Archives of Razi Institute, Vol. 77, No. 2 (2022) 853-859

DOI: 10.22092/ARI.2022.357069.1965

Original Article

Evaluation of Oxidative Stress Parameters and Antioxidant Status in Coronary Artery Disease Patients

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Received 13 December 2021; Accepted 16 January 2022
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Abstract

A surge in oxidative stress and weakened antioxidant defense contributes to the initiation and progression of Coronary Artery Diseases (CAD). The resultant burst in free radicals causes oxidation of lipoproteins mainly oxidized low-density lipoprotein (oxLDL). Further studies need to be conducted to find whether the management of CAD can be evaluated within the context of oxidant/antioxidant balance with the contribution of newer markers. This study was performed to evaluate, compare, and correlate oxidative stress parameters and antioxidant status in CAD patients with controls and evaluate and compare pro-oxidant, a pro-inflammatory enzyme, myeloperoxidase (MPO) and anti-oxidant, anti-inflammatory enzyme, and paraoxonase (PON) between CAD patients and controls. OxLDL, an oxidation product of low-density lipoprotein, malondialdehyde (MDA), an oxidative marker, and reduced glutathione (GSH), an anti-oxidant marker, and lipid profile were assessed and compared in CAD patients and controls. The activity of MPO was correlated with that of PON, and MDA level was correlated with GSH level. A total of 100 clinically proven CAD patients, in the age range of 35-70 years, were selected from the Out Patient Department (OPD) of our Institute. A total of 60 controls in the same age range and without CAD were selected after undergoing health checkups in the hospital. Based on the obtained results, oxLDL, MDA, and MPO were significantly increased in patients than in controls (P<0.05), and PON and GSH were significantly lowered in patients than in controls (P<0.05). Total cholesterol, triglyceride, and LDL were significantly high in CAD patients. A significant negative correlation was observed between MPO and PON levels and between MDA and GSH levels. Increased oxidative stress and decreased antioxidant status were observed in patients with CAD. Formation of oxLDL increased MPO and decreased PON are all additional risk factors for the development of CAD and can be targeted for future therapeutic purposes. Lifestyle modifications and treatment methods can reduce CAD risk through the reduction of oxidative stress and improvement of antioxidant status.

Keywords: Coronary artery disease, Malondialdehyde, Myeloperoxidase, Oxidized low-density lipoprotein, Paraoxonase

1. Introduction

Coronary artery disease (CAD) is one root cause of mortality worldwide. Recent studies have emphasized the role of oxidative stress in the pathogenesis and progression of CAD. Major conventional risk factors for CAD involve diabetes, hyperlipidemia, smoking, hypertension, and obesity. Oxidative stress and related inflammation are also reflected as major risk factors for CAD (1).

Oxidative stress and hyperlipidemia contribute highly to the development of CAD. The lack of balance in

pro-oxidant and antioxidant equilibrium will result in the oxidation of excess lipoproteins which further leads to the pathogenesis of CAD. Uptake of this oxidative product of lipoproteins increases foam cell formation. The formation of these oxidative products, including oxidized LDL, is mediated by enzymes such as myeloperoxidase (2).

In normal physiological homeostatic conditions, an uneven increase in reactive oxygen species (ROS) is kept under control by enzymatic and non-enzymatic antioxidant systems. The level of ROS increases in the presence of the above-mentioned traditional risk factors, such as hypertension, obesity, and diabetes. An increase in ROS concentration increases the synthesis of such pro-oxidant enzymes as myeloperoxidase (MPO) (3). MPO is released from azurophilic granules of leukocytes, mainly neutrophils and partly monocytes. When released on activation, MPO catalyzes the generation of hypochlorous acid (HOCl) in the presence of hydrogen peroxide and halides. Hypochlorous acid thus formed combines with superoxides to form hydroxyl radical which is a lipid peroxide initiator or it can interact with lipoproteins and oxidize them (2).

The enzymatic antioxidant system that has anti-atherogenic properties involves paraoxonase (PON). PON is an enzyme associated with HDL which is believed to protect both HDL and LDL from oxidation. It is produced in the liver and secreted into circulation, and then it combines with high-density lipoprotein (HDL). PON1 is typically associated with HDL in human serum and safeguards lipoproteins from oxidative damage. This defense mechanism offered by HDL is probably associated with the capacity of the PON enzyme to hydrolyze oxidized phospholipids and lipid peroxides (4).

The available epidemiological studies indicate a decreased level of antioxidants and an increased level of oxidants associated with cardiovascular diseases. However, the extent of imbalance in oxidant and antioxidant enzymes and the level of oxidant and anti-oxidant markers together are not well known. The results of some studies suggest that CAD patients show decreased PON1 activity; however, few studies are available on the relationship between MPO and PON1 together with the extent of oxidative stress. Therefore, this study was carried out to evaluate, compare, and correlate oxidative stress parameters and antioxidant status, with special reference to pro-oxidant MPO and antioxidant PON in CAD patients. It evaluates and compares pro-oxidant, a pro-inflammatory enzyme, MPO and anti-oxidant, anti-inflammatory enzyme, and PON in CAD patients and controls. Malondialdehyde (MDA) and oxLDL (both formed as a result of oxidative stress developed in CAD), reduced glutathione (GSH), as an anti-oxidant marker, and lipid profiles were assessed in CAD patients and controls. An attempt was also made to correlate MPO with PON and also correlate MDA with GSH to determine the extent of oxidative stress with reference to oxidant and antioxidant enzymes.

2. Materials and Methods

2.1. Study Subjects and Samples Collection

This study was conducted in Sreelekshmi Narayana Institute of Medical Science, Bharath Institute of Higher Education and Research. The required sample size was determined to be 49 per group with a power of 90% and a significance level of 5%, assuming a difference of 100 ng/ml of oxLDL (assumed standard deviation=150).

A total of 100 clinically proven CAD patients were selected from the OPD of the cardiology department of Sreelekshmi Narayana Institute of Medical Science, Bharath Institute of Higher Education and Research and 60 controls in the same age range and with no history of CAD were selected after undergoing health checkups in the hospital. Samples from each subject were collected by venipuncture when reported at the Out Patient Department (OPD). Blood samples were subjected to centrifugation for 10-15 min at 3000 rpm and serum was separated subsequently. Samples were stored at -80℃ until the time of analysis.

Blood samples were collected from each patient after obtaining written informed consent as per the criteria

laid down by the scientific and ethics committee of the institute. Detailed demographic, anthropometric, and other relevant information were recorded using proforma. Peripheral blood (4ml) was collected from all participants and analyzed to obtain the mentioned biochemical parameters.

2.2. Methods

Oxidized low-density lipoprotein (oxLDL), paraoxonase -1 (PON-1), myeloperoxidase (MPO), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), MDA, and GSH. VLDL was calculated from the obtained data, and oxLDL was measured using the IMTEC-oxLDL antibody IgG/ IgM ELISA kit. MPO and PON1 were detected spectrophotometrically, using the methods adopted by (1), Gur, Aslan (4), respectively. Yaghoubi, Ghojazadeh (5) method was used to estimate MDA. GSH was estimated using commercial assay kits used by Abu-Amero, Al-Boudari (6). Fully automated chemistry analyzers were used to determine plasma lipid profile levels.

2.3. Statistical Analysis

In the case of normally distributed variables, data are presented as mean±SD. The difference in baseline characteristics between the two study groups was tested using a t-test. Correlation analysis was carried out between MPO and PON and between MDA and GSH. Correlation among these parameters was assessed through the calculation of the Karl Pearson correlation coefficient. A *P*-value less than 0.05 ($P<0.05$) was considered to be statistically significant. The statistical analysis was performed using SPSS software (version 21).

3. Results

The study was conducted to assess oxidative stress parameters and antioxidant status in CAD patients. Pro-oxidant, a pro-inflammatory enzyme, MPO and anti-oxidant, anti-inflammatory enzyme, PON, oxLDL, MDA, GSH, and lipid profile were assessed in CAD patients. The values were compared with those obtained for the controls (who were in the same age group). The results indicated that MPO level was correlated with PON and MDA level had a correlation with GSH level.

A total of 100 CAD patients and 60 control individuals participated in this study. In terms of gender, out of 100 patients, 33 (33%) and 67 (67 %) patients were female and male, and out of 60 controls, 39 (65%) and 21(35%) subjects were male and female, respectively. The mean±SD age in the groups of patients and controls were 55 (age range of 35-75 years) and 55.5 years (age range of 36 -75 years), respectively. Baseline characteristics of CAD patients and controls are presented in table 1.

The distribution of the test and control groups, according to the conventional risk factors of CAD, is presented in table 2.

Table 1. Baseline characteristics of CAD patients and control group

Baseline characteristics		CAD (n=100)	CONTROL (n=60)
Age		55(35-75)	55.5 (36-75)
Gender	Male	67	39
	Female	33	21
BMI (kg/m^2)		28.24±3.24	24.07±3.17

Table 2. Distribution of CAD and control subjects according to the risk factors

RISK FACTORS		CAD	CONTROL
DIABETIC	YES	66%	22%
	NO	34%	78%
HYPERTENSION	YES	65%	24%
	NO	35%	76%
SMOKING	YES	81%	31%
	NO	19%	69%
ALCOHOL	YES	28%	16%
	NO	72%	84%

3.1. Biochemical Analysis

3.1.1. Lipid Profile

The lipid profile was compared between CAD patients and control subjects. The mean value of TC, TG, HDL, LDL, and VLDL of these two groups are presented in table 3. The level of TC, TG, LDL, and VLDL showed a significant increase in CAD patients,

compared to controls. Although the HDL value was high in controls, the difference was not statistically significant.

3.1.2. Oxidative Stress and Antioxidant Status

Oxidative stress-related enzyme MPO and antioxidant enzyme PON1 are evaluated and compared in CAD patients and controls. It was revealed that the MPO and MPO/PON1 ratio of CAD patients was significantly high compared to controls, and PON1 was significantly lowered in CAD patients compared to controls. Moreover, lipid peroxidation product MDA and endogenous antioxidant GSH levels were measured in ACS patients and control subjects. It was found that MDA was significantly higher in CAD patients than in controls, and GSH was found to be significantly low in control subjects, compared to CAD patients. The comparison of the level of oxidized LDL showed that OxLDL was significantly high in CAD patients, compared to controls. The comparison of the mean value of MPO and PON1 activity, MPO/PON1 ratio, MDA, GSH, and oxLDL between CAD patients and control subjects are presented in table 4.

Correlation analysis was performed between the oxidative stress and antioxidant markers. MPO activity was correlated with PON, and MDA was correlated with antioxidant GSH. The correlation was considered to be significant at $P<0.05$. A statistically significant negative correlation ($P=0.0002$) was observed between MPO and PON levels. Similarly, a statistically significant negative correlation ($P=0.054$) was observed between MDA and GSH levels. Correlation coefficients and *p*-values are presented in table 5.

Table 3. Lipid profile in CAD and control subjects

Parameters	CAD patients	Control	*P*-Value
Total Cholesterol mg/dl	225.49 ± 50.00	196.92 ± 32.19	0.000
Triglycerides mg/dl	173.84 ± 52.72	125.25 ± 69.57	0.05
High Density Lipoprotein mg/dl	44.29 ± 11.56	52.88 ± 11.74	0.10
Low Density Lipoprotein mg/dl	148.62 ± 36.18	128.68 ± 20.15	0.01
Very Low Density Lipoprotein mg/dl	34.76 ± 10.54	25.05 ± 13.91	0.05

Results are expressed in mean ± SD

Table 4. MPO, PON1, MPO/PON1 ratio, MDA, GSH, and oxLDL in CAD and control subjects

Parameters	CAD	Control	*P*-Value
MPO (U/ml)	278.88 ± 172.55	151.05 ± 56.95	0.049
PON (U/ml)	315.17 ± 80.05	410.56 ± 86.53	0.000
MPO/PON ratio	0.955 ± 0.68	0.374 ± 0.14	0.002
MDA (ng/dl)	2.95 ± 1.13	2.03 ± 0.44	0.010
GSH (mg/dl)	7.63 ± 2.10	8.22 ± 2.98	0.013
OxLDL (ng/ml)	40.27 ± 47.23	20.75 ± 19.05	0.03

Results expressed as mean ± SD

Table 5. Correlation analysis of MPO with PON and MDA with GSH

	CAD		
MPO	PON	Pearson Correlation Sig. (2-tailed)	-0.34 0.0002**
MDA	GSH	Pearson Correlation Sig. (2-tailed)	-0.209 0.054*

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

Huang, Mai (7), first put forward the idea of risk factors. Age, gender, race, family history, total cholesterol, HDL-C, high sensitive C-reactive protein (hsCRP), blood pressure, diabetes, and smoking status are suggested to be the strong 10-year risk predictors as per the updated risk assessment guidelines for atherosclerotic cardiovascular disease by the American College of Cardiology and the American Heart Association. Proper understanding of these conventional risk factors is critical in the prevention of disability and early death (8).

It was observed that 81% of the CAD patients were smokers and 65% were hypertensive. In a study conducted by Abu-Amero, Al-Boudari (6), smoking was described as a major risk factor for CAD and was closely linked to increased oxidative stress. An increase in oxidative stress and reduced antioxidant status has been related to the average number of cigarettes smoked per day by a particular person (9). In this study, 66% of CAD patients suffered from diabetes, which is another major cardiovascular risk factor and is associated with a higher incidence of CAD and cardiovascular deaths (10).

Oxidative enzyme MPO and antioxidant enzyme PON were estimated in our study subjects. The results showed a statistically significant increase in MPO activity in CAD patients, and PON was significantly lowered in CAD patients, compared to healthy controls. MPO/PON ratio was calculated which was significantly high in CAD patients. Based on the results of the study conducted by Bacchetti, Ferretti (11), leukocyte-derived MPO catalyzes the formation of ROS which causes alteration in the structure and function of HDL and eventually results in cardiovascular risk. On the contrary, PON coupled with HDL protects the associated lipids from oxidation and forms a defending factor against CVD. Moreover, CVD in patients is depicted by a high MPO/PON ratio (11). Current studies have indicated the formation of an efficient complex between HDL, PON1, and MPO. Here, HDL serves as a platform upon which MPO and PON1 interact during inflammation. MPO is partially inhibited by the binding of PON1, and PON1 in turn undergoes oxidative modification by MPO (12). Another study conducted by Aggarwal, May-Zhang (13) has shown that the association of MPO with HDL reduces PON1 activity to some extent via modification of PON1, and this contributes to an increased risk of CAD. Therefore, CAD risk can be reduced by hindering this type of modification (13).

OxLDL measured in our study was found to be significantly high in CAD patients. A study conducted by Huang, Mai (7) showed that the level of oxLDL was high in CAD. They have reported that the ratio of oxLDL to total cholesterol, LDL-C, HDL-C, and albumin were better biomarkers than conventional markers, such as total cholesterol, triglyceride, HDL-C, and LDL-C for differentiating CAD patients from controls. They have shown that patients with an elevated ratio of oxLDL to total cholesterol may increase the risk of developing CAD (7).

MDA level was significantly higher in CAD patients, compared to controls. A study conducted by Yaghoubi, Ghojazadeh (5) showed that serum MDA was significantly higher in CAD patients compared to controls. Based on their findings, serum MDA serves as a diagnostic marker in CAD and helps assess disease severity (5).

GSH levels were significantly low in CAD patients than in controls. A study conducted by Bastani, Rajabi (14), revealed a marked increase in MDA level and a significant decrease in GSH concentration in CAD groups compared to controls. The results of this study indicate that increased oxidative damage is more common in the acute form of CAD which also leaves a margin for the adoption of antioxidant therapy (14).

In the present study, MPO level was correlated with PON, and a significant negative correlation was observed between MPO and PON. The study conducted by Lixia, Feng (15), in 2010, observed that MPO was significantly higher and PON was

significantly lower in the CHD group, compared to the control group. Moreover, MPO increased gradually with CAD type and severity, while the PON decreased gradually (15). Many other studies reported an increase in MPO with a corresponding decrease in PON in the patients' group, compared to the control group (16, 17).

Based on the results of a study conducted by Yunoki, Naruko (18), plasma MPO has a significant inverse correlation with PON-1, especially in female stable angina pectoris (SAP) and Unstable angina pectoris (UAP) patients. Their study also suggests that an imbalance between the pro-oxidants and anti-oxidants further promotes the advancement of coronary plaque instability (18).

Zsiros, Koncsos (19), in 2016, demonstrated a significant negative correlation between MPO and PON1 activity in hyperlipidemia patients with and without vascular complications. They have suggested that PON1 activity can be an independent predictor of MPO levels. The results of this study suggest that parallel investigation of MPO and PON1 activity is a more precise indicator of atherosclerosis, and allows for earlier and advanced treatment (19).

The results of the comparison between MDA and GSH levels showed a statistically significant negative correlation between MDA and GSH. In a study conducted by Uppal, Uppal (20), MDA was significantly increased and GSH showed a significant depletion in CAD patients, compared to controls. This study showed a considerable decrease in GSH levels in the case of stable angina patients. A significant negative correlation was observed between glutathione and MDA levels. In oxidative stress, an increase in the MDA level causes lipid peroxidation, and GSH is utilized for destroying the free radicals formed during this process. The role of oxidative stress in the progression of CAD from stable angina to unstable angina to myocardial infarction can be explained by a decrease in GSH level and elevation in MDA level in unstable angina and myocardial infarction, compared to stable angina (20).

5. Conclusion

Oxidative markers and antioxidant status in CAD patients were assayed in this study with special reference to pro-oxidant and antioxidant enzymes. An increase was observed in pro-oxidant MPO and oxidative marker MDA. Antioxidant enzymes PON and GSH are lowered in the case of proven CAD patients. Based on the obtained results, it can be suggested that these two enzymes may be used to reveal the extent of inflammation around the atheroma. Detection of these enzymes can be coupled together with other inflammatory markers and provide a new source for diagnosing and forecasting CAD. External antioxidants can be supplied to reduce oxidative stress in such patients. Meanwhile, key pathways comprised of other important pro-inflammatory markers involved in atherogenesis should also be studied in detail.

Authors' Contribution

Study concept and design: S. S. A.
Acquisition of data: S. V.
Analysis and interpretation of data: S. S. A.
Drafting of the manuscript: S. V.
Critical revision of the manuscript for important intellectual content: R. V. and S. M. P.
Statistical analysis: S. V.
Administrative, technical, and material support: R. V., S. V., S. S. A., and S. M. P.
Study supervision: R. V. and S. M. P.
All authors collaborated in the conduction of this study. All authors read and approved the final manuscript.

Ethics

The study protocol was approved by the Ethical committee in the SMCSIMCH/EC(PHARM)02/02/12. Written informed consent was obtained from the participants before the start of the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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