ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Association of Oxidation of Low Density Lipoproteins with Atherosclerosis In Patients With or Without Diabetes Mellitus.

Kamna Singh[1], Ritu Singh[1], Sudhir Chandra[1,*], and Sanjay Tyagi[2].

[1]Department of Biochemistry, Lady Hardinge Medical College, New Delhi-110001, India.
[2]Department of Cardiology, G.B. Pant Hospital, New Delhi-110002, India.

ABSTRACT

Atherosclerosis is one of the chronic arterial diseases, the leading cause of deaths among low-income and middle-income countries. The present study aims to evaluate the role of oxidative stress biomarkers in atherosclerotic patients with and without diabetic mellitus as well as in control subjects in Northern Indian population. We analyzed glutathione peroxidase (GPx), superoxide dismutase (SOD), Oxidised LDL (Ox- LDL) and antioxidised LDL antibody (antiox-LDL abs) levels by Daytona autoanalyser and ELISA, respectively. The levels of GPx and SOD were significantly ($p<0.0001$) lower in Group I and Group II as compared to Group III. The Ox-LDL levels were significantly higher in Group II (7.0 ± 4.5) as compared to Group I (5.0 ± 3.6) and Group III (2.9 ± 2.9). However, antiox-LDL abs levels were significantly lower in Group II (342.0 ± 172.0) as compared to Group I (539.0 ± 166.0) and Group III (621.0 ± 109.0). The down-regulated levels of GPx, SOD, antiox-LDL abs and elevated levels of Ox-LDL in patients may be associated with the pathogenesis of atherosclerosis. The levels of oxidative stress biomarkers and antiox-LDL abs might be potential determinants of susceptibility to atherosclerosis in patients with type 2 diabetes mellitus.

Keywords: Atherosclerosis, glutathione peroxidase, superoxide dismutase, Oxidised LDL, antioxidised LDL antibody.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Cardiovascular diseases (CVDs) are known as the disorder of heart and blood vessels which includes Coronary artery disease, coronary heart disease, cerebrovascular disease, rheumatic heart disease and various other conditions. According to World Health Organisation (WHO) data sheet 2017, more than 75% deaths by CVD reported in low-income and middle-income countries, while 80% of all CVD deaths are as a consequence of heart attacks and strokes [1]. Atherosclerosis is one of the chronic arterial diseases among CVD, caused by the gradually progression of plaques and their acute rupture leading to local thrombosis and partial or total occlusion of the associated artery [2]. Recent atherosclerosis reports suggested that approximately 18 million people are suffering from this disease out of total 1.06 billion Indian populations [3].

Diabetes is one of the major and independent risk factor for cardiovascular disease [4]. Previous Framingham study had been reported about the association between glycemic control and CVD [5]. One of the mechanisms suggested that high glucose status increases the adhesion of monocytes to arterial endothelial cells through activation of NFkB pathways [6]. Monocytes are differentiated into intimal macrophages, which accumulate lipids in the artery wall and lead to fatty streak formation and further developed into advanced lesions, which become unstable and rupture, resulting in clinical appearance of CVD. Several studies have reported that the levels of non-enzymatic well as enzymatic antioxidants are important factors, associated with type 2 diabetes [7-8]. Down-regulated activity of these antioxidant enzymes may accelerate the production of ROS and oxidative injury, further progression of type 2 diabetes mellitus [9] and atherosclerosis [10]. These studies confirmed the oxidative modification hypothesis and ROS production may induce oxidation of LDL, cholesterol derived particles and modification of several proteins, which give rise to foam cell formation and atherosclerotic plaques [11]. Recent clinical study revealed that oxidation of LDL is associated with various pathological conditions and oxidative modification of LDL (Ox-LDL) involved in pathogenesis of atherosclerosis [12], and diabetes mellitus-induced atherogenesis [13]. The mechanism and function of antioxidized LDL antibodies (antiox-LDL abs) in the pathogenesis of atherosclerosis and diabetes is complex and still not clear. The levels of these antibodies have been shown to predict the development of advanced atherosclerotic lesions [14] and peripheral vascular disease [15]. Apart from altered levels of antiox-LDL abs in disease conditions, their clinical role in cardiovascular disease pathogenesis is still under research and discussion. The aim of the present study was to evaluate the role of oxidative stress biomarkers in atherosclerotic patients with and without diabetic mellitus as well as in control subjects in Northern Indian population.

MATERIAL AND METHODS

Study population

A total of 150 study subjects were recruited for the present pilot study, were residents of North India. Study population is comprised of three groups:

Group I: Angiographically proven atherosclerotic CAD patients (N=50) without diabetes mellitus (DM).
Group II: Included angiographically proven atherosclerotic CAD patients (N=50) having DM.

Inclusion criteria: i) Angiographically proven patients [Age between 35 years to 70 years], who have 70% or more stenosis of at least one of the major coronary artery. ii) CAD patients without DM iii) CAD Patients with DM. Exclusion criteria: i) Patients with less than 70% stenosis, ii) Patient with abnormal liver function test and iii) Autoimmune diseases like Rheumatoid Arthritis.

Patients were recruited from Department of Cardiology, G.B. Pant Hospital, Delhi, India in collaboration with Department of Biochemistry, Lady Hardinge Medical College, Delhi, India. All patients had stenosis (>50%) that was severe enough to require intervention, assessed by coronary angiography. The American Diabetes Association (ADA) recommendation and criteria was used for selection of CAD patients with type-2 diabetes mellitus [16-17].

Group III: Included patients (N=50) found to have no or insignificant atherosclerosis, considered as controls.

Inclusion criteria: No or insignificant atherosclerosis, when angiography was done.

Exclusion criteria: i) Patients with diabetes mellitus or autoimmune disease like rheumatoid Arthritis and ii) Patient with CAD.

Group I & II were considered as cases, whereas group III was considered as controls. Cases and controls were matched for age and sex. The study was conducted in accordance with the guidelines of the Helsinki Declaration and written informed consent was obtained from all the participants recruited for the study. A detailed history of study subjects was obtained, followed by a complete clinical examination. Patients already undergoing angiography as advised by Cardiologist were included as study group. An approval of ethics committee of G.B. Pant Hospital, Delhi, India and Lady Hardinge Medical College, Delhi, India was obtained prior to the study.

Sample collection and processing

Two millilitres (2 ml) of blood were taken in plain and EDTA vials. Serum was used for Ox- LDL, antiox-LDL abs estimation, while whole blood EDTA samples were used for Gpx and SOD levels. The venous blood sample taken without anti-coagulant was allowed to clot, centrifuged and serum was separated. Serum and whole blood EDTA samples were stored at -70^{o}C and -20^{o}C for estimation of Ox-LDL, antiox–LDL abs and Gpx and SOD levels, respectively.

Glutathione Peroxidase (GPx) and Superoxide dismutase (SOD) levels

The levels of antioxidants such as GPx and SOD were estimated by Daytona autoanalyser using commercially available kits Ransel and Ransod (RANDOX Laboratories Ltd) respectively according to the manufacturer's protocol.

Oxidised LDL (Ox- LDL) and antioxidised LDL antibody (antiox-LDL abs) levels

Ox- LDL and antiox-LDL levels were estimated by ELISA using biomedical kits according to the manufacturer's protocol.

Statistical analysis

All statistical analysis was performed with the IBM SPSS statistical package 20. A one-way-ANOVA and post-hoc scheffe test was performed for the comparisons of continuous variables between the groups. Results were expressed as mean ± standard deviation (SD).

P <0.05 considered to be significant.

RESULTS

Glutathione Peroxidase (GPx) and Superoxide dismutase (SOD) levels

The levels of GPx were significantly (p<0.0001) lower in Group I (293.6 ± 112.0) as compared to Group III (686.0 ± 115.0) as well as in Group II (197.0 ± 74.0) as compared to Group III. Similarly, SOD levels were also significantly (p<0.0001) lower in Group I (256.8 ± 75.3) as compared to Group III (487.0 ± 110.0) as well as in Group II (138.0 ± 31.0) as compared to Group III **(Table 1)**.

Oxidised LDL (Ox-LDL) and antioxidised LDL antibody (antiox-LDL) abs levels

The Ox-LDL levels were significantly higher in Group II (7.0 ± 4.5) as compared to Group I (5.0 ± 3.6) and Group III (2.9 ± 2.9). However, antiox-LDL abs levels were significantly lower in Group II (342.0 ± 172.0) as compared to Group I (539.0 ± 166.0) and Group III (621.0 ± 109.0) **(Table 1)**.

ISSN: 0975-8585

Table 1: Oxidative stress biomarkers levels in study subjects

Parameters	CAD (Group I) (N=50)	CAD with DM (Group II) (N=50)	Controls (Group III) (N=50)	*p-Value between groups
GPx (U/l)	293.6 ± 112.0	197.0 ± 74.0	686.0 ± 115.0	I Vs II = 0.0001 I Vs III = 0.0001 II & III = 0.0001
SOD (U/ml)	256.8 ± 75.3	138.0 ± 31.0	487.0 ± 110.0	I Vs II = 0.0001 I Vs III = 0.0001 II & III = 0.0001
Ox-LDL (µg/ml)	5.0 ± 3.6	7.0 ± 4.5	2.9 ± 2.9	I Vs II = 0.037 I Vs III = 0.025 II Vs III= 0.0001
antiox-LDL abs (MU/ml)	539.0 ± 166.0	342.0 ± 172.0	621.0 ± 109.0	I Vs II = 0.0001 I Vs III = 0.028 II Vs III = 0.0001

antiox-LDL abs, antioxidised low density lipoprotein antibody; GPx, Glutathione peroxidase; Ox-LDL, Oxidised low density lipoprotein; SOD, Superoxide dismutase; MU/ml, milli units/milliliter; U/l, Unit/liter; U/ml, Unit/milliliter; µg/ml, microgram/milliliter.
Patients group were compared with controls with analysis of variance (ANOVA); *$p < 0.05$ is considered to be significant.

DISCUSSION

The association between diabetes and CVD has been well established [4-5]. An elevated glucose level induces diacylglycerol accumulation and activation of protein kinase C in vascular cells, resulting in upregulated glucose flux via aldose reductase pathway. During elevated glucose levels, up-regulated expression of inflammatory cytokines was observed [18], associated to the production of reactive oxygen species (ROS) and glucose dependent oxidative stress [19]. An increase in ROS production and reduced antioxidant potential may lead to the various complications in diabetic patients including oxidative DNA damage and insulin resistance conditions [20]. The levels of antioxidants play an important role in type 2 diabetes [7-8], their reduced activity promotes oxidative stress and ROS generation leading to the development of atherosclerosis and other CVDs.

In our study, we found that the levels of antioxidants such as GPx and SOD were significantly reduced in CAD patients with DM as compared to CAD patients and controls subjects. GPx-1 and SOD have both been reported as major antioxidants which help to protect and prevent against oxidative damage in patients with CAD [21]. Previous studies also reported that levels of SOD and GPx were lower in diabetic patients than the controls [9]. Reduced activity of GPx-1 may correlate with the progression of atherosclerotic plaques and severe lesions, an important factor for the cause of atherosclerosis in humans [10]. Our results are in consistent with the previous study in which SOD activity decreased with the development of CAD [22]. Similar results have also been observed by Pytel et al. (2013), suggested that SOD activity in CAD patients was 17% lower when compared with healthy individuals [23]. SOD catalyzes the dismutation of oxygen free radical to H_2O_2, which can further be reduced by catalase and GPx. The potential role of SOD is to inhibit the oxidative changes caused by oxygen free radical, help in the prevention against progression of type 2 diabetes and atherosclerosis.

During hyperglycemia, production of ROS is remarkably increases via multiple processes. Glucose can undergo nonenzymatic reactions forming gluco-oxidants and glycated products, which results in the modification of LDL (oxidised and glycated LDL). In addition to classical LDL receptors, macrophages derived from circulating monocytes can take up modified LDL via scavenger receptors. Scavenger receptors recognize chemically and biologically modified lipoproteins, typically oxidized LDL [24]. Ox-LDL potentially promotes atherogenesis, in addition to the ability to be taken up rapidly by macrophages to form foam cells. In our study, Ox-LDL levels were significantly increased in CAD and CAD with DM group as compared to controls. Our

results are inconsistent with the previous studies showed that Ox-LDL levels were significantly higher in patients with coronary artery disease [25] and diabetes [26] as compared to controls. We observed a significant decrease in antioxidised LDL antibodies (antiox-LDL abs) titre in cases as compared to controls. It has been hypothesized that the physiological role of antiox-LDL abs is to remove Ox-LDL from the circulation by mean of soluble antigen-antibody complexes [27]. The reduced levels of antiox-LDL abs may be correlated with the insufficient scavenging and increase in Ox-LDL species, ultimately lead to the progression of atherosclerosis and in diabetic patients [28]. Several hypotheses have been raised to explain the inverse relationship between antibody against Ox-LDL titres and atherosclerosis. Ox-LDL seems to be an immunogenic molecule that stimulates the induction of antiox-LDL abs. Antiox-LDL abs is present in healthy individuals as well as in CAD patients possessing the dual role, by virtue of either protective or pathogenic. The major limitation of our study is small sample size. Further studies in extended sample size are needed to understand the patho-physiological role of oxidative stress biomarkers in atherosclerotic patients with and without diabetic mellitus.

CONCLUSIONS

The down-regulated levels of GPx, SOD, antiox-LDL abs and elevated levels of Ox-LDL in patients may be associated with the pathogenesis of atherosclerosis. The levels of oxidative stress biomarkers and antiox-LDL abs might be potential determinants of susceptibility to atherosclerosis in patients with type 2 diabetes mellitus. However, a detail study in extended sample size with patients follow-up is warranted to confirm the significance of oxidative stress biomarkers in atherosclerosis.

ACKNOWLEDGEMENTS

This work was supported by Lady Hardinge Medical College, Delhi, India.

REFERENCES

[1] World Health Organisation. Global Hearts: a new initiative to prevent Heart attacks and Strokes. The World Health fact sheet 2017; New York.
[2] Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. Circ Res 2014; 114: 1852-1866.
[3] https://www.omicsonline.org/india/atherosclerosis-peer-reviewed-pdf-ppt-articles/. Accessed on 08.09.2017.
[4] American Diabetes association. Consensus Statement: Role of cardiovascular risk factors in prevention and treatment of macrovascular disease in diabetes. Diabetes Care 1993; 16: 72-78.
[5] Kannel WB, and McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. Diabetes Care 1979; 2: 120-126.
[6] Piga R, Naito Y, Kokura S, Handa O, Yoshikawa T. Short-term high glucose exposure induces monocyte-endothelial cells adhesion and transmigration by increasing VCAM-1 and MCP-1 expression in human aortic endothelial cells. Atherosclerosis 2007; 193: 328-334.
[7] Bigagli E, Raimondi L, Mannucci E, Colombi C, Bardini G, Rotella CM, et al. Lipid and protein oxidation products, antioxidant status and vascular complications in poorly controlled type 2 diabetes. The British Journal of Diabetes & Vascular Disease 2012; 12: 33-39.
[8] Likidlilid A, Patchanans N, Peerapatdit T. Lipid peroxidation and antioxidant enzyme activities in erythrocytes of type 2 diabetic patients. Journal of the Medical Association of Thailand 2010; 93: 682-693.
[9] Hisalkar PJ, Patne AB, Fawade MM, Karnik AC. Evaluation of plasma superoxide dismutase and glutathione peroxidise in type 2 diabetic patients. Biology and Medicine 2012; 4: 65-72.
[10] Lapenna D, de Gioia S, Ciofani G, Mezzetti A, Ucchino S, Calafiore AM, Napolitano AM, Di Ilio C, Cuccurullo F. Glutathione-related antioxidant defenses in human atherosclerotic plaques. Circulation 1998; 97: 1930-1934.
[11] Yu XH, Fu YC, Zhang DW, Yin K, Tang CK. Foam cells in atherosclerosis. Clin Chim Acta 2013; 424: 245-52.
[12] Yang H, Mohamed AS, Zhou SH. Oxidized low density lipoprotein, stem cells, and atherosclerosis. Lipids Health Dis 2012; 11: 85.

[13] El-Bassiouni EA, Helmy MH, El-Zoghby SM, El-Nabi Kamel MA, Hosny RM. Relationship between level of circulating modified LDL and the extent coronary artery disease in type 2 diabetic patients. Br J Biomed Sci 2007; 64: 109-116.

[14] Inoue T, Uchida T, Kamishirado H, Takayanagi K, Hayashi T, Morooka S. Clinical significance of antibody against oxidized low density lipoprotein in patients with atherosclerotic coronary artery disease. J Am Coll Cardiol 2001; 37: 775–779.

[15] Monaco C, Crea F, Niccoli G, Summaria F, Cianflone D, Bordone R, Bellomo G, Maseri A. Autoantibodies against oxidized low density lipoproteins in patients with stable angina, unstable angina or peripheral vascular disease; pathophysiological implications. Eur Heart J 2001; 22: 1572–1577.

[16] Consensus development conference on the diagnosis of coronary heart disease in people with diabetes: 10-11 February 1998, Miami, Florida. American Diabetes Association. Diabetes Care 1998; 21: 1551-1559.

[17] American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; 33: S62-S69.

[18] Dasu MR, Devaraj S, Jialal I. High glucose induces IL-1beta expression in human monocytes: mechanistic insights. Am J Physiol Endocrinol. Metab 2007; 293: E337 E346.

[19] Venugopal SK1, Devaraj S, Yang T, Jialal I. Alphatocopherol decreases superoxide anion release in human monocytes under hyperglycemic conditions via inhibition of protein kinase C-alpha. Diabetes 2002; 51: 3049-3054.

[20] Lodovici M, Giovannelli L, Pitozzi V, Bigagli E, Bardini G, Rotella CM. Oxidative DNA damage and plasma antioxidant capacity in type 2 diabetic patients with good and poor glycaemic control. Mutat Res 2008; 638: 98-102.

[21] Brown DI, Griendling KK. Regulation of signal transduction by reactive oxygen species in the cardiovascular system. Circ Res 2015; 116: 531-549.

[22] Gupta S, Sodhi S, Mahajan V. Correlation of antioxidants with lipid peroxidation and lipid profile in patients suffering from coronary artery disease. Expert Opin Ther Targets 2009; 13: 889–894.

[23] Pytel E, Olszewska-Banaszczyk M, Koter-Michalak M, Broncel M.. Increased oxidative stress and decreased membrane fluidity in erythrocytes of CAD patients. Biochem Cell Biol 2013; 91: 315–318.

[24] Platt N, Gordon S. Scavenger receptors: diverse activities and promiscuous binding of polyanionic ligands. Chem Biol 1998; 5: R193-203.

[25] Tanaga K, Bujo H, Inoue M, Mikami K, Kotani K, Takahashi K, Kanno T, Saito Y. Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. Arterioscler Thromb Vasc Biol 2002; 22: 662-666.

[26] Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovasc Res 2004; 63: 582-592.

[27] Ylä-Herttuala S, Palinski W, Butler SW, Picard S, Steinberg D, Witztum JL. Rabbit and human atherosclerotic lesions contain IgG that recognizes epitopes of oxidized LDL. Arterioscler Thromb 1994; 14: 32-40.

[28] Orchard TJ, Virella G, Forrest KY, Evans RW, Becker DJ, Lopes-Virella MF. Antibodies to oxidized LDL predict coronary artery disease in type 1 diabetes: a nested case-control study from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes 1999; 48: 1454-1458.