



Malondialdehyde: An oxidative stress biomarker evaluation in patients with coronary artery disease, diabetes and subjects with obesity

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Abstract

Oxidative stress is known to involve in the pathogenesis of various disease including coronary artery disease (CAD). Diabetes mellitus and obesity are known to be the risk factors for CAD. Hence in the present study we investigated the levels of oxidative stress marker, Malondialdehyde (MDA) which is a breakdown product of peroxidation of long chain fatty acids in patients with diabetes mellitus or obesity to identify the possible link between oxidative stresses in the occurrence of CAD in diabetes mellitus or obesity. Blood samples were collected from patients with CAD, DM and obese subjects and analyzed for MDA estimation by HPLC. Results show that there was a significant increase in serum MDA levels with CAD, DM and obese subjects when compared to healthy controls. In conclusion, based on the result obtained it may be presumed that increased oxidative stress seen in diabetes mellitus and obese subjects is one of the risk factors for CAD.

Keywords: oxidative stress, coronary artery disease (CAD), diabetes mellitus (DM), obesity, malondialdehyde (MDA), high performance liquid chromatography (HPLC)

1. Introduction

Coronary Artery Disease (CAD) occurs within the walls of arteries whose function is to supply blood to the heart. These arteries will show plaque development as a result narrows the arteries over time, which could partially or totally block the blood flow^[1,2] leading to atherosclerosis. Important factors responsible for cardiovascular disease, in addition to smoking and diabetes mellitus, is obesity- a growing worldwide health problem that is associated with reduced life span^[3,4], which generally tends to be the leading cause for morbidity and mortality. During the period 2010-2013, in countries like India the overall frequency of deaths was raised to 23% of which 32% were adult deaths.

Diabetes mellitus is a metabolic disorder in which the pancreas have reduced capacity to produce or respond to insulin which leads to improper levels of glucose in the blood. This when continued for a long period of time results in hyperglycemia? In most cases type II diabetes causes CAD. Despite the latest advancement in diagnosis and treatment of cardiovascular disease (CVD) the risk of diabetes mellitus continues to increase over a past few decades^[5,6]. As per the statistics of WHO, in the year 2012 diabetes became the root cause for 1.5 million deaths, making it the 8th leading cause of mortality. According to the International Diabetes Foundation. India was said to have more diabetic patients compared to the other countries in the world.

Obesity on the other hand is a serious medical condition. The scale of obesity is measured beyond 30 kg/meter² of BMI. According to the

WHO Statistics Report 2012, globally one out of six adults is obese and the death rate accounts to nearly 2.8 million individuals each year. India being the second most populous country in the world is experiencing a rapid transition with increasing obesity cases.

Oxidative Stress plays a major role in the development of atherosclerosis as it takes up smooth muscle cells and macrophages which helps in formation of foam cells which is a vital step in the progress of the pathological state^[7,8]. An imbalance between free radicals and antioxidants (Pro-oxidant and anti-oxidant imbalance) may lead to lipid peroxidation and tissue damage by attacking proteins and DNA due to production of highly reactive and unstable free radicals and cause prolonged oxidative stress^[9,10]. Oxidative stress also has its adverse effect on diabetes mellitus, where increased ROS production induces higher oxidative damage in the circulation and reduces antioxidant defense mechanisms. Increased lipid peroxidation and fat accumulation in human has been associated with oxidative stress biomarkers. Malondialdehyde (MDA) is a possible biochemical marker for coronary artery disease (CAD). Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde (Fig: 1). In recent years, oxidized LDL has attracted attention as a blood marker that is also associated with CAD. Oxidized LDL plays important roles in the formation and development of the primary lesions of atherosclerosis. The purpose of this study is to investigate the role of oxidative stress using MDA as biomarker in patients with CAD, Obesity and Diabetes Mellitus.

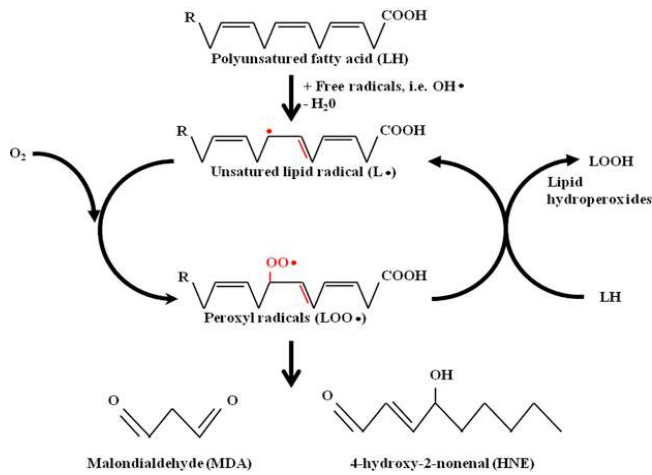


Fig 1: Formation of MDA from polyunsaturated fatty acids through lipid peroxidation and ROS ⁽¹¹⁾.

2. Materials and Methods

A total 400 subjects were recruited in the present study, which includes 100 angiographically documented CAD patients, 100 diabetic subjects, 100 obese subjects and 100 healthy controls from NIMS (Nizam’s Institute of Medical Science Hyderabad, India). After 12hrs overnight fasting, 5 ml blood was collected in plain vacutainers. The serum is separated by centrifugation at 2500 rpm for 20 min. Thus collected serum is stored at -20°C for further biochemical analysis. Institutional Ethical Committee has approved this project and informed consent was obtained from all the subjects. We analyze malondialdehyde by reverse phase HPLC in human serum.

2.1 Reagents Required

Rinsing Solution - 50% Methanol, 5M Potassium Hydroxide (KOH), 50 mM Potassium di hydrogen Phosphate in water (50mm KH₂PO₄), pH 6.5, MOBILE PHASE: 50mM KH₂PO₄ in water (pH 6.5): Methanol (60:40 Ratio), Solid phase extraction of sample 0.2% Orthophosphoric Acid (0.2% OPA), 50% glacial acetic acid, 0.6% Thiobarbituric Acid (TBA) and dissolve in 100mL of 50% glacial acetic acid.

Stock Solutions: TEP (1, 1, 3, 3 Tetraethoxy Propane) Working Stock Solution (10µM/mL)

Dissolve 25 µL of TEP stock solution in 10mL of distilled H₂O. Working Standard Solutions: Using distilled H₂O as diluents prepare working solution as below table 1.

Table 1: Preparation working standards by serial dilution

ID	Conc. (nM/mL)	Volume of STD (mL)	Volume of Water (mL)	Final Volume (ml)
Working Stock (10 µM/ml)	10000	0.010	0.990	1.000
STD 1	100	0.500	0.500	
STD 2	50	0.400	0.600	
STD 3	20	0.500	0.500	
STD 4	10	0.500	0.500	
STD 5	5	0.400	0.600	
STD 6	2	0.500	0.500	
STD 7	1	0.500	0.500	

Concentrations of 100nM/mL, 50nM/mL, 20nM/mL, 10nM/mL, 5nM/mL, 2nM/mL, 1nM/mL and 0.5nM/mL were taken as

Standard 1 to standard 8 for analysis.

2.2 Sample Preparation

Take 50 µL of serum standards into micro centrifuge tube (1.5ml tube), add 400µL of 0.2% OPA solution and vortex, add 100 µL OF 0.6% TBA solution and vortex, incubate at 100°C (heating blocks or water bath) for 30 min, Immediately place in ice bath for at least 10 min, centrifuge at 12000 rpm at 10°C for 5 min, transfer the supernatant into HPLC vials and place in auto sampler.

2.3 Statistical Analysis

Quantitative variables were expressed as mean ± standard deviation using Student t-test and qualitative variables as percentages. P value less than 0.05 (p<0.05) was considered statistically significant.

3. Results & Discussion

From the demographic details in table 2 we can conclude that the mean age of all the 209 CAD patients is said to be 53.79 ± 11.64 years, the mean age of all the 100 diabetic subjects is 53.27 ± 11.39 years and the mean age of all the 82 obesity subjects is 36.79 ± 9.19 years when compared to the mean age of the controls which is 47.25 ± 12.15. The percentage of ratio of males is to females from (fig-2), in CAD patients is 59.42%:40.57%, in diabetic subjects is 61%:39%, in obese subjects it is 70.73%:29.26% and that of control subjects is 54.05%:49.94%. The BMI average of CAD subjects is 25.40 ± 5.13 kg/m², of diabetic subjects is 24.51 ± 2.68kg/m², of obese subjects is 32.90 ± 2.41kg/m² when compared to the controls having BMI of 22.34 ± 4.93. The percentage of smoking and alcohol in CAD subjects is 53.6 % and 32%, of diabetic subjects is 28% and 37%, of obese subjects is 46% and 30% and the percentage of controls are said to be 44% and 28%.

The lipid profiles average of TC, TG, HDL and LDL from fig-3, in CAD is 199.81 ± 106.39 mg/dL, 188.73 ± 107.05 mg/dL, 37.95 ± 16.49 mg/dL and 91.43 ± 49.67 mg/dL, in diabetic subjects it is 159.1 ± 42.42 mg/dL, 159.1 ± 42.42mg/dL, 43.42 ± 12.88mg/dL and 82.43 ± 36.03mg/dL, of obese subjects is 158.64 ± 43.99 mg/dL, 176.59 ± 99.64 mg/dL, 42.59 ± 13.58 mg/dL and 81.14 ± 37.51mg/dL and that of controls is 172.2 ± 10.6mg/dL, 137.9 ± 20.1mg/dL, 38.5 ± 2.3mg/dL and 102 ± 8.9mg/dL. In the present study there was a statistically significant increase in the levels of MDA in patients of CAD, Diabetes and obesity (9 ± 10.35, 8.4 ± 5.33 and 14.5 ± 8.62 nmol/ml, p<0.0001) as compared to controls (4.6 ± 3.013nmol/ml). The high concentration of MDA in all the disease groups indicates increased membrane lipid peroxidation.

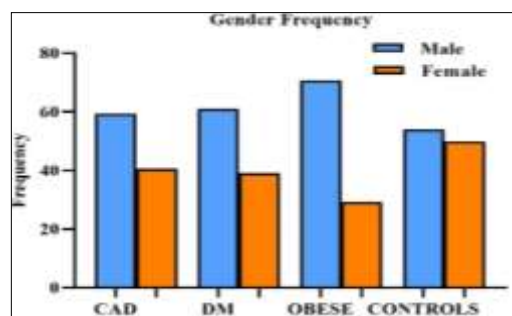
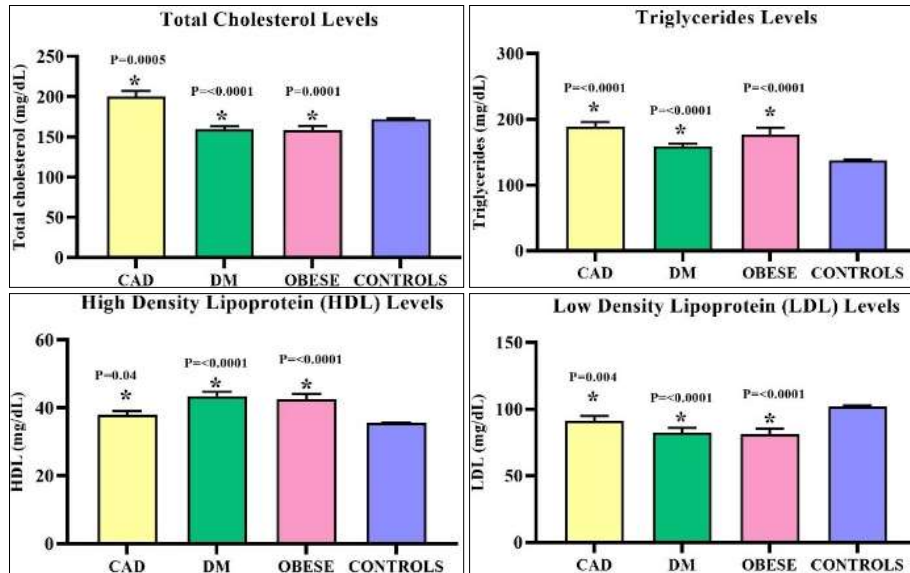


Fig 2: Percentages of males is to females taken in the study under each category

Table 2: Demographic and clinical characteristics of the study subjects

Variables	Cad patients (n=207)	Diabetis subjects (n=100)	Obesity (n=82)	Controls (n=185)
Mean age (yrs)	53.79±11.64*	53.27±11.39*	36.79±9.19*	47.25±12.15
Gender				
Male	123(59.42%)	61(61%)	58(70.73%)	100(54.05%)
Female	84(40.57%)	39(39%)	24(29.26%)	85(49.94%)
Bmi kg/m ²	25.40±5.13*	24.51±2.68*	32.90±2.41*	22.34±4.93
Smokers (%)	53.6 %	28%	46%	44%
Alcohol intake (%)	32%	37%	30%	28%

Values are expressed in Mean ± SD; BMI- Body mass index



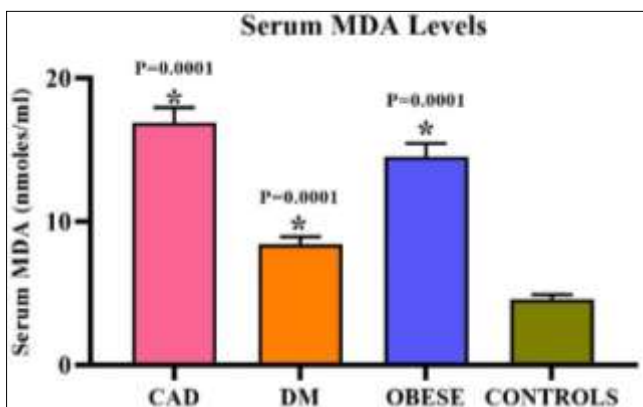
TC - Total cholesterol; TG - Triglycerides; HDL - High density cholesterol; LDL - Low density lipids.*p<0.05 is considered as significant

Fig 3: Lipid Profile in Cad, Dm, Obese & Control Subjects

Table 3: Serum malondialdehyde levels in controls, CAD, DM and obese subjects

Disease Groups	MDA (nmoles/ml)	P value
Controls (n=92)	4.6±3.013	---
CAD (n=100)	16.9±10.35	0.0001*
Diabetes mellitus (n=95)	8.4±5.33	0.0001*
Obesity (n=82)	14.5±8.62	0.0001*

*Patients vs Controls. *P value <0.05 considered as significant



Values are expressed as the mean ± SD. Statistically significant difference, p= 0.0001, control versus case groups

Fig 4: Serum levels of malondialdehyde among healthy control vs CAD, T2DM subjects and Obesity.

4. Discussion

Coronary Artery disease also termed as atherosclerosis. Oxygen is said to be a major factor of cardiac gene expression, and a critical contributor in the formation of reactive oxygen species (ROS). Oxidative stress plays a substantial role in the pathogenesis of coronary atherosclerosis and its complication. Oxidative stress arises when ROS evade or overwhelm antioxidants^[12]. Due to their highly reactive and non-specific nature, ROS can attack almost all biomolecules including lipid membranes^[13]. Lipid peroxides are derived from the oxidation of polyunsaturated fatty acids of membranes and are capable of further lipid peroxidation by a free radical chain reaction^[14]. Malondialdehyde (MDA) is a breakdown product of peroxidation of long chain fatty acids which accumulates when lipid peroxidation increases^[15]. MDA is used as an index of oxidative damage^[16] reported a significant rise in MDA levels and lipid peroxidation with a decrease in antioxidants in patients with unstable angina and chronic heart failure. In other study high levels of MDA in atherosclerotic patients was observed as compared to controls^[17]. In the present study we have found significant increase in MDA levels in CAD patients when compared to controls. Some complications of diabetes are associated with increased activity of free radical-induced lipid peroxidation and accumulation of lipid peroxidation products^[18]. It is also

involved in oxidative stress, which in turn exaggerates diabetes associated complications. Beta cells of the pancreas and vascular endothelium are highly susceptible to oxidative stress. This is due to the fact that many biochemical pathways strictly associated with hyperglycemia increase the production of free radicals. Serum levels of MDA were high in diabetic subjects whereas in normal healthy subjects (4.6 ± 3.013). Significantly higher levels of MDA were observed ($p = 0.0001$) in diabetic patients in comparison to healthy controls. In recent decades, the prevalence of obesity has increased alarmingly, making it a significant health problem in not only high-income countries, but low and middle-income countries as well [19]. Biomarkers of oxidative damage are higher in individuals with obesity and correlate directly with BMI. In the present study we also found significant increased serum MDA levels ($p=0.0001$) as compare to the controls Lipid peroxidation is thought to be a component of obesity-induced pathology [20]. Obesity is associated with derangements in the lipid profile, which further increases the risk of coronary heart disease, diabetes mellitus, stroke and certain cancers [20]. The risk of CAD appears to be more in males compared to females.

5. Conclusions

Obesity and type 2 Diabetes mellitus are serious and growing health care problems worldwide, leading to an increased risk for coronary artery disease. The elevated levels of serum MDA in CAD, Type 2 Diabetes mellitus and Obesity Groups in present study clearly reflects the increasing burden of oxidative stress and MDA serves as a good indicator of oxidative stress. In the clinical practice, there is the need for affordable, accessible and safe biomarkers for the early detection of diseases and also to identify high risk subjects who might benefit from increased screening surveillance. Free radical induced damage can be prevented by supplementation of antioxidants and antioxidants rich fruits including green tea etc. Fruits and green tea contains phytochemicals possess strong antioxidant properties.

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