ABSTRACT

Heart is an organ with many functions. Like any organ, heart and the blood vessels are subject to malfunctions and diseases (CVD). There are over 60 different disorders of the cardiovascular system and hence the cause of CVD depends on the disease is in question. Many of the risk factors are divided in to modifiable risk factors such as age, gender and family history.

Mortality and morbidity due to cardiovascular diseases (CVD) has been on the increase in the past half century, reaching epidemic proportions. Hence several theories were put forth to explain the risk in the incidence of CVD. The Diet-Heart theory has dominated our understanding of what causes heart disease. The inflammation hypothesis of heart diseases is actually older than the Diet-Heart theory, but was ignored for almost a century. The response to injury hypothesis is of more recent origin, and response to retention hypothesis, is even more recent.

A common theme in all these theories is the oxidation of lipoproteins; particularly low density lipoprotein (LDL). Much of the research in the last half a century was focused on the LDL molecule as the major cause of CVD. Consequently all therapeutic approaches were focused at reduction of Low density lipoprotein cholesterol (LDL-C), as a method for reducing the risk of CVD. It is now increasingly becoming clear that the only negative risk factor for CVD is high density lipoprotein associated cholesterol (HDL-C). The cardio protective functions of HDL were believed to be because of its ability to bring cholesterol from peripheral tissues to the liver for degradation to bile acids and excretion.

With the advances in proteomics, many proteins were identified in the HDL particle which had no role to play in reverse cholesterol transport, instead these proteins were antioxidants and antiinflammatory in function. Several recent studies have identified ‘dysfunctional HDL’ which has lost or reduced its functionality.

The current dogma that oxidation of lipoproteins are necessarily atherogenic may not be true concerning the HDL oxidation. HDL is the major carrier of oxidized lipids in the plasma. This may be because the HDL removes them from other oxidized lipoproteins to detoxify them. HDL has many antioxidant enzymes and proteins which
can reduce lipid hydroperoxides. Since HDL accepts oxidized lipids for detoxification, HDL was found to have many oxidized lipids and products of oxidation. This was attributed to oxidative damage and hence it was thought to be a mechanism for rendering HDL dysfunctional. In support of this hypothesis, methionine residues and tyrosine residues in HDL protein were found to be oxidized particularly in atherosclerosis.

In contrast to these observation there are several reports that show that oxidation of HDL enhances its functions rather than inhibiting them. The apparent controversy was resolved by showing that mild oxidation of HDL increases its functionality while extensive oxidation inhibits it. Hence this study was taken up to investigate the role of oxidation on HDL function.

The ability of lipoprotein to oxidize dichlorofluorescence (DCFH) was used as a method to evaluate the oxidizability of the lipoprotein. Oxidized lipoprotein will have lipid hydroperoxides which can oxidize DCFH. LDL and HDL were both able to oxidize DCFH in a dose dependent manner. On equal molar proportions of cholesterol, HDL was able to oxidize DCFH more than the LDL. When HDL was added to LDL, the oxidizability of the mixture was equal to their individual abilities. When HDL or LDL was oxidized further their ability to oxidize DCFH decreased. This was probably because the oxidation modified the protein in addition to the lipids forming more Thiobarbituric acid reactive substances (TBARS) and decreasing the conjugated diene content measured by 234nm absorption. When antibody to oxidized HDL was added to the oxidized serum, a precipitate was obtained with very little ability to oxidize DCFH. However the supernatant had increased ability to oxidize DCFH.

The oxidized HDL precipitated by the antibody could competitively inhibit the activity of HDL. Interestingly, when antibody was raised against oxidized Apo A-I it cross reacted with oxidized LDL more than either apo A-I or oxidized HDL suggesting that the antibody was raised against oxidation specific epitopes. This was confirmed by the observation that the preimmune antibody was also able to bind the oxidized HDL.
HDL or oxidized HDL did not stimulate the production of TNF-α in adipocytes. However when Lipopolysaccharide (LPS) was added, the HDL was able to potentiate the action of LPS in a time dependent manner.

Oxidation of HDL decreased the two promiscuous activities namely the phenyl acetate hydrolysing activity and the paraoxonase activity. However the thiolactonase activity was increased. When the three activities determined in the serum of 242 subjects, the three activities did not correlate among themselves. These results suggest that the variability of substrate specificities among individuals is probably due to the variability of the pro and antioxidant activities in the blood in addition to the variability introduced through genetic polymorphism is the enzymes.

The highest oxidizability of HDL seen in our study may not be a pathological change but a normal physiological phenomenon which account for its antioxidant activity. In fact oxidation enhanced its lactonase activity. Experiments with raising antibodies to oxidized HDL suggest that the oxidation phenomenon may be more common than expected and may give raise to natural antibodies to oxidation specific epitopes. This mechanism could potentially reduce the harmful effects of oxidized proteins and other molecules.

Thus the HDL molecule may have more complex function than discovered to date and may have a significant role in diseases besides cardio vascular diseases.