INTRODUCTION

In spite of our expanding knowledge about atherosclerosis, it remains to be the leading cause of death. Hypercholesterolemia represents one of the very important and recognized risk factor for atherosclerosis (Daugherty et al., 2004). The relationship between dietary cholesterol and coronary heart disease (CHD) has been a topic of intense research. The concept that dietary cholesterol contributes to hypercholesterolemia and CHD risk has been a fundamental part of public health policy. High fat diet feeding leads to cholesterol deposition in the arterial wall (Brown and Goldstein, 1984; Castro et al., 2005). Strong association between coronary risk and lipoproteins concentration in plasma has been well documented in several epidemiological studies (Castelli et al., 1977; Simon et al., 2004). These lipoproteins are believed to act in the arterial wall to initiate or propagate complex inflammatory reactions leading to intimal accumulation of cholesterol-laden macrophages, foam cells and the progressive development of atherosclerotic plaque (Palmer et al., 2004).

Hypercholesterolemia is further associated with an elevation of plasma low-density lipoproteins (LDL). LDL is the major carrier of cholesterol in the blood and is most significantly associated with atherosclerotic plaque formation (Auwerx et al., 1989; Gouni-Berthold and Sachinidis, 2004). Several epidemiological studies correlated high concentration of LDL cholesterol in plasma to the incidence of coronary heart disease (Castelli et al., 1990; Mabuchi et al., 2004). In the genetic disorder, Familial Hypercholesterolemia (FH), basically the patients develop massive LDL concentrations and frequently die within the second decade of life from complications of coronary artery atherosclerosis (Fredrickson et al., 1972; Hogue et al., 2004). Increased LDL level in the body is accumulated in the intima, where it is oxidized to form oxidized LDL (oxLDL). This oxLDL play a major role in initiation of atherosclerotic lesions (Holvoet, 2004; Takahashi et al., 2005).
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Basically, the low-density lipoprotein receptor (LDL-R) mediates the removal LDL and remnant lipoproteins from circulation by binding to apolipoproteins (Brown and Goldstein, 1986; Ouguerram et al., 2004). Liver demonstrates the highest level of LDL-R activity (Rudel et al., 1986). The LDL-R deficient subjects have modestly elevated levels of plasma cholesterol levels. When fed with high cholesterol diet they accumulate much higher cholesterol levels than normal subjects and in turn develop atherosclerosis (Ishibashi et al., 1993). So, LDL-R plays a critical role in the regulation of plasma cholesterol levels in the body. Patalay et al., (2005) demonstrated that lowering of lipid levels following a weight reduction program was due to increased expression of LDL-R gene in their subjects. Nomura et al., (2004) concluded that LDL-R gene therapy to high cholesterol diet fed LDL-R deficient mice resulted in the reduction of plasma cholesterol levels. Studies have shown that down regulation of LDL-R mRNA expression on high cholesterol diet feeding was the principle reason for hypercholesterolemia (Liu et al., 1997).

Further, apolipoprotein B (apoB) plays the central role in the interaction between LDL and LDL-R. It is the major structural apolipoprotein found mainly in the atherogenic lipoproteins LDL and VLDL. It is synthesized in the liver (Thomas et al., 1989). Basically apoB contains the ligand-binding domain for the binding of LDL to LDL-R site, which enables the removal of LDL from circulation. During Familial Defective ApoB-100 (FDP) high level of LDL is accumulated in the body. Basically mutation in the apoB gene leads to this defect (Innerarity et al., 1990; Kaiser et al., 2002). Whitfield et al., (2004) demonstrated that mutations in the apoB gene is responsible for abnormal lipid metabolism which is further associated with hypercholesterolemia. In addition to LDL-receptor binding, apoB is known to interact with proteoglycans (Williams and Tabas, 1995). Proteoglycans are present on the endothelial cell surface and facilitate the lipoprotein entry into the vascular intima and accelerate the atherosclerotic plaque progression. Various studies suggested that apoB is better
indicator of atherosclerotic status in the body than total cholesterol and LDL levels (Simon et al., 2004).

Alterations in cellular cholesterol homeostasis are related to the development of atherosclerosis. In addition to LDL-R, 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase also maintains the cellular cholesterol homeostasis. This enzyme catalyzes the rate-limiting step in cholesterol biosynthesis (Ness and Chambers, 2000). Cholesterol uptake by the LDL-R is generally considered to be regulated via transcriptional control mechanisms, whereas HMG-CoA reductase activity is regulated at many levels from transcriptional control to degradation of the enzyme (Cuthbert and Lipsky, 1992). Ness and Gertz (2004) have shown a conspicuous association between dietary cholesterol and expression of HMG-CoA reductase in liver. They demonstrated that when high cholesterol was fed to the animals in the diet, the HMG-CoA reductase expression decreased to compensate for the excess of cholesterol through diet. So, endogenous cholesterol biosynthesis is down regulated to compensate for the excess of dietary cholesterol.

Involvement of thyroid hormones in cardiovascular system is also an established fact now (Pingitore et al., 2005). Both hypothyroidism and hyperthyroidism are related to cardiovascular risk (Canturk et al., 2003; Jung et al., 2003). Thyroid hormone level was found to be decreased during myocardial infarction (Franklyn et al., 1984). Several studies concluded that lipid levels were significantly higher during hypothyroidism (Frank et al., 2004). Thyroid hormone replacement therapy improved the cardiac function in several patients (Bettendorf et al., 2000; Siegmund et al., 2004). Various studies suggested that hypothyroidism is associated with a lower HDL cholesterol level (Caron et al., 1990). HDL is involved in reverse cholesterol transport to the liver. Therefore it helps in reducing the cholesterol level in the blood. Thyroid hormone replacement results in a significant increase in the HDL cholesterol level (Diekman et al., 2000). Hypothyroidism leads to decreased LDL-R mediated catabolism of
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lipoproteins (Abrahm et al., 1981). Staels et al., (1990) demonstrated a 50% reduction in LDL receptor mRNA levels in rats during hypothyroidism. Thompson et al., (1981) suggested a significant improvement in the receptor-mediated catabolism of LDL during T4 replacement therapy. Basically, T4 acts as an antioxidant and it protects LDL from oxidation (Hanna et al., 1993). T3 is known to increase the low-density lipoprotein receptor (LDL-R) level, which has long been proved to have a protective role against cholesterol induced atherogenesis (Shepherd and Packard, 1984).

In normal subjects, thyroid is the unique source of T4, but it secretes only 20% of the whole T3 in the body (Engler & Burger, 1984). All the metabolic and developmental effects of thyroid hormones are mediated by T3. Almost 80% of T3 is produced from T4 by 5'-deiodination in peripheral tissues (Geyten et al., 2005). This reaction is catalyzed by type-I 5'-iodothyronine deiodinase (5'-DI). Liver and kidney provide most of the plasma T3, but skeletal muscle may also contribute significantly to the plasma pool of T3 (Leonard and Visser, 1986; Alvarez et al., 2005). Behne et al., (1990) have concluded that type-I 5'-iodothyronine deiodinase is a selenoprotein and selenium is situated at its active site as selenocysteine. It is an integral membrane protein and is located in the cytosolic side of endoplasmic reticulum in liver (Korhle, 1994), while in the kidney it is present in plasma membrane (Leonard and Rosenberg, 1978a; Leonard et al., 1991). Keeping in view the important role of thyroid hormone status in the cardiovascular disorders and its association with 5'-DI, further investigation into the study of thyroid hormone levels and deiodinase activity during experimental hypercholesterolemia needs attention.

One of the major breakthroughs in atherogenesis research has been the realization that oxidative modification of LDL might be a crucially important step in the development of atherosclerotic plaque (Witztum et al., 1997). The formation of foam cells from monocyte-derived macrophages in early atherosclerotic lesions is not caused by native LDL but only after the modification of LDL by various
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chemical reactions such as oxidation. Oxidation of LDL is a process initiated and propagated by free radicals (Heinecke et al., 1997). Involvement of antioxidants as the preventive agents in cardiovascular diseases has been implicated. One such well-known potent antioxidant is the trace element, selenium (Se). Studies have demonstrated the association of Se deficiency with coronary heart disease (Salonen et al., 1988; Huang et al., 2002).

Several studies related hypercholesterolemia with selenium deficiency (Huang et al., 2002; Lee et al., 2003). Nassir et al., (1997) have demonstrated that selenium deficiency lead to increased HMG-CoA reductase activity, which in turn resulted in increased endogenous cholesterol synthesis. Vijaya et al., (2000) have reported that selenium deficiency is associated with cardiomyopathy resulting in congestive heart failure. On the contrary, selenium supplementation proved beneficial during myocardial ischemia (Poltronieri et al., 1992).

Basically selenium acts through its dependent enzymes, glutathione peroxidase (GSH-Px) and 5′-DI. In fact, reduced expression of glutathione peroxidase has been shown to increase cell-mediated oxidation of low-density lipoprotein (Guo et al., 2001). In addition, glutathione peroxidase activity is decreased or absent in carotid atherosclerotic plaques. The lack of glutathione peroxidase activity in atherosclerotic lesions appears to be associated with the development of more severe lesions (Lapenna et al., 1998). As 5′-DI (a selenoprotein) converts T₄ to T₃, so selenium plays an important role in the control of thyroid hormone metabolism. The 5′-DI activity decreased in hypothyroid state and elevated in hyperthyroidism (Larsen et al., 1981; Wassen et al., 2004). Berry et al., (1990) observed that type-I deiodinase mRNA levels correlated with thyroid hormone (T₃/T₄) status.

Several studies concluded that selenium deficiency leads to downregulation of 5′-DI expression (Behne et al., 1990; Kahrle, 1999). The expression was recovered on selenium supplementation to the selenium deficient animals (Arthur et al., 1990). Levels of 5′-DI activity decreased in liver and kidney but were
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maintained in brain, thyroid and placenta (Chanoine et al., 1993; Meinhold et al., 1993). It has been suggested that the ability of a tissue to maintain deiodinase activity is a function of the extent to which it can maintain its local selenium concentration (Buckman et al., 1993; Vadhanavikit and Ganther, 1993). This tissue specific differential behavior of 5'-DI expression suggests that there is some compensatory mechanism that might get activated during selenium deficiency.

Further, owing to the findings, which indicate that 5'-DI activity rapidly decreases during selenium depletion. It is evident that Se is playing a direct role in maintaining the T₃ levels and it can not be produced in any quantity without deiodination of T₄ being carried out through 5'-DI. Thus selenium being an inherent component of 5'-DI is directly regulating the T₃ levels. Further T₃ is directly involved in regulation of the LDL-R levels at transcriptional level. So, it all depicts that there is some important link between 5'-DI enzyme and lipid metabolism.

Relationship between thyroid hormone status and cardiovascular complications has been a much discussed topic in research now. Also various researchers have linked hypercholesterolemia with selenium deficiency. But there was a basic lacuna in literature that no one has ever tried to explore the behavior of 5'-DI in relation to hypercholesterolemia. Though this enzyme being a selenoprotein and important for circulating T₃ levels in the body. Selenium as such, as a trace element as well as an antioxidant has been much studied for its preventive efficacy against cholesterol induced hypercholesterolemia. Furthermore, T₃ and T₄ being directly involved in the regulation of LDL-R activity but the effect of selenium status on LDL-R activity as well as mRNA expression under experimental hypercholesterolemia has not been much studied in literature.

Hence, keeping in view all the above stated findings, present study was aimed to understand the role of selenium status in modulation of its dependent enzyme, 5'-DI and LDL-R expression at transcriptional as well as
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posttranscriptional level during experimental hypercholesterolemia in SD male rats.

To achieve this, in the present studies animals of different selenium status i.e. deficient, adequate and excess were created by feeding respective diets initially for 10 days to attain the required selenium status. Each Se-status group was further divided into two subgroups i.e. control and high cholesterol diet (HCD) fed groups and respective diets were then fed for 1, 2 and 3 months. After the completion of diet feeding schedule, to know the selenium status of the animals, the selenium levels in liver and serum and GSH-Px levels in liver were estimated. Cholesterol, triglyceride and LDL level were estimated to check the status of hypercholesterolemia. T₃ and T₄ levels were estimated in serum to access the role of 5'-DI in a better way. Apolipoprotein B level was estimated by ELISA and western immunoblot as it is involved in the binding of LDL to LDL-R. 5'-DI activity by radioimmunoassay and mRNA expression was done by RT-PCR. LDL-R activity was estimated in vivo and mRNA expression was studied by RT-PCR. HMG-CoA reductase expression to study the endogenous cholesterol biosynthesis was done by RT-PCR.