p<0.001). The variations in the levels of MDA in normal healthy individuals and patients were statistically highly significant (p<0.001) with the level of MDA significantly higher in patients as compared to normal healthy individuals.

DISCUSSION

There is now a consensus that atherosclerosis represents a state of heightened oxidative stress which is characterized by lipid and protein oxidation in the vascular wall. In the light of above facts the present study was conducted in 100 subjects divided into two groups.

Group I – patients of CAD and Group II Normal Healthy individuals. Both the groups were analyzed to compare the difference in lipid peroxidation product MDA and antioxidant status of GSH to predict the role of oxidative stress in CAD. The variations in the levels of GSH in patients of CAD and normal healthy individuals depicting the decrease in antioxidant status in CAD patients as compared to healthy individuals were studied in the present study (TABLE I). The variations in the levels of GSH in normal healthy individuals and patients were statistically highly significant (p<0.001). These finding suggest that depressed GSH levels may be associated with enhanced protective mechanism to oxidative stress in AMI[21].

The variations in the levels of MDA in normal healthy individuals and patients were also statistically highly significant (p<0.001) (TABLE I) with the level of MDA significantly higher in patients as compared to normal healthy individuals. This increase in MDA despite neutralization of free radicals by GSH depicts the persisting oxidative stress and depletion of protective mechanisms leading to persistent damage by free radicals[22].

Although abnormalities in lipoprotein metabolism is one of the key factors in atherogenesis, an attempt to optimize the predictive capacity of lipid profile, several lipoprotein ratios or "atherogenic indices" have been defined. The total/high density lipoprotein (HDL) cholesterol ratio, known as the atherogenic or Castelli index and the TG/HDL ratios are two important components and indicators of vascular risk, the predictive value

of which is greater than the isolated parameters. Prostasio Lemos et al showed that the ratio of TG/HDLcholesterol ratio correlates inversely with the plasma levels of small, dense LDL. They found that an TG/HDL ratio > 4 is the most powerful independent predictor of CAD development[23]. Similar findings were also observed in the Helinski Heart study by Manninen V, Tenkanen L, Koskinen P et al in 1992[24]. The ratio of TG/HDL, initially proposed by Gaziano et al is an atherogenic index proven to highly significant independent predictor of MI even stronger than TC/HDL or LDL/HDL[25]

According to some studies, Urate can directly scavenge singlet oxygen, OH and peroxyl radicals, certain oxidants produced by enzymes and CO_3 and NO_2 derived from ONOO- [14]. In the present study the role of Uric acid as an antioxidant was studied. TABLE I shows that the difference in the levels of UA in patients of CAD and Group II was statistically significant with the $p<0.05$ indicating the role of uric acid as an antioxidant[26].

The lower levels of S. Bilirubin in patients as compared to controls was statistically highly significant ($p<0.001$) (TABLE 1). These finding suggest that depressed S. Bilirubin levels may be associated with enhanced protective mechanism to oxidative stress in CAD[8].

TABLE 2 shows the coefficient of correlation between GSH and MDA in both patients of CAD and normal healthy individuals. The results show a negative significant correlation between GSH and MDA with coefficient of correlation being -0.335. This may be due to utilization of GSH in quenching free radicals and still persisting oxidative stress causing increase in MDA levels due to increased lipid peroxidation. Stevuljevica JK et al observed similar results with levels of MDA showing significant negative correlation with GSH in patients of CAD as compared to normal healthy individuals in a study conducted University of Belgrade in 2006[22].

S. Uric Acid shows a positive correlation between GSH whereas a negative correlation was found between UA and MDA in patients of CAD (TABLE 3). Similar results were obtained in a study conducted by Joseph T. et al on uric acid.

TABLE 4 shows a positive correlation was observed between S. Bilirubin, GSH and UA whereas a negative correlation was found between S. Bilirubin and MDA. This predicts the antioxidant role of S. Bilirubin in neutralizing free radicals just like Reduced Glutathione.

Human and animal atherosclerotic lesions are characterized by the presence of oxidized lipids thought to result from LDL oxidation. The latter, of course, has been proposed to cause foam cell formation, a critical part of the initiation of atherosclerotic lesion formation. In light of this hypothesis S. LDL when correlated with various oxidation markers and antioxidants (TABLE 5), correlated positively with lipid peroxidation marker MDA. This indicated that more the free radicals to cause lipid and membrane peroxidation, and higher the S. LDL levels, more the OxLDL levels leading to increased foam cell formation and there is increased risk of CAD. Assman G et al concluded that the association between high serum cholesterol level, especially high LDL-C, and CAD is causal and independent of other risk factors[27].

CONCLUSIONS

The distinct inverse correlation between lipid peroxidation marker MDA and various antioxidant (Reduced Glutathione, Serum Total bilirubin and UA) concentration may have an important clinical and diagnostic implication. The clinical relevance relates to potential preventive and therapeutic approach including the increased intake of antioxidants in the diet, whereas the diagnostic relevance stresses the plasma bilirubin concentration as a provisional new marker of atherogenic risk that can be measured easily in the clinical laboratory and applied in the medical practice.

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