Small Dense LDL: Risk Factor for Coronary Artery Disease (CAD) and its Therapeutic Modulation

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Pathogenesis of coronary artery disease (CAD) is multi-factorial and many risk factors are associated with development of CAD. LDL-C has been an important target for therapeutic interventions and has been extensively studied. But, various studies have indicated that estimation of LDL-C is not enough to assess the risk. Moreover, LDL particles vary in their content, density and size which have different physico-chemical properties. In this paper, the role of small dense (sd) LDL in risk assessment for CAD and its response to different therapeutic modalities available have been reviewed.

Keywords: sd LDL, Coronary artery disease, Hypertriglyceridemia, Life-style modification, Dietary modulation, Drug therapy

Introduction

Coronary artery disease (CAD) is one of the commonest causes of mortality and morbidity the world over. According to WHO, it was one of the leading causes of deaths in 2008, being responsible for 7.3 million deaths all over the world1. There has been an alarming increase in incidence of CAD in younger individuals more so in developing nations2. Relative risk of all lipoproteins in CAD has been extensively studied and the principle target for cardiovascular preventive strategies has been low density lipoprotein cholesterol (LDL-C)3. With the advent of new therapeutic modalities and strategies, identification and management of dyslipidemia has gained importance for primary as well as secondary prevention of recurrent events.

Pathogenesis of atherosclerosis and thus CAD is multi-factorial and include conventional risk factors like hypertension, diabetes mellitus, smoking, dyslipidemia and central obesity at a younger age. New risk factors include elevated lipoprotein(a), high sensitivity C reactive protein (hs-CRP), fibrinogen and hyper-homocysteinemia etc. Moreover, certain metabolic diseases/syndromes also have a bearing on the final outcome of the disease and its management. One of the important clinical syndrome which often is associated with CAD is metabolic syndrome which comprise of insulin resistance (IR), hypertension, hyperlipidemia and central obesity though IR in isolation, is also considered to play an important role in cardiovascular diseases3,4. In addition, dyslipidaemia and smoking are important modifiable risk factors and smokers tend to have higher incidence of deranged lipid profile. We observed that elevated LDL-C is the most important lipid derangement consequent to smoking and smokers have increased ratio of LDL/HDL (high density lipoprotein) making them more susceptible to atherosclerosis5.

Estimation of LDL-C is not enough

Although various interventions have already proved to lower the risk of CAD and various randomized controlled trials have shown the benefits of lowering LDL-C with HMG-CoA reductase inhibitors (statins), treatment for LDL-C levels alone
attributes to 30-37% decrease in CAD, rest continued to have clinical events e.g. graft progression, arteriographic progression even after adequate reduction of LDL-C by medications. Thus, other factors have been sought to explain the additional risk. A growing number of studies suggest that hs-CRP, a marker of inflammation, is an independent risk factor for atherosclerotic vascular disease. Many studies have demonstrated an increase in serum hs-CRP levels in CAD patients of all age groups. The hs-CRP is believed to be directly and actively involved in atherogenesis by binding to LDL particles in atherosclerotic plaques leading to activation of complement.

Plasma triglyceride (TG) levels are also associated with CAD, independently of LDL and HDL cholesterol levels. Furthermore, LDL-TG levels are more strongly associated with CAD than LDL-C concentration. High levels of LDL-TG, but not LDL-C, are also associated with high levels of inflammatory markers i.e. C-reactive protein and serum amyloid A. The Interheart study comprising 15,000 individuals from 52 countries has demonstrated the Apo B/Apo A1 ratio is a major predictor of CAD in men and women all over the world. We found that smokers too tend to have significantly higher Apo B levels compared to non-smokers. Since each VLDL and LDL particle has one molecule of Apo B in it, number of Apo B particles in principle provides indication of the number of LDL particles present. But, the LDL particle may vary in their content of cholesterol and TGs.

Several studies suggest that it is the content of LDL that has an important bearing on progression of atherosclerosis and CAD. In the Framingham study, the levels of LDL-C in most cases of premature cardiovascular disease (CVD) have been found to be similar to those who do not develop premature disease. It is the TG content of LDL particles that is associated with systemic inflammation. This study has also indicated that LDL-TGs have positive correlation with vascular adhesion molecules like ICAM-1 and VCAM-1. Moreover, many individuals with LDL-C levels apparently within the normal range suffer from CVD. These individuals have visceral obesity and are characterized by a typical atherogenic dyslipidemia. Atherogenic lipoprotein phenotype is characterized by elevated levels of TG and sd-LDL particles and reduced HDL cholesterol (HDL-C).

The hypertriglyceridemia, low HDL, high LDL along with central obesity is frequently seen in individuals with insulin resistance or type 2 diabetes or individuals with familial combined hyperlipidemia (FCHL). This conglomerate of biochemical parameters also constitute metabolic syndrome. Metabolic syndrome is now well known to be associated with sd LDL which has emerged as an important risk factor for CAD. In a prospective study on pre-eclamptic women, it has been demonstrated that high TG levels resulted in a significant shift in lipid profile towards sd LDL.

What is sd LDL

Various studies have clearly indicated that LDL can be fractionated into various sub-fractions by using various methods like density gradient, ultracentrifugation, PAGE, NMR etc. As many as seven distinct LDL subpopulations depending on their densities, which differ in their metabolic behaviour and pathological roles, have been identified. LDL particles are shown to have bimodal distribution and thus two phenotypes are identified — pattern A with LDL $>25.5$ nm (large and buoyant) and pattern B with LDL $\leq 25.5$ nm (small and dense). Pattern B is prevalent in 30% adult men, 5-10% in young population (men and women less than 20 yrs) and 15-25% in post-menopausal women.

Although sd-LDL particles are known to co-exist with other atherogenic risk factors like increased plasma TGs and Apo B and low HDL cholesterol concentration, their association with certain diseases i.e. familial combined hyperlipidemia, hyper $\beta$-lipoproteinemia and hypoalphalipoproteinemia indicate a possible genetic basis. Two independent studies have clearly demonstrated a correlation of inheritance of LDL particle size from parent to offspring as well as siblings. Even though LDL size is genetically linked, environmental factors also play an important role. Abdominal obesity, use of oral contraceptives, smoking and diet high in carbohydrates and low fat content favours sd LDL. Distribution of sd LDL also varies with sex. Male have smaller and more dense LDL than do female. A nine year follow up study in Japanese men and women has indicated that number of small LDL particles varies between two sexes. It increases in women with age, although weight gain leads to an increase in small LDL particles in both sexes. However, weight reduction is more effective in men in reducing number of small LDL particles.
Size of LDL depends on how much lipid is present in the core; and lipid content in turn determines its density. Small and dense LDL particles are formed largely as a response to high levels of TGs. Small dense (sd) LDL particles are the products of the intravascular remodelling of TG-rich VLDL particles after interaction primarily with lipoprotein lipase, hepatic lipase and cholesterol ester transfer protein. Cholesterol ester transfer protein-mediated cholesterol ester/TG exchange between VLDL to LDL along with hepatic lipase-mediated hydrolysis results in transformation of LDL to sdLDL. Austin et al found that pattern B is associated with a two-fold increase in plasma TGs, higher plasma Apo B, reduced HDL cholesterol and ApoA-I concentration, i.e., sd LDL does not appear in isolation from other plasma lipid abnormalities. The critical value of TGs above which sd LDL predominate is 15 mmol/l though some authors consider it to be 13 mmol/l. A positive correlation has also been found between plasma TG and LDL-TG content among phenotype B subjects. Recently, it has been demonstrated that glycation of LDL by methylglyoxal reduces the size of LDL particle, providing an important link between various metabolic factors and increased incidence of CAD in diabetic patients.

Atherogenicity of sd LDL

Atherogenicity of sd LDL is due to its high oxidizability, owing to low cholesterol and high PUFA and ApoB content. Furthermore, this molecule is depleted of vitamin E, which accounts for its susceptibility to oxidation. In addition, sd LDL is cleared slowly by receptors as compared to large buoyant LDL and thus has a long residence period in plasma, allowing more time for them to be oxidized and taken up by macrophages in extra-vascular spaces. Low uptake by receptors has been attributed to decrease binding affinity to receptors due to conformational change brought about in ApoB by increase in TG content or decrease in size of LDL. sd LDL shows higher preponderance for binding to arterial proteoglycans as well as higher permeability through endothelial barrier, resulting in formation of foam cells. Soran et al in their review article have argued that it is the sd LDL fraction rather than the buoyant fraction which is preferentially glycated in diabetics. This glycation of LDL results in reduced uptake by receptors as well as increase its property of aggregation and binding to arterial proteoglycans, thus making it more atherogenic.

Apart from increased atherogenesis, increased levels of circulating small dense LDL particles are associated with elevated fibrinogen concentrations (>2.90 g/L). This association is independent of other risk factors associated with hyperfibrinogenemia, i.e. BMI, age, insulin resistance and serum lipid concentrations. Association of hyperfibrinogenemia with hypertriglyceridemia may provide a metabolic link between fibrinogen levels and sd LDL, since both entities are associated with sd LDL, but exact mechanism is still unknown.

sd LDL is also found to be associated with increased concentration of plasminogen activator inhibitor protein-1 (PAI-1), which is an inhibitor of fibrinolysis and thus increases the risk of CAD. Even this association is independent of plasma TGs, BMI, fasting insulin and insulin resistance. Oxidised LDL is also shown to stimulate PAI-1 release from endothelial cells. Since sd LDL is oxidised easily, it may increase atherogenicity through PAI-1 also. sd LDL also impairs endothelial dysfunction and the probable reason may be the association of sd LDL with high TG levels which correlate with LDL-TG levels and are causing vascular damage.

sd LDL and risk of CAD

The National Cholesterol Education Program (NCEP)-ATP III has already identified sd LDL as a risk factor for CAD, as is evident from inclusion of metabolic syndrome in its guidelines for risk stratification. Although ATP III considers small and dense LDL as a lipid risk factor, many studies have given conflicting evidence against the importance of sd LDL to assess the risk of CAD. In EPIC-Norfolk study, it is found that relationship between small LDL-C and CAD is not significant after adjustment for TGs and HDL-C levels and elevated TG levels in serum and decreased HDL-C concentrations can provide enough information in assessing the risk of CAD. Capell et al have also reported that after adjusting LDL-TG for plasma TG concentration, the LDL-TG is similar in the phenotype A and B subjects. The study has shown that high LDL-CE and LDL-TGs in phenotype B is due to increased LDL number in these individuals and not due to the change in the composition of LDL particles.

However, there are a number of prospective studies which have demonstrated that the small LDL particle is the better predictor of CAD and an important risk factor for atherosclerosis. In the 13 year follow-up study...
of the Quebec Cardiovascular Study comprising 2072 men of 46 to 75 yrs of age, it is concluded that small LDL is a strong and independent predictor of CAD, particularly for short-term follow-up. Since the individuals with familial hypercholesterolemia having higher concentration of large LDL particles are also at high long-term risk of developing CAD, Quebec Cardiovascular Study has indicated that in addition to size, LDL particle number, determined by Apo B content has its own importance in assessing risk of CAD. In addition, EPIC Norfolk study, though has argued that after making adjustments for TGs and HDL-C, estimation of sd LDL does not provide any extra advantage in assessing the risk, the study has clearly shown that small LDL is associated with the reduced survival rates. Considering all the studies, either for or against, it appears that sd LDL does have implication in risk identification and prevention/management of CAD because different therapeutic modalities affect sd LDL in different ways.

Therapeutic modalities

Conventional treatment of atherogenic lipid profile aims at reducing total cholesterol levels and LDL cholesterol or increasing HDL cholesterol by using different treatment modalities available, which include statins, fibrates, niacin, resins, and even lifestyle modifications. In subsequent discussion, we will try to analyze different studies showing the effect of the available therapeutic modalities on sd LDL.

Combination of inappropriate diet with physical inactivity results in unfavourable lipid concentrations, high BMI, obesity, diabetes and hypertension, all being important risk factors for CAD. Therapeutic life style modification includes diet as an essential element. ATP III has recommended that 25-35% of total calories should be from fats, out of which not more than 7% should be constituted by saturated fats, while the remainder must be from PUFA and MUFA. Intake of cholesterol is recommended to be < 200 mg/day. On the other hand, a very low fat content in diet can increase TGs and can increase sd LDL.

Including soy protein in the diet can reduce CV risk by reducing requirement to animal food, thereby decreasing saturated fatty acids (SFA) intake and leading to reduction of LDL-C. It may also prevent LDL oxidation, increase LDL particle size, as well as reduce cholesterol synthesis and increase bile secretion. Antioxidants like α-tocopherol and β-carotene prevent oxidation of LDL, but supplementation with these have not resulted in any reduction in risk of CVD. Exercise has been shown to increase the peak particle size of LDL. Intensive lifestyle changes reduce CAD risk by altering traditional risk factors, as well as by increasing LDL particle size and reducing number of small LDL particles. However, men seem to derive more benefit than women. Recently, diet containing mixture of rice bran oil and palm oil has been proposed to reduce CVD risk by virtue of reduction of sd-LDL cholesterol levels as well as its oxidation.

Drug therapy includes use of statins, fibrates, niacin, resins, ezetimibe etc Statin group of drugs inhibit HMG CoA reductase, a key regulatory enzyme of cholesterol biosynthesis. 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 and particle number, their effect on particle size is variable. Both fluvastatin and atorvastatin have been shown to shift the LDL profile toward more buoyant particles but pravastatin and simvastatin have not shown any such result. Rather a study has indicated that pravastatin causes a change in the LDL particles to a more dense form. Fluvastatin treatment also improves endothelial dysfunction by decreasing the atherogenic sd LDL fraction in post-menopausal overweight female population.

Fibrates that include drugs like fenofibrate, gemfibrozil and bezafibrate act by stimulating peroxisome proliferator activator receptor α. They are most effective for treating patients with hypertriglyceridemia and reduced HDL-C. These drugs although are less effective in reducing LDL-C, but shift the LDL particle to more buoyant size. Moreover, treatment with fenofibrate markedly reduces post-prandial increase in TG levels and small LDL particles in hypertriglyceridemic subjects. Similarly, VA-HIT trial has reported that gemfibrozil causes a 20% reduction in small LDL particles. However, another study has reported that gemfibrozil does not alter the levels of sdLDL in patients of FCHL even though it reduces TGs.

Niacin reduces Lp(a), LDL-C and TG and increases HDL-C and LDL particle size, but has
major adverse effects like hot flashes, gastric irritation and hepatotoxicity, which limits its use in the clinical practice. Niacin has been used very effectively in combination with statins like lovastatin to reduce LDL-C and TGs and increase HDL-C. 

Ezetimibe, a newly approved drug interferes with the absorption of cholesterol and reabsorption of cholesterol secreted into bile and the enterohepatic circulation of endogenously produced cholesterol. Although it reduces LDL-C by 15-20%, it is used more effectively with statins. Combining ezetimibe with fenofibrate does not improve effect of fenofibrate on sd LDL, suggesting that ezetimibe may not alter sd LDL quantitatively or qualitatively.

In type-2 diabetes, thiazolidinedione group of drugs form the essential part of therapy. The two commonly used drugs are rosiglitazone and pioglitazone. In addition to their effect on the glycemic status, these drugs are observed to have some beneficial effects on lipid levels also, probably through their action on PPARγ. Pioglitazone appears to be more effective in reducing TG and LDL-C and increasing HDL-C than rosiglitazone. Rosiglitazone also increases LDL size, while pioglitazone has effect on both number and size of LDL. So, it appears that these drugs might be useful in individuals with metabolic syndrome. Even though these drugs seem to have beneficial effect on lipids, their role in reducing CHD risk has still not been documented, but combination of pioglitazone and simavastatin does have synergistic effect on lowering cholesterol levels in LDL3 subtype.

Elevated LDL cholesterol, TGs and reduced HDL cholesterol may be present in different individuals in varying combinations. The picture is complicated by presence of small dense LDL, more so in case of metabolic syndrome and type-2 diabetes. Moreover, patient with different LDL sub-fractions respond differently to different therapy. Although we previously discussed some studies which pointed out that considering high TGs and low HDL, measurement of sd LDL does not provide any further advantage in risk assessment, its importance is reflected in deciding the treatment modalities. This is clearly indicated by a study which has demonstrated that although gemfibrozil reduces triglycerides, it does not alter the levels of sdLDL.

ATP III advocates the use of combination therapy, depending upon whether goal for non-HDL-C is achieved or not. Statins can be combined with fibrates or niacin for further improving the risk profile.

It is evident from the above discussion that treatment should be based on presence of different risk factors, as well as that all risk factors cannot be modified by similar therapeutic modalities in all individuals. Thus, individualised treatment is required for proper risk management, depending upon the risk factors present.

Conclusion

The therapeutic modulation of sd LDL has been shown to significantly reduce cardiovascular risk and weight reduction. Therapeutic lifestyle modification like increased physical activity and dietary modulation may constitute first-line therapy. In addition, lipid-lowering drugs are able to favourably alter these particles. Treatment of patients with sd LDL particles often requires the use of combined lipid-altering drugs to decrease the number of particles and to convert them to larger, more buoyant LDL, more so in individuals who have high TGs and low HDL. Since lipid lowering agents are being used for both primary and secondary prevention of CAD, each individual should be assessed for the risk factors and drugs be used along with lifestyle modification in such a manner, so that they target risk profile. The rational combination of drugs along with modulation in physical activity and diet must be justified for medical, financial and ethical reasons.

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