Small Dense LDL: New Marker for Cardiovascular Risk Assessment and its Therapeutic Inflection

M Salman Khan*

Department of Biotechnology, Integral University, Lucknow-226026, India

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Cardiovascular diseases (CVD) encompass any medical conditions related to the heart and blood vessels. CVD is the main cause of disability and premature death worldwide, and is projected to remain the leading cause of death. An estimated 17.5 million people died from this disease in 2005, representing 30% of all global deaths. Of these deaths, 7.6 million were due to coronary heart disease (CHD) and 5.7 million because of stroke. If immediate and proper attention is not paid, by 2015 an estimated 20 million people will die from CVD, including stroke [1]. In terms of attributable deaths, the leading cardiovascular risk factor globally, is raised blood pressure (to which 13% of global deaths is attributed), followed by tobacco use (9%), raised blood glucose (6%), physical inactivity (6%) and overweight and obesity (5%). CVD also accounts for about 60% of all mortality in people with diabetes.

Persons with high levels of cholesterol in blood, a condition called hypercholesterolemia, are more prone to atherosclerosis. It is not a disease but a metabolic derangement that can be caused by many diseases, notably cardiovascular disease. “Hyperlipidemia” (elevated levels of lipids in the blood) and “hyperlipoproteinemia” (elevated levels of lipoproteins in the blood) are the other factors responsible for CVD. Elevated cholesterol in the blood is due to abnormalities in the levels of lipoproteins, the particles that carry cholesterol in the bloodstream.

Cholesterol contains various subtypes that include very-low-density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). The normal cholesterol range in human body should be below 200 mg/dl and is good for body to function normally and anything over 240 mg/dl indicates risk for developing CVD. Relative risk of all lipoproteins in CVD has been extensively studied and till now LDL-C, known as bad cholesterol, is considered as a marker for cardiovascular risk assessment [2]. The LDL-C level in human body should be below 130 mg/dl. High density lipoprotein cholesterol, which is known as good cholesterol and have antioxidant property, should range between 35-40 mg/dl and the high level of HDL-C (~60 mg/dl) has the benefit of reducing the chances of heart attack. Moreover, there are several amendable risk factors that include hyperlipidemia, dyslipidemia and have higher incidence of deranged lipid profile. The various study established that elevated LDL-C and increased ratio of LDL/HDL (high density lipoprotein) is the most important lipid derangement making those subjects more susceptible to atherosclerosis [3].

Small dense LDL: A New Marker

Modern research on CVD decode several new risk factors that include elevated lipoprotein(a), high sensitivity C reactive protein (hs-CRP), fibrinogen and hyper-homocysteinemia etc., an important one of which is estimation of small dense LDL cholesterol (sd LDL-C).

Low density lipoproteins are composed of distinct subclasses that differ in size, density, chemical composition, and their association with cardiovascular disease. Numerous studies have clearly demonstrated the various sub-fractions of LDL by using different methods like density gradient, ultracentrifugation, PAGE, NMR etc [4-6] and seven distinct LDL subspecies were identified depending on their densities, which differ in their metabolic behavior and pathological roles [7,8]. Two phenotypes of LDL were identified-pattern A with LDL >25.5 nm (large and buoyant) and pattern B with LDL ≤ 25.5 nm (small and dense).

Lipoprotein profiles that are relatively rich in sd-LDL particles are associated with up to a 3-fold greater risk of myocardial infarction than those mainly consist of large buoyant (lb)-LDL particles. Currently published report established that predominance of sd-LDL-C is a major component of an atherogenic lipoprotein phenotype, and a source of increased risk for coronary heart disease. The ratio of sd-LDL-C to LDL-C plays an important role in assessing CVD. Researches established that sd-LDL cholesterol and lipoprotein level in normolipidemic condition is ~30% of total LDL-C in blood. In hyperlipidemic animal and human subject this ratio is increased by several folds depending upon the degree and severity of disease [9-11]. It has been shown that not only the prevalence of sd-LDL, but its concentration was substantially increased in healthy subjects with various types of hyperlipidemia such as hypercholesterolemia(LDL), hypertriglyceridemia, combined hyperlipidemia and chylomicronemia, hyperlipidemia with CHD, type 2 diabetic patients with and without CHD [9]. Similarly, Quebec cardiovascular study has confirmed that a predominance of sd-LDL is a strong and independent predictor of CHD in the first seven years of follow-up [12]. Another report specifies that progression of CHD was closely linked not to the LDL particle size, but to the concentration of sd-LDL [13].

Atherogenic Properties of Oxidized LDL and Small Dense LDL

Evidence suggests that common risk factors for atherosclerosis increase the risk of the production of reactive oxygen species (ROS), not only from the endothelial cells, but also from the smooth muscle cells and the adventitial cells. That include: hypercholesterolemia or dyslipoproteinemia, diabetes mellitus (DM), arterial hypertension, smoking, age etc. According to the theory of oxidative stress,
atherosclerosis is the result of the oxidative modification of LDL in the arterial wall by ROS.

Oxidative modification of lipoproteins is believed to play a central role in the pathogenesis of atherosclerosis [14]. Because plasma contains several antioxidants and lipoproteins with oxidative damage have been isolated from atherosclerotic lesions, lipoprotein oxidation generally is considered to occur in the vessel wall [14]. Research has shown that human atherosclerotic plaques contain massive amounts of lipid peroxidation products, despite the presence of large quantities of α-tocopherol (vitamin E) and ascorbate Suarna et al. [15]. Therefore, it is unclear whether oxidized lipoproteins originate in the arterial wall or are produced in the circulation and then enter the intimal space. Although LDL is a major risk factor for CAD, experiments have shown that incubation of macrophages with native LDL does not result in foam cell formation, a characteristic feature of atheromatous lesions [16]. However, after chemical modification of LDL, rapid uptake occurs, leading to cholesterol accumulation in the macrophage with subsequent foam cell formation, which suggests that LDL modification is a necessary step in atherogenesis. Oxidized (OX) LDL can induce foam cell formation, and oxidative modification of LDL is now recognized as an important process that occurs in vivo Steinberg [14]. Several lines of research support the occurrence of LDL oxidation in vivo [14].

Many cell types are capable of oxidizing LDL, including monocytes, macrophages, neutrophils, endothelial cells, smooth muscle cells and fibroblasts. However, cell types that are involved in the atherosclerotic lesion in which OX LDL is found, i.e. macrophages, endothelial cells and smooth muscle cells, would seem to be the most likely to contribute to LDL oxidation in vivo. Once initiated, oxidation of LDL is a free radical driven lipid peroxidation chain reaction. Lipid peroxidation is initiated by free radical attack on double bond associated with a polyunsaturated fatty acid (PUFA). This results in the removal of a hydrogen atom from a methylene (CH₂) group, the rate of which determines the rate of initiation, a key step in lipid peroxidation. Molecular rearrangement of the resulting unstable carbon radical results in a more stable configuration, a conjugated diene. The conjugated diene reacts very quickly with molecular oxygen, and the peroxyl radical thus formed is a crucial intermediary. A PUFA peroxyl radical in LDL may abstract a hydrogen atom from an adjacent PUFA to form a hydroperoxide and another lipid radical, a reaction which results in chain propagation. Removal of hydrogen atoms by the peroxyl radical from other lipids, including cholesterol, eventually yields oxysterols. Lipid hydroperoxides fragment to shorter-chain aldehydes, including malondialdehyde and 4-hydroxyxenonal. These reactive aldehydes in turn may bind to ε-amino groups of apo B-100, giving the protein an increased net negative charge. The classical LDL receptor recognizes a specific domain of positive charges from lysine, arginine and histidine residues on apo B. Alteration of this domain results in failure of binding by the apo B/E receptor and an increase in negative surface charge on apo B-100 results in increased recognition by the scavenger receptor. In vitro LDL oxidation has verified the existence of "lag phase", during which significant oxidation of LDL cannot be detected, prior to the onset of the "propagation phase", presumably after the endogenous antioxidants have been consumed. A steady increase in the detectable byproducts of oxidation continues, until the substrate, i.e. the PUFAs, has been depleted, and a plateau phase is reached. Assessment of resistance of LDL to oxidation has generally involved in measurement of the duration of the lag phase including other variables, such as the rate of propagation, are also noted.

The increased atherogenicity of LDL is associated with a preponderance of small dense (sd-) LDL subpopulation that is more prone to oxidative modification than large buoyant (lb-) LDL [17]. Lipoprotein profiles that are richly associated in sd-LDL particles are associated with up to a 3-fold greater risk of myocardial infarction than those mainly consist of lb-LDL particles [18,19]. However, the mass of sd-LDL is independently associated with disease risk [19,20], suggesting that these particles are more directly atherogenic than lb-LDL. It has been suggested that in comparison to lb-LDL or LDL, sd-LDL are highly atherogenic as a result of their enhanced susceptibility to oxidative modification, higher penetration in the arterial wall, their lower binding affinity for the LDL-receptor, and prolonged plasma half life [21]. Differences in oxidative susceptibility between lb-LDL and sd-LDL have been attributed to differences in their physicochemical properties. Relative to lb-LDL, sd-LDL have a reduced content of antioxidants and free cholesterol [22-25], and are enriched with polyunsaturated fatty acids [22] and possibly, hydroperoxides [26]. Antioxidant concentrations and free cholesterol content have been shown to predict differences in the oxidative susceptibility of LDL density subfraction [21,22,25]. Therefore, in view of above evidence, sd-LDL has now been highlighted as a new powerful and useful marker for the risk of hyperlipidemia with and without CVD/CHD, type 2 diabetic patients with and without CVD/CHD.

**Therapeutic approaches: Role of Natural Products**

Conventional treatment of atherogenic lipid profile aims at reducing total cholesterol levels and LDL cholesterol or increasing HDL cholesterol by using different treatment available. The most important and widely used drugs for the treatment of hypercholesterolemia are Statins. They reduce the cholesterol level by inhibiting HMG-CoA reductase enzymatic activity, the rate limiting enzyme in biosynthetic pathway of cholesterol. These drugs decrease intracellular cholesterol, increase LDL receptors and accelerate removal of LDL-C and TGs-rich lipoprotein and constitute the first line of treatment for reducing LDL-C. Though statins are superior to all other medications for reducing LDL-C concentration but their effect on sd-LDL-C is variable [27,28]. Both fluvastatin and atorvastatin have been shown to shift the LDL profile towards more buoyant particles but pravastatin and simvastatin have not shown any such result. In addition, in hyperlipidemic patients with type 2 diabetes, low doses of pitavastatin (1 mg) and fenofibrate (100 mg) were both effective in decreasing sd-LDL-C concentration but via a different mechanisms: the former decreases total LDL including sd-LDL, while the latter decreased sd-LDL specifically [29]. Furthermore, in diabetic patients with mixed dyslipidemia, combination therapy with simvastatin and fenofibrate had a greater positive effect on LDL cholesterol size profile, with a shift from more atherogenic sd-LDL-C to less atherogenic lb-LDL-C, than either fenofibrate or simvastatin monotherapy [30].

Although statins are relatively safe and well tolerated, there are still significant numbers of patients who cannot tolerate them because of their high costs, especially in India, and lots of side effects, such as, Liver Enzyme Elevation (AST, ALT), muscle pain and weakness, headaches or fevers, loss of memory and brain function, stomach pain, weight loss, vomiting etc. In order to exonerate these side effects and to develop new drugs that are not only capable of reducing cholesterol level but also inhibits LDL and sd-LDL oxidation, existing research are now focused on natural products which have no side effects, cost effective and easily available. Search of new agents that have both lipid lowering as well as antioxidant property are going on.
There are some natural antioxidant enzymes as well as low molecular weight antioxidants, which are subdivided into lipid-soluble antioxidants (tocopherol, carotenoids, quinones, bilirubin and some polyphenols) and water soluble antioxidants (ascorbic acid, uric acid and polyphenols). These delay or inhibit cellular damage mainly for treating oxidative stress and atherosclerosis such as, Vitamin A, C, E, K, quercitin, curcumin, retinol, catechin, thymoquinone, lutin, lycopene etc. Preclinical and clinical studies showed that some of these compounds possess antiatherogenic activity but detailed investigation regarding their mechanism of action is not done, except for Vitamin E that contains tocotrienol and tocopherol. Tocotrienols are now considered to be more potent antioxidant and hypolipidemic agent than tocopherol [10,11]. Like statins, tocotrienols also works by suppressing HMG-CoA reductase enzymatic activity.

The disappointing results of antioxidant strategies, in both the prevention and limitation of atherogenesis and cardiovascular complications in humans, raise questions about the role of oxidative modification of LDL in atherosomatosis. Numerous reasons could explain the failure of antioxidants in the prevention and treatment of cardiovascular diseases. There are points that need further examination, such as the fact that random generation of free radicals cannot explain the progression rate of atherosclerosis, the level of oxidative modification of LDL, in what way the oxidative process contributes to the reaction of atherosclerotic plaque and why atherosclerotic lesions occur at specific sites on vessel wall, which antioxidants best inhibit endothelial dysfunction, proliferation of smooth muscle cells, hypertrophy and lipid oxidation, which patient groups respond better to antioxidant treatment, and what are the required doses of antioxidants and in what way may the side-effects, such as DNA damage after the high dose administration of vitamin C be confronted.

From the above discussion it is clear that sd-LDL is now recognized marker and therapeutic target for CVD risk assessment and management. Moreover, there is a large body of evidence connecting the effects of oxidative stress with atherogenesis. However, so far we have not determined a specific causative relation between oxidative events in general and oxidative modification of LDL and sd-LDL in particular with respect to atherosclerosis. Further, with the contribution of molecular cardiology and pharmacogenetics, to elucidate the molecules as well as the inhibiting mechanisms that interfere in the oxidative process is needed. Thus, the therapeutic inflection of sd-LDL often require the combination of different treatment of drugs, lifestyle modification and dietary intake, which may constitute both primary and secondary prevention of CVD. In addition, individualized treatment is required for proper assessment of various risk factors that promotes CVD, particularly sd-LDL. In last, the lipid lowering agent whether they are natural or chemically synthesized may constitute the first line of therapy along with life style modification.

References
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