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Research Article

DYSLIPIDAEMIA AND FREE RADICALS ARE INDEPENDENT RISK FACTORS IN CAUSATION OF CORONARY HEART DISEASE IN NIDDM

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ABSTRACT
Abnormalities that characterizes lipoprotein metabolism in non-insulin dependent diabetes mellitus (NIDDM) patients, fasting concentration of triglyceride rich lipoprotein especially very low density lipoprotein (VLDL) are higher, and those of HDL, commonly measured as HDL-c, are lower than among people without diabetes, which leads to increased triglyceride HDL-c ratio and insulin resistance. This type of diabetic dyslipidemia is major cause of oxidative stress and thus, coronary heart disease (CHD). The study was carried at the Central Clinical Laboratory MIMSR Medical College Latur, NIDDM patient were selected after attending Medicine OPD MIMSR Medical College Latur, the lipid profile and total serum lipid peroxides (malanodialdehyde) of 50 diabetic patients and 50 healthy subjects were determined and compared. In the control group I mean values of total cholesterol were 175.55 ± 29.87 mg %, LDL-c 110.60 ± 28.73mg%, serum triglycerides were 108.39 ± 39.62 mg %,HDL-c were 44.45 ± 6.7 mg% and In the group II serum triglycerides 145.68 ± 68.94 mg% and were significantly increased as compared to group I. The serum HDL-c group II 41.46 ± 6.6 mg % were significantly decreased as compared to group I (P is<0.05). The serum lipid peroxides in the group 2nd (217.9±19.1) were significantly increased as compared to group 1st (180.21±18.13). A characteristic feature of NIDDM is the elevated triglycerides and lowered HDL-c levels, which leads to insulin resistance oxidative stress damage to endothelium and end organ damage CHD.

Keywords: *NIDDM, Serum Triglycerides, HDL-c, Insulin Resistance Oxidative Stress Lipid Peroxides Malanodialdehyde and CHD*

INTRODUCTION
The major independent risk factors for the development of atherosclerosis are the plasma cholesterol concentration, cigarette smoking, hypertension and diabetes, which are by them self's risk factor for coronary heart disease (Ross, 1986).
Despite recent decline in cardiovascular mortality, atherosclerotic disease is still major health problem facing Western society. Incidence of coronary heart disease has shown upward trends in Indian in last decade (Dewan *et al.,* 1974; Sarvothan and Berry, 1968). A large amount of epidemiological evidences also supports the relationship between serum low density lipoprotein cholesterol (LDL-c) and coronary artery disease (CAD) in Indians (Enas *et al.,* 1996).
Serum high densitylipoprotein-cholesterol (HDL-c) level has been found to have inverse relationship with the CAD (Castelli and Andreson, 1986). Diabetes mellitus is a common among Indians with CHD both in their land of origin and abroad (Bhoraskar and Raheja, 1997). Individuals with NIDDM are more likely to have multiple risk factors for CHD than age matched non diabetic subjects. Peoples with diabetes have a risk of CHD two to five times that of nondiabetic individuals (World Health Organization, 1994; Panzram, 1987; Meigs *et al.,* 1997).
Abnormalities that characterizes lipoprotein metabolism in non insulin dependent diabetes mellitus (NIDDM) patients, fasting concentration of triglyceride rich lipoprotein especially very low density lipoprotein (VLDL) are higher, and those of HDL, commonly measured as HDL-c, are lower than among people without diabetes (Taskinen, 1990; Howard, 1987). NIDDM is an integral component of the metabolic syndrome (Zimmet, 1993).

In addition to triglycerides levels, overweight and plasmatriglyceride to HDL-c ratio of three or greater as a reliable indicator of insulin resistance (Mc Laughlin *et al.,* 2003).
In fact Mc Laughlin & Colleagues (2003) suggested that an elevated TG to HDL-c ratio may be "Clinically appealing marker because of its robust association with Cardio vascular disease (CVD) (Gaziano *et al.,* 1997).
NIDDM is associated with increase in plasma triglyceride and decrease in plasma HDL-c concentration i.e. dyslipidaemia changes that have been identified as the increasing risk, of CHD. Hyperglycemia, a hallmark of diabetic condition depletes natural antioxidants and facilitates the production of reactive oxygen species (ROS) which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, DNA etc and exert cytotoxic effects on cellular components (Dincer *et al.,* 2002).
Thus, increased ROS and impaired antioxidant defense contributes for initiation and progression of micro and macro vascular complications in diabetics (Maritim *et al.,* 2003; Jialal *et al.,* 2002; Ceriello *et al.,* 1998).
Since the classic risk factors do not account for the excess risk of atherosclerosis in NIDDM, we need new approaches to explain the connection of this risk factor and accelerated vascular disease. Thus, the aim of our study was to investigate role of altered lipoprotein-triglyceride metabolism abnormalities and oxidative stress in progression of CHD events in NIDDM.

MATERIALS AND METHODS

Methods

We studied 50 healthy and 50 diabetic patients matched for age and body mass index. Subjects were selected from medical, paramedical staff and general public who were around 40 to 60 year of age. All subjects were belonged to the Latur district of Marathwada region.
Patient belonging to group II were selected after attending medicine OPD of MIMSR Medical College, Latur and diagnosed as diabetic.
The healthy subjects were nonsmokers, nonobese, nonalcoholic and free from any disease and not taking any drugs that alter lipid and carbohydrates metabolism.
All patients belonging to group II had NIDDM. Criteria of diagnosis of diabetic are: Fasting blood sugar levels not less than 140.0 mg %. All subjects after taking informed consent was interrogated and detailed examination was done. Blood samples drawn after an overnight fast. After serum separation the analysis was done on the same day.
We estimated serum triglycerides by enzymatic method (Autopack Siemens kit) and total cholesterol by enzymatic methods (Autopack Siemens kit) HDL-c measured by phosphotung state method (Autopack Siemens kit).
LDL-c and VLDL- c values were calculated by Friedwald's equation (Levy and Fridickson, 1972). We also estimated the total serum lipid peroxides by reaction with thiobarbituric acid using the method of Nadiger and Chandrakala (1986).
Reaction involved in this method is malanodialdehyde combines with thiobarbituric acid to form malanodialdehydethiobarbituric acid complex which is measured colorimetrically at 530nm. The total serum lipid peroxides were calculated by using the coefficient of malanodialdehyde 1.5x10 and was expressed as malanodialdehyde /dl serum.

RESULTS AND DISCUSSION

Results

In the control group I mean values of total cholesterol were 175.55 ± 29.87 mg %, LDL-c 110.60 ± 28.73 mg%, serum triglycerides were 108.39 ± 39.62 mg %, HDL-c were 44.45 ± 6.7 mg% and in the group II serum triglycerides 145.68 ± 68.94 mg% and were significantly increased as compared to group I. The serum HDL-c group II 41.46 ± 6.6 mg % were significantly decreased as compared to group I (P is<0.05). The serum lipid peroxides in the group 2^{nd} (217.9±19.1) were significantly increased as compared to group 1^{st} (180.21±18.13).

Table 1: Serum Cholesterol and Other Biochemical Parameter in Normal Healthy Subject (Group I) and Diabetic Patient (Group II)

	175.55±29.87	•183.24±41.94
	108.30±39.92	*145.68±68.94
	44.45±6.70	*41.46±7.67
	110.63±28.73	•111.77±35.37
	21.73±8.23	•111.77±35.37
	2.53±0.83	•2.79±1.06
	180.21±18.3	*217.9±19.1

N=50 in each group Comparison between group I and II
•P>0.05, * P <0.05

Discussion

The catabolism of triglyceride-rich lipoproteins is initiated by lipoprotein lipase, an endothelial enzyme that hydrolyses the triglyceride moiety of chylomicrons and VLDL, and releases fatty acids for energy production in muscle and for storage in adipose tissue.

The activity of this enzyme is generally lower in NIDDM patient than in non diabetic people of similar age and degree of adiposity: The difference is more striking for patient with both NIDDM and coronary artery disease (CAD) (Kahari *et al.,* 1995).

Lipoprotein lipase activity is low in untreated or poorly controlled NIDDM and increase with improved glycaemic control (Taskinen, 1987). In NIDDM passage of triglyceride- rich lipoproteins through the lipolytic cascade is delayed for two reasons: there is a shortage of catalytic sites on lipoprotein lipase, and over production of triglyceride saturates the sites that are available. Both mechanisms promote hypertriglyceridaemia.

The two components of diabetic dyslipidaemia, high concentrations of triglyceride-rich lipoproteins and low concentrations of HDL, are closely interwoven. Hypertriglyceridaemia contributes to low HDL concentrations in one or combination of following reasons. 1) The first process involves the transfer of surface remnants'-redundant phospholipids and apolipoproteins from lipolysis of triglyceride – rich lipoproteins – to HDL particles.

Because lipoprotein lipase activity is decreased and lipolysis impaired in NIDDM, there are fewer surface remnants available to be incorporated into the HDL particle. 2) The large amount of triglyceride-rich lipoproteins and their prolonged residence time in the circulation increased the exchange (mediated by cholesteryl-ester transfer protein) of esterified cholesterol from HDL to triglyceride-rich lipoproteins and of triglyceride to HDL particles.

The result is enrichment of the HDL particle core with triglyceride. Enriched HDL has a faster catabolic rate than normal HDL which leads to a lower number of circulating HDL particles (Figure 1). Furthermore, the HDL particles in NIDDM are smaller owing to a high hepatic lipase activity-another feature of NIDDM.

Hepatic lipase has a great avidity for triglyceride-rich HDL and hydrolyses the triglyceride in the HDL core, which leads to a smaller HDL particle size. Small dense HDL and LDL particles are components of the dyslipidaemia of NIDDM (Taskinen *et al.,* 1996). The lipolytic process itself of triglyceride enriched HDL may lower HDL particles number.

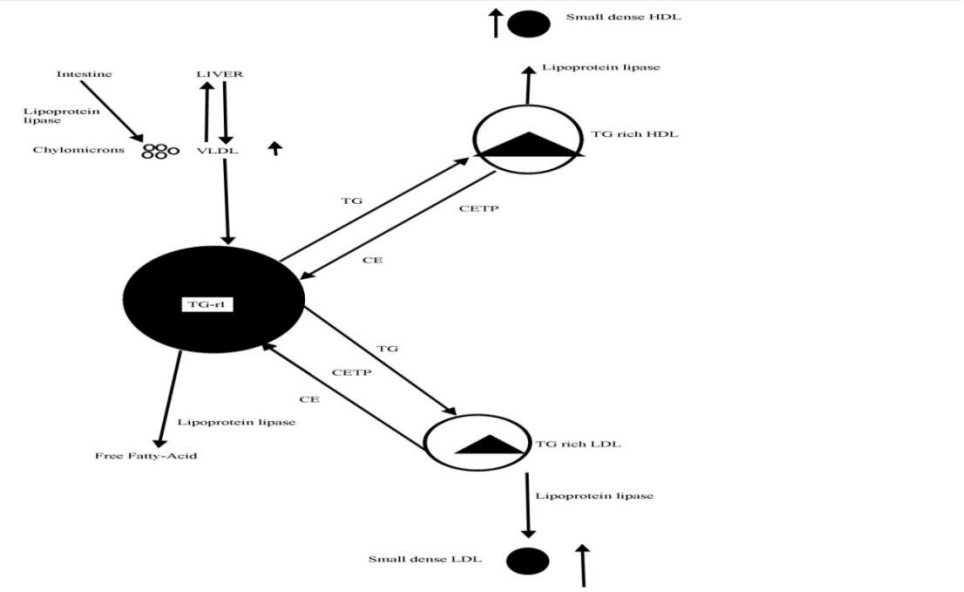

Figure 1

Figure 1: Shows increased production of VLDL by the liver and low lipoprotein lipase activity lead to raised concentration of triglycerides-rich (TG-rich) lipoprotein in plasma. High TG-rich lipoprotein concentration increase the transfer of TG to LDL and HDL and a concomitant transfer of cholesterol esters (CE) from LDL and HDL to TG-rich lipoproteins, all these reaction are mediated by cholesterol-esters transfer protein (CETP).

Lowered plasma HDL-c and elevated plasma triglyceride levels are features of NIDDM dyslipidaemia (TG/HDL-c ratio above 3) which is a reliable predictor of insulin resistance. Insulin resistance a syndrome that favors atherosclerosis and thus, CAD (figure 2). Relying on LDL-c or total cholesterol alone can be misleading. It is also proved that people with obesity, metabolic syndrome or diabetic lipid disorders often have raised triglycerides, low HDL-c and normal or closed to normal LDL-c. Free radicals controls oxygen transport, require for cytochrome P-450 activity, prostaglandin cascade, phagocytosis, blood pressure regulation and detoxication processes. Under certain normal conditions oxygen may accept only one electron (usually in the electron transport chain accepts four electron and get converted to water) and this results in the formation of oxygen derived free radical superoxide. (O_2^-) which may initiate the chain reaction of free radical formation. Malanodialdehyde is stable products of lipid peroxidation. Malanodialdehyde levels are indicative of lipid peroxidation which is a oxidative degeneration of polyunsaturated fatty-acids. Malanodialdehyde is regarded as a marker of inflammation induced by free radical injury on membrane lipids. Proposed pathway for free radical formation and development of complications due to free radical stress is shown in (figure 3). Diabetes contributes to atherosclerosis through various mechanism such as accelerated formation of reactive oxygen species due to decreased in activity superoxide dismutase, glutathione peroxidase (Giugliano *et al.,* 1995). Oxidation of lipoproteins and decreased levels of HDL. Some studies have shown that diabetes mellitus may result from oxidative injury to the islets due to free radical production catalyzed by decompartmentalized transition metals such as iron and copper (Mukaopadhya, 1994). Elevated levels of free radicals in NIDDM oxidizes the lipoproteins. Oxidized lipoproteins particularly oxdized LDL is more atherogenic**.** Several studies have suggested that the LDL may oxidized in arterial wall and thus, initiate and promote atherosclerosis (Witztum and Steinbberg, 1991). A short lag phase for the oxidation of LDL is associated with coronary atherosclerosis in patients with CHD (Regnstrom *et al.,* 1992; Cominacini *et al.,* 1993). Susceptibility of LDL to oxidation has been related to progression of atherosclerosis in carotid and femoral arteries and a

higher proportion of partially oxidized LDL was found in patients with progression of atherosclerotic plaque (Andrews *et al.*, 1995). In addition to this, insulin at physiological level has antiatherogenic effects in vasculature (Feener and King, 1997). Many reports show that in NIDDM activity of insulin to induce vasodilation is low due to insulin resistance (Velloso *et al.*, 1996).

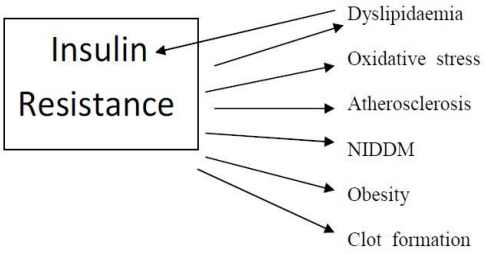

Figure 2: Biochemical and Pathological Consequences of Insulin Resistance

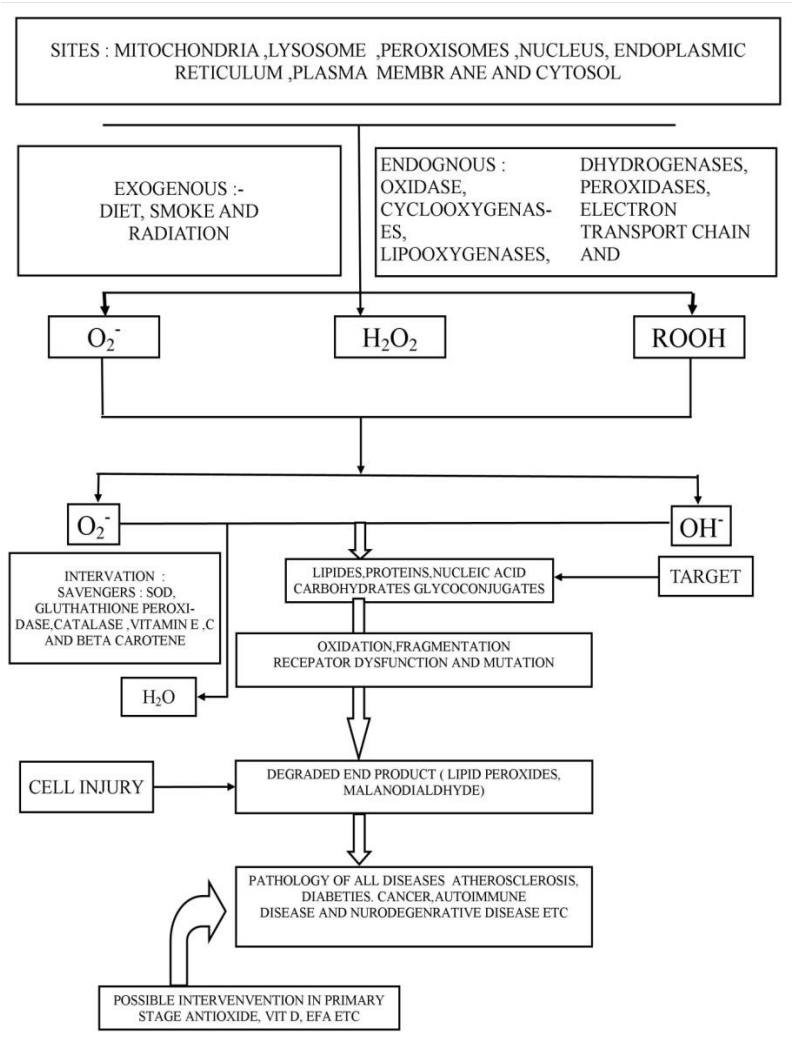

FIGURE 3 : PROPOSED PATHWAY FOR FREE RADICAL FORMATION, OXIDATIVE STRESS AND PATHGENESIS OF ATHEROSCLEROSIS AND OTHER CHORNIC DISEASES.

Conclusions

The lipid profile in NIDDM is abnormal, with hypertriglyceridaemia and low HDL-c concentration as the dominant features. This is because of insulin resistance or leads to insulin resistance and this disturb or impair lipoprotein metabolism. The TG enriched HDL and LDL are atherogenic (Grundy and Vega, 1992). Further these may get oxidized by oxidative stress of NIDDM and oxidized LDL is again more atherogenic. This atherogenic conditions and cell damage (plaque) needs to be a corrected before the development of end organ damage as both are independent risk factors for CHD (Figure2).

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