REVIEW ARTICLE

Oxidized lipoproteins as the diagnostic target for cardiovascular diseases

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Low HDL-cholesterol and high LDL-cholesterol in plasma have long been associated with cardiovascular disease (CVD) risk. The quantity of cholesterol associated with these lipoproteins is being traditionally used to predict CVD risk. However, recent studies have suggested that the quality and functionality of these lipoproteins are more important. The lipoproteins – HDL and LDL – undergo both enzymatic and non-enzymatic modifications which impair their functional capability and hence, test of such modification which reflects the quality of HDL can be a good predictor of CVD risk. The present article discusses oxidation-associated dysfunctionality of lipoproteins and their potential in laboratory diagnosis of CVD.

Keywords: Cardiovascular disease, cholesterol, diagnostic target, oxidized lipoproteins.

CARDIOVASCULAR disease (CVD), a major cause of mortality and morbidity, has become a leading public health problem. According to the statistical update (2017) by the American Heart Association, CVD is responsible for more than 17.3 million deaths per year and expected to increase to more than 23.6 million globally by 2030 (ref. 1). More than 75% of such deaths are taking place in developing countries[2]. India is one of the countries having the highest number of CVD deaths[3]. Multiple approaches to diagnose this condition have been included to study its prevalence in India. The most common predictive factors of future cardiovascular event are age, sex, smoking, diabetics, lipid profile, various risk scores and variations in electrocardiogram of the patient[4]. Recently, various cardiovascular risk prediction biomarkers are being incorporated and seem to be effective in forecasting CVD risk. The drastic increase of CVD risk among the young population also demonstrates the urgent need of more accurate and consistent diagnostic biomarkers for early diagnosis. This will help to monitor disease status, initiate early management and thereby reduce further complications of CVD[5,6].

The balance between lipid storage and its removal from cells and tissues has a major impact on cardiac health. The role of lipoproteins in maintaining cholesterol homeostasis is apparent as they mediate the transport of lipids from intestine to liver and between liver and extra hepatic cells. The low density lipoprotein (LDL) is involved in the distribution of triacylglycerol and cholesterol to various extra hepatic systems as it binds to specific receptors on the cells surface. Apolipoprotein B100, the main protein part of LDL, is essential for the stabilization of its structure and receptor-mediated endocytosis at the target cells[7]. The LDL receptors at the hepatic tissues are chiefly responsible for the elimination of LDL particles from the circulation, ensuring its serum level in normal physiological range. However, increase in serum LDL weakens the receptor-mediated uptake and initiates its accumulation in the arterial wall where it gets modified[8,9]. The modifications in LDL are found to promote atherosclerosis as it binds to scavenger receptors – SR-A1 – rather than native LDL receptors in tissues. SR-A1, predominantly expressed in macrophages, can specifically interact with oxidized LDL (ox-LDL)[10].

ox-LDL is considered as one of the major contributors of atherosclerosis lesion[11–13]. In the presence of lipid hydroperoxides, the transition metal ions and metal ions binding to heme protein can initiate the oxidation of LDL non-enzymatically[14]. The enzymes lipoxygenase and myeloperoxidase can also initiate oxidation of LDL. Myeloperoxidase can generate potent oxidants which in turn modify the major protein apolipoprotein B100 of LDL[15–17]. Since the fatty acid composition of LDL influences plaque formation in the artery wall, diet may influence oxidative damage of LDL[18]. The macrophage SR-A1 has the ability to recognize this ox-LDL leading to the formation of foam cells, a characteristic feature of atherosclerotic lesions (Figure 1)[19]. It has been shown that the highly ox-LDL is resistant to lysosomal proteases-mediated degradation, leading to its accumulation in the macrophages; as a consequence, foam cells with pro-inflammatory property are formed[20,21].

High density lipoprotein (HDL) plays an important role in reverse cholesterol transport (RCT), a process of carrying cholesterol from the cells of the artery wall back to the liver for its excretion, which is mediated by the membrane lipid transporter adenosine-triphosphate-binding cassette transporter A1 (ABCA1)[22]. It has been reported that HDL can inhibit the oxidation of LDL and contribute to cardiac protection[23]. These processes are governed by

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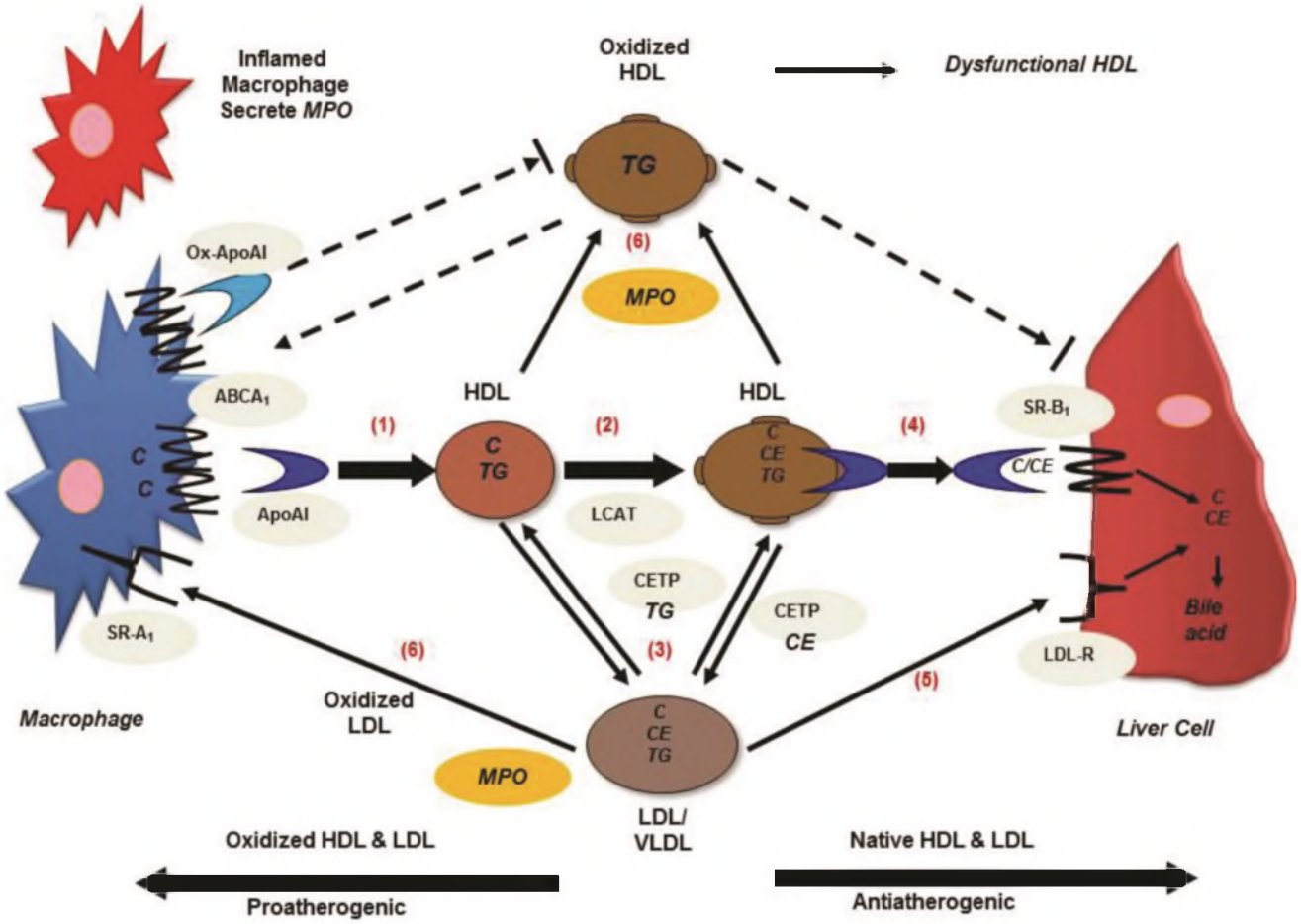

Figure 1. Oxidized lipoproteins HDL and LDL are dysfunctional and proatherogenic. Cholesterol present in macrophages is transported across the plasma membrane by ATP-binding cassette transporter ABCA1. Nascent apolipoprotein (Apo) AI, a major component of HDL, interacts with ABCA1, extracts cholesterol from macrophages and develops into discoidal HDL (1). HDL activates an enzyme LCAT and converts cholesterol into cholesteryl ester (2). In this process native discoidal HDL matures into HDL particles. These HDL particles constantly exchange cholesterol/cholesteryl ester for triglycerides with LDL/VLDL (3). In addition, these HDL particles transport cholesterol and cholesteryl ester to the liver through scavenger receptor and convert them into bile which is excreted in faeces (4). Thus HDL lowers the accumulation of cholesterol in extra hepatic tissues and in blood vessel such as the arteries. This process is known as reverse cholesterol transport and it is cardio-protective. LDL also transports cholesterol and cholesteryl ester. They bind to LDL-receptor in various cell types of the body and supply cholesterol required for the various functions of a cell (5). ApoB100 is a major component of LDL. Under chronic inflammatory conditions, macrophages and neutrophils secrete myeloperoxidase (MPO). This enzyme, in the presence of hydrogen peroxide, oxidizes HDL and/or LDL and converts them to oxidized lipoprotein particles (6). These lipoprotein particles are not only dysfunctional, but also proatherogenic in nature. This dysfunctional HDL/LDL allows cholesterol build-up in macrophages and converts it to foam cells, which results in chronic inflammation. As a result atherosclerotic plaques are formed leading to increased risk of cardiovascular disease (CVD) and stroke. Thus, the quality of lipoprotein particles determines the health of the cardiovascular system. The oxidized lipoprotein particles in blood, especially HDL, have been suggested to serve as a diagnostic marker of CVD risk. ABCA1, ATP-binding cassette transporter A1; Apo1 AI, Apolipopprotein A1; ApoB100, Apolipoprotein B100; HDL, High density lipoprotein; LCAT, Lecithin cholesterol acyl transferase; LDL, Low density lipoprotein; LDLR, LDL receptor; MPO, Myeloperoxidase; SRA1, Scavenger receptor A1; SRB1, Scavenger receptor B1; C, Cholesterol; CE, Cholesteryl ester; TG, Triglyceride; CETP, Cholesteryl ester transfer protein.

apolipoprotein A1, a major protein part of HDL. Apart from the RCT activity and antioxidant property, HDL possesses anti-inflammatory, anti-thrombotic and healing activities in endothelial cells[24,25]. It has long been recognized that the LDL–HDL profiles have tremendous impact on the risk of atherosclerosis[26]. HDL is also susceptible to modifications in its structure during atherosclerosis, which enhances the progression of this process[27,28]. Figures 1 and 2 indicate the key steps responsible for pro-atherogenesis and anti-atherogenesis. The focus of this article is to provide an overview on dysfunctional lipoproteins (LDL and HDL), the underlying mechanism and its impact on CVD. Understanding dysfunctional lipoproteins and their causes may provide biomarkers for potential diagnosis and management of CVD.

Enzymatic and non-enzymatic modifications of LDL

Many lines of evidence suggest that enzymatic and non-enzymatic oxidation of LDL can be considered as a

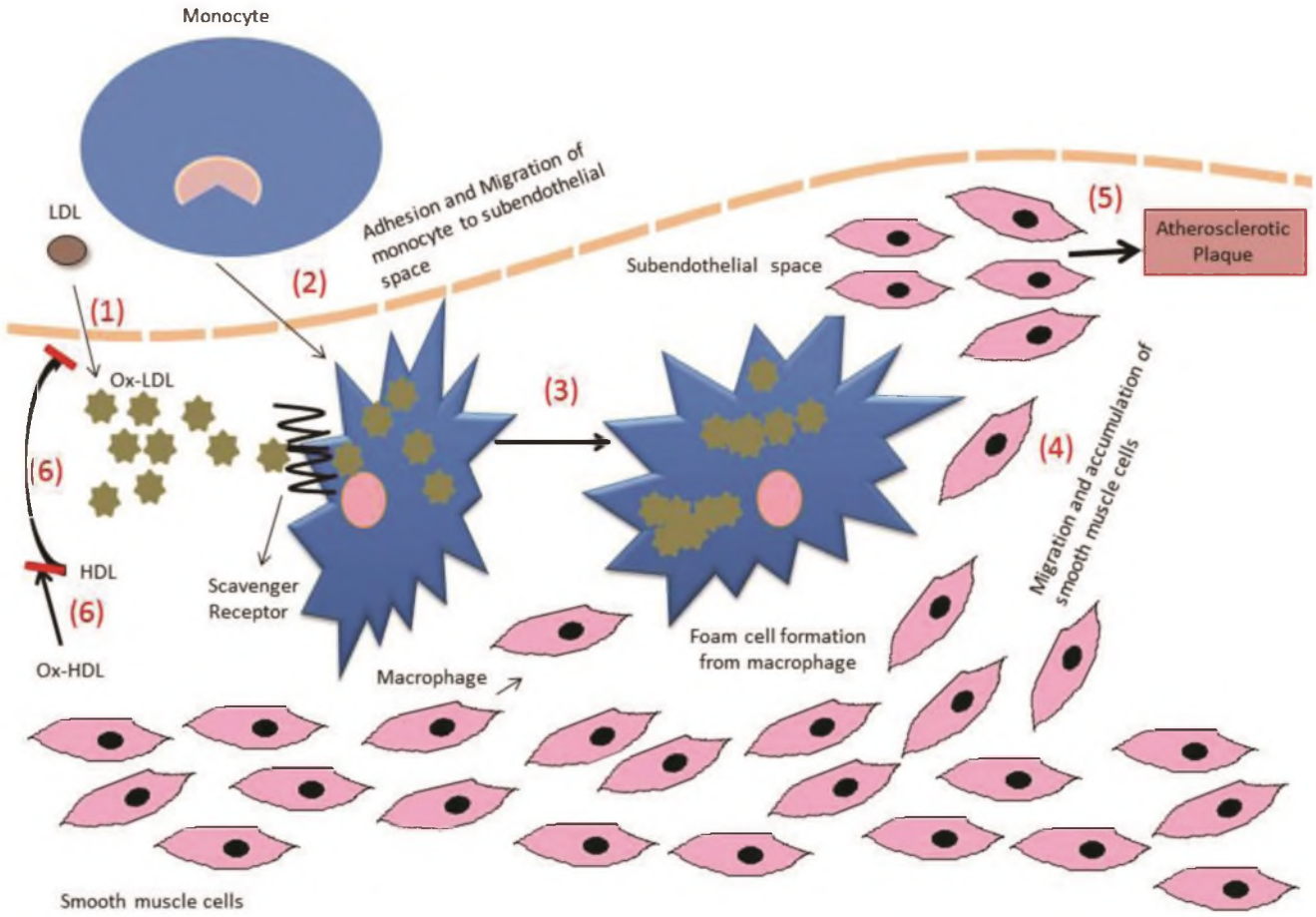

Figure 2. Oxidized LDL and HDL initiate atherosclerosis. LDL gets oxidized in the sub-endothelial space (1). During inflammation, monocyte adheres to the artery wall and migrates to the sub-endothelial space where it gets differentiated into macrophages (2). The ox-LDL gets attracted to scavenger receptors of the macrophages and forms foam cells (3). The smooth muscle cells migrate to this region and initiate matrix formation (4). Steps 2 and 3 together form major components of the atherosclerotic plaque (5). The oxidation of LDL is usually prevented by the antioxidant property of HDL. However, the oxidation of HDL makes it dysfunctional (6).

corner stone in atherogenesis. The peroxidation of lipids in LDL by arterial smooth muscle cells was determined through its increased electrophoretic mobility and the presence of malondialdehyde, an oxidation product. The transition metal ions such as iron (Fe) and copper (Cu) are found to contribute to oxidative modifications of LDL in human arterial smooth muscle cells. Chelating agents of these metals were found to block metal ions-mediated LDL modifications under *in vitro* conditions[29,30]. Bicarbonate in the buffer system was also found to enhance the formation of reactive free radicals which facilitate LDL oxidation[31]. Acidic pH in the atherosclerotic lesion might have a role in extensive LDL modifications by the cells and transition metal ions[32,33]. Metal ions catalyse oxidative damage on the surface of LDL to generate minimally ox-LDL, which has an affinity towards normal LDL receptor rather than scavenger receptors of macrophages. However, highly ox-LDL binds to scavenger receptor and initiates atherosclerotic lesion. The oxidation at the core of LDL changes the function of minimally ox-LDL and enhances its binding affinity to macrophage scavenger receptors with noticeable alterations in apolipoprotein B[34,35].

Lipoxygenase (LOX) is one of the cellular oxygenation enzymes found to incorporate oxygen in polyunsaturated fatty acids[36]. The inhibitors of this enzyme are shown to reduce 70–80% LDL oxidation, indicating the role of LOX in oxidative modifications of LDL[37]. Based on the location of oxygenation in arachidonic acid, four LOXs (5-, 8-, 12- and 15-LOX) were identified in mammalian tissues. Though a variety of cells such as blood cells, cardiomyocytes, adipocytes and macrophages are found to produce 12/15 LOX, macrophages-produced LOX is reported to have dominant potential in the progression of atherogenesis[38].

The role of 12/15-LOXs seems to be double-faced as these enzymes showed contradictory results in different animal models[39]. Overexpression of monocyte/

Table 1. Effect of 12/15-LOX in atherosclerosis in transgenic animals

Gene (knock-in or knock-out)	Status of atherosclerosis
12/15-LOX (human LOX transgenic rabbit)	Overexpression of monocyte/macrophage 12/15-LOX gene protects transgenic rabbits from atherosclerosis
15-LOX (knock-out mice)	Absence of 15-LOX protects mice from atherosclerotic plaque formation
12/15-LOX and ApoE-/- (double knock-out mice)	Absence of 12/15-LOX in ApoE-deficient mice protects them from atherogenesis
12/15-LOX and LDL receptor (double knock-out mice)	Disruption of 12/15-LOX in LDL receptor-deficient mice protects them from atherogenesis

macrophage-specific 15-LOX in transgenic rabbits showed anti-atherosclerotic effects[40,41]. Similar results showing atheroprotective effects of 15-LOX were observed in knock-out mice[42]. However, disruption or absence of 12-/15-LOX noticeably reduced atherosclerotic lesion in apoE-/- and LDL receptor-deficient mice, showing the prominent role of these enzymes in atherosclerosis[43,44]. Table 1 summarizes studies in transgenic animals with regard to the effect of 12-/15-LOX in atherosclerosis. Sukhanov *et al.*[45] showed that insulin-like growth factor-1 negatively regulates lipoprotein oxidation through macrophage-specific 12-/15-LOX in apoE-/- mice. Genetic studies in humans did not show consistency in the results. This may be due to the presence of different isoforms of 12-LOX and 15-LOX that may show difference in expression and substrate specificity[46].

Myeloperoxidase (MPO), a heme-containing peroxidase enzyme, is related to both oxidative stress and inflammatory reactions. Granulated neutrophils and monocytes express MPO abundantly when exposed to inflammation, which ultimately results in the generation of reactive oxygen species. The MPO expressed in macrophages has been involved in the beginning and progression of atherosclerosis, and initiates new possibilities in the management of the same. Epidemiological studies have shown the relationship of higher levels of MPO with CVD risk[47–49].

MPO is considered as one of the enzymes catalysing the formation of highly reactive superoxide radicals. A major part of the catalytic activity of MPO is assigned to the microenvironment of sub-endothelial space. The highly cationic enzyme, MPO, can interact with LDL and enhance its oxidation. MPO can also enhance the oxidative potential of H_2O_2 in reaction with chlorinating and nitrating agents. This reaction would generate reactive species such as hypochlorous acid (HOCl), tyrosyl radicals, chloramines and nitrogen dioxide that oxidize LDL. Most of these oxidation products are unstable, which catalyse further oxidation of LDL and are responsible for its accumulation in the artery wall. The stable oxidation products generated can be used as biomarkers for MPO-mediated LDL oxidation. Best among them are 3-nitrotyrosine and 3-chlorotyrosine[50,51].

MPO is known to catalyse the formation of cytotoxin HOCl from substrates H_2O_2 and chloride. The HOCl initiates 3-chlorotyrosine formation from protein-bound or free tyrosine, and MPO is considered as the unique human enzyme which catalyses these reactions. The HOCl produced by MPO/H_2O_2/chloride system is found to generate oxidative modifications in the major protein part of LDL, apoB100, in vascular intima, and this could be proatherogeneic[52,53]. The reaction product of superoxide and nitric oxide, peroxynitrite, is involved in the formation of 3-nitrotyrosine[15]. MPO and its stable product 3-chlorotyrosine are noticeably expressed in human atherosclerotic lesions[54,55], which authenticates the possibility of these stable oxidation products as diagnostic markers of CVD.

Malondialdeyde (MDA), a lipid peroxide product formed from prostanoids can react with exposed ε-amino groups of apo B-100 lysine residues and cause oxidation of LDL under oxidative stress. This MDA–LDL (collectively referred as ox-LDL) can induce lipid accumulation in macrophages leading to the formation of foam cells (Figure 2). Thus, this ox-LDL in circulation is proposed to be the biomarker of CVD risk in humans[56,57]. Amino acids such as methionine, tyrosine and tryptophan are oxidized in both *in vitro* and *in vivo* conditions, resulting in the generation of ox-LDL. In Table 2, only those amino acids of apo B-100 isolated from haemodialysis patients are shown[58]. Since a large number of methionine, tyrosine and tryptophan are post-translationally modified, it is difficult to pinpoint which of them will be critical for diagnostic or prognostic purposes in the clinic. Recently, there has been increased interest in understanding the role of oxidized HDL, as the quality of HDL is affected and has high potential in predicting CVD risk in clinical settings.

Modified HDL and atherosclerosis

High plasma HDL levels have a solid negative influence on atherosclerotic CVD through reverse transport of cholesterol from arteries to hepatic tissue. An imbalance between cholesterol deposits in the arterial wall and its removal for excretion leads to atherosclerotic lesion[59]. Macrophages are primary cell types responsible for atherosclerosis; hence, the cholesterol efflux from macrophages by HDL is known as macrophage RCT. HDL biosynthesis and its metabolism are mainly maintained by the cholesterol efflux from non-macrophage tissues. The major protein involved in RCT is ABCA1, which uses the

Table 2. Post-translational modifications of ApoB-100 found in circulation

Oxidized amino acids	Amino acid position in apo B-100
3-Hydroxy-tyrosine	76, 249, 425, 693, 713, 1579, 1753, 1747, 1965, 1972, 2189, 2405, 2732, 3206, 3268, 3533, 3653, 3744, 4055, 4057, 4184, 4205, 4242, 4424
Chlorinated-tyrosine	125
Oxidized methionines (methionine sulphoxide)	507, 1022, 1162, 1239, 1560, 2481
Oxidized (mono) tryptophan	936 and 4063
(Di)oxidized trytophan	1114 and 3536

These modifications are found in ApoB-100, a major component of LDL isolated from haemodialysis patients who had elevated levels of MPO and MPO–LDL as reported by Delporte *et al.* No such exhaustive information is available on oxidized modifications of LDL in other metabolic diseases such as diabetes, CVD and obesity. However, the above information provides putative sites of ApoB-100 where it could get potentially modified by MPO in diabetes, CVD and obesity.

Table 3. Amino acid modifications of HDL-ApoA1

Type of modification	Amino acid ApoA1 modified	Functional consequences
Chlorination of tyrosine[100,101]	Tyr-166 and Tyr-192	Loss of cholesterol acceptor activity
Nitration of tyrosine[100,101]	Tyr-166 and Tyr-192	Loss of LCAT activation
Oxidation of methionine[101,102]	Met 86, Met 112 and Met 148	Loss of cholesterol acceptor activity and of LCAT activation Maturation of HDL particles
Hydroxylation of tryptophan[103]	Trp-8, Trp-50, Trp-72 and Trp-108	Loss of cholesterol acceptor activity
Carbamoylation of lysine[104]	Lys 225	Generates proatherogenic and pro-inflammatory particles

These modifications of ApoA1 are found in atherosclerotic plaques and also generated during *in vitro* biochemical reactions of ApoA1 amino acids with MPO.

energy source ATP for transport of substrates. The transport of cellular cholesterol and phospholipid to apolipoprotein A1 (ApoA1), the major initial acceptor, is mediated by the ABCA1 transporter and hence, this protein is considered as representative of rate-regulatory step in RCT[60–63].

Apart from cholesterol transport, HDL possesses anti-atherogeneic, anti-inflammatory and antioxidant activities[64]. Coronary artery disease (CAD) patients with high HDL level showed less anti-inflammatory activity compared to HDL from healthy controls, indicating that the lipoprotein is dysfunctional in CAD patients[65]. The antioxidant activity of HDL is mainly due to its interaction with human serum enzyme paraoxonase 1 (PON1). HDL-bound PON1 protects the lipoproteins – LDL and HDL – from oxidation[66]. Shih *et al.*[67] showed that PON1 knock-out mice fed with high-fat diet were more prone to atherosclerosis, providing evidence for the antioxidant property of PON1. The injection of recombinant PON1 in mice enhances the anti-atherogeneic potential of HDL and macrophages[68,69].

Alterations in the composition of protein and lipid part of HDL might make it dysfunctional. The oxidative modifications of the protein ApoA1 are the dominant factor responsible for the dysfunctionality of HDL. Like LDL, Cu ions mediate the oxidation of HDL, which leads to the proteolysis of ApoA1 subsequently reduces the ability of HDL in RCT[70]. The major contributor MPO, secreted by macrophages, generates HOCL in reaction with H_2O_2 which has the capacity to chlorinate the tyrosine residues, especially at position 192 (Tyr-192) in ApoA1 to 3-chlorotyrosine[71,72]. 3-Nitrotyrosine, another product of oxidative modification mediated by the MPO–HOCL system derived from nitric oxide, was also found in atherosclerotic lesion[73]. Apart from site-specific modifications in tyrosine residue, MPO modifies amino acids methionine, lysine and tryptophan residues of the ApoA1 to methionine sulphoxide, carbamoylated lysine and mono-/di-hydroxytryptophan respectively (Table 3)[74–76]. Modified ApoA1 in HDL is found to reduce its reverse cholesterol efflux and become atherogeneic[77]. Even though both 3-chlorotyrosine and 3-nitrotyrosine are reported to be higher in CVD patients, the former shows more negative correlation with reverse cholesterol efflux capacity of HDL[78]. Recently, two studies have shown that nitrated ^{166}tyrosine and hydroxylated ^{71}tryptophan in HDL–ApoA1 are abundant in atherosclerotic plaques and these modified HDLs are dysfunctional[79,80].

Oxidized products of MPO in atherosclerosis: Studies in knock-out and knock-in mice

Although there is a clear-cut enhancement of oxidation of ApoA1 in human atherosclerotic plaques and in plasma/serum, the extent of ApoA1 modifications is less clear in mouse models. For instance, both acute and chronic manifestation of atherosclerosis seems to occur due to the action of pro-oxidants developed by MPO. The inhibition of MPO *in vivo* by a variety of drugs has been

Table 4. Lessons learnt from mouse models of atherosclerosis regarding the oxidation of lipoproteins

Gene	Knock-in/knock-out	Status of atherosclerosis and CVD risk	Conclusion
Myeloperoxidase (murine MPO knock-out mice)[82]	Murine MPO knocked-out in: (a) LDL receptor-deficient mice fed with high-fat diet (b) ApoE-deficient mice fed with high-fat diet	Lack of oxidized products in atherosclerotic plaque Increased atherosclerotic plaque	Mouse MPO protects against murine atherosclerosis, unlike human atherosclerosis
MPO (human MPO transgenic mice expressing MPO in macrophages)[83]	Human MPO was knocked-in for LDL receptor-deficient mice fed with high-fat diet	Atherosclerotic plaques larger in size and abundant oxidized product of MPO in atherosclerotic plaque and circulation	Human MPO promotes atherosclerosis
MPO (human MPO transgenic mice expressing MPO in macrophages)[84]	Human MPO was knocked-in for LDL receptor-deficient mice fed with high-fat diet	Promotes atherogenesis, builds high levels of cholesterol and triglycerides. Oxidation products in atheroma	Human MPO promotes atherogenesis and increases CVD risk in murine models

found to reduce the atherosclerotic plaque size and number[81].

Various mouse models have provided key insights into the development of atherosclerosis; however, some discrepancies have been noticed. This includes detection of very low levels of characteristic products of MPO that have been identified in human atherosclerotic lesions and the development of atherosclerosis even in MPO knock-out mice[82]. Further, the inhibition of murine MPO did not have any consequence in the development of atherosclerosis in LDLR-deficient mice. In contrast, transgenic mice expressing human MPO gene have attained greater importance in the studies related to macrophage-specific MPO and associated atherosclerosis. LDL receptor-deficient transgenic mice expressing human MPO in macrophages are found to enhance atherosclerosis when fed with high-fat diet[83,84]. Thus, there are evidences available regarding the human myeloperoxidase enzyme directly playing a role in the oxidation of both LDL ApoB-100 and HDL ApoA1 (Table 4). Such oxidation renders these lipoproteins dysfunctional. Moreover, some of these modifications also render these lipoprotein particles proinflammatory and proatherogenic. Thus, these oxidized modifications of both ApoB-100 and ApoA1 may serve as a useful marker for predicting CVD risk in humans.

Role of lipids in cardiomyopathy and myocardial infarction

Cardiomyopathy refers to the diseased heart muscles leading to heart failure and arrhythmias (irregular heart beat), resulting in the inability of heart to pump blood required for various parts of the body. There has been a sharp increase in death due to heart failure associated with aging, diabetes and obesity[85,86]. In general, individuals with diabetes mellitus carry 2–4 times higher risk of cardiomyopathy and myocardial infarction[86,87]. The clinical outcome of myocardial infarction and cardiomyopathy in diabetic patients is far worse than in patients without diabetes[86]. The causes have been attributed to a variety of factors. For myocardial infarction, the oxidation of lipoproteins and its consequences have been extensively discussed in this article. With regard to diabetic cardiomyopathy, a number of factors such as hyperglycaemia and mitochondrial dysfunction along with increased fatty acid and triacylglycerols in circulation have been reported to collectively contribute to poor functioning of heart muscle and eventually to death[85].

Dyslipidaemia refers to the abnormal lipid levels, which include triacylglycerols, free fatty acids, cholesterols, phospholipids, sphingolipids, etc. This abnormality is often associated with coronary heart diseases, diabetes, and obesity which are linked by inflammation. Often dyslipidaemia is one of the major factors for both myocardial infarction and cardiomyopathy, which are highly prevalent in diabetic patients. This is further complicated by the oxidation of lipids by LOX, etc., wherein liver, skeletal muscle and adipose tissue do not utilize both glucose and fatty acids for storage as glycogen and triglycerides respectively. Moreover, the presence of higher levels of triglyceride-enriched lipoproteins such as VLDL and chylomicrons in the circulation results in lipotoxicity[89]. Increased levels of both glucose and lipids in diabetes mellitus causes glucolipotoxicity to pancreas, adipose tissue, liver, heart, etc., resulting in increased risk of coronary artery diseases and cardiomyopathy.

A number of modified fatty acids and lipids have also been observed. For instance, there has been increased prevalence of esterified fatty acid peroxides (13-hydroxylinoleic acid 13-hydroperoxy octadecadienoic acid), esterified fatty acid hydroperoxide (13-hydroxylinoleic acid, 13-hydroxy octadecadienoic acid); prostaglandin-like products such as isopentane and its esterified forms, aldehydes such as malondialdehyde (MDA), aldehydes containing esterified lipids, etc. modified lipids such as lysophosphatidyl choline, 7-keto cholesterol and internally modified lipids such phosphatidyl serine and phosphatidyl ethanolamine products (reviewed in Parthasarathy *et al.*[13]). The precise roles of these oxidized lipids have not been well defined. However, it is observed that these oxidized lipids have been found to prevent proper insulin signalling and energy homoeostasis. It has also been identified that the oxidized phospholipids such as oxidized phosphatidyl choline and phosphatidyl inositol generated during LDL oxidation by the reactive species, play a significant role in proatherogenic effects of ox-LDL[89].

Recently, there has also been increased interest in understanding the role of various sphingolipids, especially sphingomyelin, glycosphingolipids, ceramides and sphingosine 1-phosphate. The levels of sphingomyelin, certain glycosphingolipids and ceramides are found to be elevated in blood plasma of patients with myocardial infarction and diabetics. In contrast, lysosphingolipid and sphingosine 1-phosphate, which are usually associated with HDL, are reduced. There has been tremendous increase in understanding the cellular, physiological and pathophysiological roles of two bioactive sphingolipids, viz. ceramide and sphingosine-1-phosphate[90,91].

It is clear that sphingosine 1-phosphate activates at least five G-protein coupled receptors (GPCRs) known as S1P receptors ($S1PR_{1-5}$), and they have a cardioprotective role. Sphingosine 1-phosphate is known to activate endothelial nitric oxide synthase, insulin sensitivity, vascular integrity, immune cell trafficking and reducing inflammation through $S1PR_1$, etc.[91]. However, ceramides are known to inhibit phosphoinositide signalling, and also play a role in the inhibition of Akt, and increasing insulin resistance. The circulating fatty acids, especially saturated fatty acids, are known to be channelled into the ceramide pathway[91]. Since these lipids are known to be associated with both LDL and HDL, they may play a role in the pathogenesis as well as disease outcome in clinical settings. Phosphoinositides, ceramides, and sphingosine 1-phosphate have clear biological roles and there is an increased need for understanding the role of these lipids in clinical settings in order to be effectively utilized as diagnostic or prognostic markers of diabetes, obesity and CVD.

Although various forms of familial cardiomyopathy are seen, most predominant forms are dilated cardiomyopathy and hypertrophic cardiomyopathy. In both these cases muscles of the ventricles are thickened and the ventricle wall is enlarged. Due to this, there is lack of proper blood flow in and out of the ventricle often leading shortness of breath, dizziness, increased arrhythmias, etc. In familial cardiomyopathies, the modified lipids are found to be accumulated and contributed for the patho-physiology of cardiac muscles. These inherited disorders are mostly due to defective lipid metabolism (either synthesis or degradation). In cardiac muscle disorders, cardiolipin – a major mitochondrial lipid – synthesis is affected. In contrast, in lipid storage diseases, the gene(s) responsible for certain lipids are defective resulting in excessive accumulation of various lipids in heart muscles[92].

In primary cardiac muscle disorders, collectively a number of genes are found to be defective which results in both dilated and hypertrophic cardiomyopathy, in addition to alterations in various other tissues, including liver. Primarily, in these inherited muscle disorders, the accumulation of carnitine esters, and fatty acids along with defective beta-oxidation of very long chain fatty acids and decreased cardiolipin were observed. A number of lipid storage diseases have been attributed to familial cardiomyopathy, which includes Fabry's disease (accumulation gangliosides), gangliosidosis (accumulation of glycosphingolipids), mucolipidosis (phospholipids accumulated in vacuoles); Gaucher disease (accumulation of glucocerebroside), neutral storage lipids with cardiomyopathy (accumulation of triglycerides) and neutral lipid storage disease with ichthyosis (accumulation of triglycerides)[92,93]. Often the symptoms of various cardiomyopathies arising out of complications due to diabetes and obesity or inherited disorders can be distinguished based on the familial history and accordingly treatment regimens could be devised and administered to patients.

Patients with myocardial infarction, a very acute condition, experience extreme chest pain without any prior indication. However, the situation is different in the case of cardiomyopathy, since these patients experience chest pain as well as other abnormalities characterized by altered lipid metabolism. These abnormalities in lipids can be correlated with the dysfunctionality of lipoproteins such as LDL and HDL. Thus, the measurement of dysfunctional LDL and HDL along with the levels of lipoproteins and triacylglycerol in circulation may also indicate heart failure, which may provide options for better treatments.

Diagnostic potential of dysfunctional LDL/HDL

The increased prevalence of CVD events in the age group between 35 and 60 years has become pronounced with major public health and socio-economic consequences. Numerous risk scores have been proposed to predict CVD risk. The most common risk prediction is made by Framingham ten-year risk score (FTRS), which predicts events over the next ten years as a primary preventive

method. In addition, FTRS offers a practical approach to identify patients at higher risk of CVD using clinical information, basic biochemical analysis of biomarkers such as troponin I, B-type natriuretic peptide and high-sensitivity C-reactive protein as well as more specialized tests such as coronary artery calcium scoring and carotid ultrasound[94].

Since both HDL and LDL are the major lipoproteins involved in cholesterol homeostasis, their oxidative modifications may be valuable for finding subjects at risk of CVD. The plasma level of MPO is positively correlated with CVD risk and subsequent cardiac events[95,96]. Likewise, increase in modified ApoA1 with chlorotyrosine and nitortyrosine has been detected in serum and atheroma samples of CVD patients by mass spectroscopy, indicating that it can also be a predictor of adverse cardiac event[97]. Presence of both chlorotyrosine and nitrotyrosine indicates the extent of loss of function of HDL. However, quantification by mass spectrometry is not suitable for routine clinical use as it is tedious, requires sophisticated equipment and trained manpower and it is not amenable for high throughput screening of patient samples. Therefore, more sensitive and usable diagnostic method must incorporate the identification of specific modified amino acids and MPO related to dysfunctional lipoproteins[98]. Clinical assays based on high-affinity monoclonal antibodies produced against MPO or oxidized amino acids of ApoA1 can be useful to detect their levels in plasma of subjects at CVD risk[78,94,99]. Early diagnosis of abnormalities in these lipoproteins – HDL and LDL – using immunoassays can be a good predictor of future CVD risk. Thus, successful validation of measuring MPO and oxidized ApoA1 levels in the blood in high-throughput clinical assays is the immediate need. If this is achieved, both MPO and oxidized ApoA1 could be included in the routine diagnostic methods to identify CVD risk at an early stage and improve disease management.

Conclusion

The absence of specific and sensitive diagnostic markers in routine clinical practice has been a major hurdle in the early diagnosis of CVD. To reduce the mortality and severity of CVD, better early diagnostic markers are essential. The functionality of LDL and HDL has to be considered since there are cases with exceptions in the association of these lipoproteins and CVD risk. Identification of more specific serum markers like MPO and modified apolipoproteins is valuable for early detection of CVD. More studies focusing on the development of antibody-based systems to examine these specific serum markers are essential.

Conflict of interest: The authors declare that they have no conflict of interest.

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ACKNOWLEDGEMENT. K.V. acknowledges the support of BIRAC–CEFIPRA–French Embassy grant.

Received 30 January 2018; revised accepted 22 May 2018

doi: 10.18520/cs/v115/i7/1276-1286