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Research Paper

OXIDATIVE STRESS STATUS IN CORONARY ARTERY DISEASE PATIENTS

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Coronary artery disease (CAD) may be caused by or cause of oxidative stress. In the present study the predictive values for oxidative stress, oxidant stress, oxidant status and antioxidant status were investigated in coronary artery disease (CAD) patients (n = 31) and in healthy age- and sex- matched controls (n = 19). Malondialdehyde (MDA) and total oxidant status levels were measured as markers of oxidative stress while total antioxidant status was determined to assess the antioxidant protection in their blood-sera samples. Lipid profiling was also carried out for each individual. The MDA, total cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins and total oxidant levels were found significantly higher though HDL-cholesterol levels were significantly decreased in the patients. The significant (p < 0.000) increase in lipid peroxidation (MDA) and oxidant status and a significant (p < 0.000) decrease in HDL-cholesterol and antioxidant status implies an imbalance of the oxidant: antioxidant status in CAD patients as indicated by the significantly raised oxidative stress index and requires rectification to prevent other co-morbidities.

Keywords: Oxidative Stress, Total Antioxidant Status, Malondialdehyde, Coronary Artery Disease

INTRODUCTION

Coronary artery disease (CAD) is one of the major causes of mortality and morbidity in both, developed and developing countries. Risk factors for CAD include hypertension, hyperlipidemia, diabetes, family history, smoking, gender, age, obesity and physical inactivity (Kasap *et al.,* 2007). Dyslipidemia is a well established risk factor for coronary artery disease and lowering of high density lipoprotein (HDL)-cholesterol is a common phenomenon observed in coronary artery disease patients (Ghosh *et al*., 2006). Besides these, oxidative stress is also a strong contender in the development of coronary artery disease. Oxidative stress arising as a result of imbalance between free radical production and

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antioxidant deficiency, can cause damage to a wide range of macromolecules i.e., lipids, proteins and nucleic acids (Mohora *et al*., 2006). Malondialdehyde (MDA) is a breakdown product of peroxidation of long chain fatty acids and it is considered an important oxidative stress biomarker as is the total antioxidant status (Locatelli *et al*., 2003). The total antioxidant status (TAS) provides a measure of plasma defenses against reactive oxygen species (ROS); a significant relation between plasma TAS levels and extent of coronary artery disease has been reported (Lopresti *et al*., 2008). Though some studies on various oxidative stress markers in CAD patients have been documented in literature, yet no reports on patients from this region have come to attention. Keeping in mind that the array of risk factors associated with CAD may vary in different population sub-groups on account of differing genetic make-up, life-styles/habits and dietary patterns, in the present study, oxidative stress in blood serum samples of CAD patients visiting the local hospitals for treatment was estimated.

MATERIALS AND METHODS

The study group (n=50) comprised CAD patients (n-31) and 19 healthy age- and sex-matched controls. Written informed voluntary consent was taken and the study proposal was approved by the Institutional Ethics Committee.

Inclusion Criteria

The patients were diagnosed as having CAD by clinical cardiologists on the basis of clinical symptoms, treadmill test and echocardiography results.

Exclusion Criteria

Patients with renal, thyroid disorders and with negative TMT were excluded from the study.

Data and Sample Collection

On a pre-designed questionnaire the demographic and disease-related information of the subjects was obtained during a face-to-face interview. Anthropometric variables (height, weight) were ascertained using the method of Weiner and Lourie (1981) to calculate Body Mass Index (BMI) and Waist Hip Ratio (WHR) as markers of obesity. Physiometric measurements (blood pressure readings) were averages of three readings taken at an interval of 10 min after the subject had rested. The mean arterial blood pressure (MBP) was calculated by using the formula MBP=DBP + (SBP-DBP)/3 (Perusse et al., 1989). Blood samples (5ml) were collected by venipuncture after overnight fasting and analyzed for oxidative stress parameters (TOS, TAS, MDA) and lipid profile markers using standard methods.

The blood samples were transported on ice to the laboratory; the samples were allowed to clot and the separated serum was analyzed for lipid profile parameters, lipid peroxidation (MDA) and oxidative stress index (TAS and TOS). Serum MDA levels were measured spectrophotometrically as per the method of Buege and Aust (1978). Serum lipids (total cholesterol, triglycerides and HDL-cholesterol) were determined on a semi-automated clinical chemistry analyzer (Erba Chem-7) using commercially available kits (Erba). Serum LDL and VLDL levels were calculated (LDL cholesterol = Total cholesterol - HDL cholesterol - Triglycerides/5 and VLDL = Triglyceride/5) according to Friedewald's formula (Friedewald *et al*., 1972). Serum TAS levels were measured by an automated colorimetric method (Erel, 2004) based on the bleaching of the characteristic colour of a stable 2 2'-azino-bis [(3-ethylbenzthiazoline-

6- sulfonic acid) (ABTS)] radical cation by antioxidants. TAS levels were measured on the microplate reader at 660 nm and the results were expressed in mmolTrolox equivalent /l. For determining the total antioxidant status (TOS) in the serum, the method of Erel (2005) was used. The oxidants in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium and the intensity of colour is measured at 560 nm and relates to the total amount of oxidant molecules in the samples. The assay was calibrated with hydrogen peroxide (H_2O_2) and the results were expressed in terms of (µmol H_2O_2 equivalent/l).

Oxidative Stress Index (OSI)

This index is the ratio of TOS to TAS levels (Harma *et al.,* 2006). For this, the units of TAS were converted to mmol/l. OSI in arbitrary units was calculated as TOS (µmol H_2O_2 equivalent/l)/ TAS (mmol Trolox equivalent/l).

Statistical Analysis

For the patient and control groups, the lipid profile parameters were expressed as mean ± SEM, TAS levels as millimolar Trolox equivalent per liter, TOS levels as millimolar hydrogen peroxide equivalent per liter and OSI as arbitrary units. In order to find the significance of results, the Student's test was performed using Statistical Package for the Social Sciences (SPSS) version 16.0. Values were compared for patients and controls. Level of significance was taken as $p \leq 0.005$.

RESULTS

The demographic details of the patients (age-range 51-66y) and of controls (age-range 47-61y) are given in Table 1. The patients were in different occupations and comprised 17 females (mean age 57.13 ± 0.92y). On the basis of general adiposity determined as BMI (WHO, 2004), there were 11 obese patients (BMI ≥25.0kg/m^2) and 12 were overweight (BMI 23.0-24.9 kg/m^2). Waist-hip-ratio (abdominal adiposity) observation revealed 13 male and 17 female patients as obese (Misra *et al.,* 2009). Alcohol drinking was observed in 20 male patients and in 6 male controls. However there were no smokers and the socioeconomic status was obtained as per Kumar *et al.* (2007). The body mass index (BMI) of patients (25.05 ± 0.68kg/m^2) was significantly higher ($p<0.004$) than of controls (22.30 ± 0.33kg/m^2) as was the waist-to-hip ratio (1.06±0.01 of patients versus 0.98 ± 0.01 in controls; $p<0.001$). The blood pressure parameters (systolic blood pressure, diastolic blood pressure and mean arterial blood pressure) were also very highly significant ($p<0.000$) in patients compared to controls. A comparison between genders for BMI, WHR and blood pressure values revealed no statistical significance between female and male controls (Table 2). However a comparison between males of patient and control groups revealed significantly more BMI and blood pressure values ($p<0.001$) while in females, WHR (<0.05) and blood pressure values were significantly higher ($p<0.05$) than in healthy controls. In Table 3 the results of oxidative stress indices are presented. Serum MDA levels of CAD patients were highly significant ($p<0.000$) as compared to values in control individuals. TOS levels were also significantly higher in patients ($p<0.000$) whereas TAS was significantly lower in patients ($p<0.000$).The OSI consequently was also significantly higher in the patient group. A comparison between genders for MDA, TOS, TAC and OSI values revealed no statistical significance between male and female patients. The lipid

Table 1: Demographic Information of CAD Patients and Healthy Controls

Variables	Category		Patient Group (n=31)	Control Group (n=19)
Age (years)	30-55		11	12
	56-81		20	7
Gender (%)	Male		22(70.97)	13(68.42)
	Female		9(29.03)	6(31.58)
BMI[a] (kg/m^2)	Underweight <18.0		1	0
	Normal 18-22.9		7	14
	Overweight 23-24.9		12	5
	Obese ≥25		11	0
WHR[b]	Men	<0.95	1	3
		>0.95	13	10
	Women	<0.80	0	0
		>0.80	17	6
Blood pressure [c](mmHg)	Pre-Hypertensive (≤120-129/80-84 ≤130-139/85-89		19	19
	Hypertensive ≥140/90		9	-
	Stage I ≥140-159/90-99		3	-
	Stage II 160-179/110-109 ≥180/110		-	-
MAP[d] (mmHg)	86-106		19	19
	107-127		12	0
Socioeconomic status[e]	Upper I		4	3
	Upper Middle II		24	14
	Lower Middle III		3	2
Alcohol drinking	Yes		20	6
	No		11	13

Note: [a] BMI as per WHO 2004; [b] Misra *et al.,* 2009; [c] IHG-II., 2007; [d] Perusse *et al., 1989;* and [e] Kumar *et al.,* 2007.

profile (Table 4), revealed significantly higher ($p<0.000$) serum triglycerides and lower ($p<0.000$) HDL-C levels in patients. Total cholesterol, low density lipoprotein cholesterol (LDL-C) and very

Table 2: Comparison of Various Parameters Between CAD Patients and Controls

	Total			Patients			Males			Females		
Parameters	Patients	Controls	p value	Male	Female	p value	Patients	Controls	p value	Patients	Controls	p value
Age(y)	57.13±0.71	54.21±0.92	**0.015**	–	–	–	–	–	–	–	–	–
BMI(kg/m^2)	25.05±0.68	22.30±0.33	**0.004**	26.33±1.22	23.99±0.65	0.085	26.33±1.22	22.51±0.02	**0.007**	23.99±0.65	21.86±0.78	0.088
WHR	1.06±0.01	0.98±0.01	**0.001**	1.03±0.01	1.08±0.02	**0.006**	1.03±0.01	0.98±0.02	0.106	1.08±0.02	0.95±0.02	**0.007**
SBP(mm/Hg)	143.23±3.21	116.95±1.29	**0.000**	143.00±4.89	143.41±4.38	0.950	143.00±4.89	116.00±1.72	**0.000**	143.41±4.38	119.00±1.53	**0.004**
DBP(mm/Hg)	87.29±0.93	79.26±0.87	**0.000**	87.86±1.45	86.82±1.21	0.591	87.86±1.49	80.46±0.79	**0.000**	86.82±1.21	76.67±1.84	**0.000**
MAP(mm/Hg)	105.94±1.54	91.82±0.66	**0.000**	106.24±2.40	105.69±2.06	0.862	106.24±2.40	92.31±0.76	**0.000**	105.69±2.06	90.78±1.28	**0.000**

Note: Values in bold are significant ($p \leq 0.05$, $p \leq 0.001$; student's t-test).

Table 3: Levels of Oxidative Stress Markers in CAD Patients and Controls

	Patients			Controls			Total		
	Males	Females	p value	Males	Females	p value	Patients	Controls	p value
MDA(μmol/l)	4.42±0.49	4.13±0.45	0.668	2.16±0.26	1.76±0.36	0.400	4.27±0.33	2.04±0.21	**0.000**
TOS(imol H2O2 Eq/l)	47.48±6.83	52.17±7.10	0.642	14.40±1.67	18.64±5.05	0.322	50.05±4.90	15.74±1.93	**0.000**
TAS(mmolTroloxEq/l)	6.23±0.43	7.02±0.49	0.250	9.51±0.54	9.26±1.06	0.813	6.66±0.34	9.43±0.48	**0.000**
OSI(AU)	8.08±1.29	7.98±1.18	0.950	1.58±0.21	2.18±1.34	0.226	8.03±0.86	1.77±0.22	**0.000**

Note: Values in bold are significant ($p \leq 0.05$; student's t-test).

Table 4: Comparison of Biochemical Changes in CAD Patients and Controls

	Patients			Controls			Total		
Parameters	Males	Females	p value	Males	Females	p value	Patients	Controls	p value
Total Cholesterol(mg/dl)	233.00±1.95	231.65±2.07	0.644	172.54±5.51	191.17±9.82	0.093	232.26±1.42	178.42±5.14	**0.000**
HDL Cholesterol(mg/dl)	25.21±0.69	24.00±0.99	0.344	61.46±4.56	50.00±5.13	0.150	24.55±0.63	57.84±3.66	**0.000**
VLDL Cholesterol(mg/dl)	36.58±0.41	36.56±0.051	0.975	27.83±0.66	29.37±1.31	0.256	36.57±0.33	28.31±0.61	**0.000**
LDL Cholesterol(mg/dl)	207.79±2.15	207.65±2.06	0.963	111.08±5.18	141.17±12.52	**0.016**	207.71±1.46	120.58±6.07	**0.000**
Triglycerides(mg/dl)	182.93±2.05	182.82±2.55	0.975	139.15±3.28	146.83±6.57	0.256	182.87±1.65	141.58±3.07	**0.000**

Note: Values in bold are significant ($p \leq 0.05$; student's t-test).

low density lipoprotein cholesterol (VLDL-C) levels were also significantly higher ($p<0.000$) in patients. However, a comparison between genders for total cholesterol, higher density lipoprotein cholesterol, triglycerides, very low density lipoprotein and low density lipoprotein levels revealed no statistical significance between male and female patients.

DISCUSSION

The significantly elevated levels of oxidative stress in CAD patients as ascertained from TAC, TOS and MDA levels and the higher degree of dyslipidemia observed in blood serum samples of these patients are in accordance with reports in literature. Oxidative stress generated by

reactive oxygen species may play a causative role in the pathogenesis of coronary artery disease (Chisolm and Steinberg, 2000). Antioxidant defense comprising enzymatic and non-enzymatic moieties can inactivate or remove the reactive species. Serum MDA, a biomarker of lipid peroxidation, has been extensively used to investigate oxidative stress in CAD patients (Mutlu *et al*., 2005; Serdar *et al*., 2006). The plasma TAS and TOS levels and the OSI reflect the redox balance between oxidant and antioxidant status. The increased lipid peroxidation (MDA) also occurs as a consequence of oxidative stress when the balance between pro-oxidant and oxidant:antioxidant status is impaired. MDA is a product of auto-oxidation of polyunsaturated fatty acids and is used as an index of oxidative damage (Cavalca *et al*., 2001). Dubois *et al.* (1994) reported a significant rise in MDA levels and lipid peroxidation with a decrease in antioxidants in patients with unstable angina and chronic heart failure. Tamer *et al.* (2002) also observed high levels of MDA in atherosclerotic patients as compared to controls. Elevated levels of total cholesterol and LDL-cholesterol have also been found to be directly related to the incidence of coronary heart disease (Gupta *et al.,* 2001). The present study observed a significant decrease in HDL levels and a significant increase in total cholesterol, LDL-cholesterol and triglyceride levels in CAD patients. Our results are similar to those of Surekha *et al.* (2007). They also observed a significant decrease in HDL values and significant increase in total cholesterol and LDL values among CAD patients in an Indian population. The significant increase (2X) in MDA levels in CAD patients in the present study is similar to the results of Kaur *et al.* (2008). They observed a significant increase (2X) of MDA levels among CAD patients in Punjabi population. Verma *et al.* (2005) had also demonstrated a significant drop in antioxidant levels whereas lipid peroxides were significantly higher in acute myocardial infarction Indian patients. In our study, TAS levels were also found significantly lower (4X) in CAD patients as compared to that in controls, and are similar to the results of Nojiri *et al.* (2001) had also observed significantly low levels of TAS in CAD patients. While Fazendas *et al*. (2000) had reported that in patients with myocardial infraction, plasma total antioxidant status was decreased constituting a risk factor for coronary artery disease. In the present study, the TOS and OSI levels were significantly higher ($p<0.000$) in patient compared to values in control group indicating increased oxidative stress.

CONCLUSION

The results of the present study show a significant increase in lipid peroxidation in patients with coronary artery disease. A significant increase in total oxidant status and oxidative stress index and significant decrease in total antioxidant status were also observed in these patients. This indicates an imbalance between oxidant and antioxidant molecules in coronary artery disease requiring rectifications as it has ramifications in terms of causing other co-morbidities.

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