Evaluation of Oxidative Protein Damage in Patients with Hypercholesterolemia

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Abstract: Background: Hypercholesterolemia is highly prevalent in Indian population and known to contribute towards increased mortality and morbidity related to cardiovascular and cerebrovascular disorders. An antioxidant defence system consisting of enzymatic and non-enzymatic compounds prevents oxidative damage of lipoproteins in the plasma. When the activity of this system decreases or the reactive oxygen species (ROS) production increases, oxidative stress may occur.The –SH group (reduced thiols) bound to proteins (protein thiols) play a major role in maintaining the antioxidant status of the body. Protein thiols acts as major extracellular antioxidant, they react with reactive oxygen species (ROS) and prevent LDL oxidation. Such thiols have been studied in different disease conditions and found to be decreased compared to healthy control samples. Reduced concentration of protein thiol found to have positive correlation with increase serum level of LDL cholesterol. In the current work we have measured the level of serum protein thiols along with lipid profile in newly diagnosed hyperlipidemic patients and we tried to establish the relationship between serum protein thiols and lipid profile parameters. Objective: To study the level of protein thiols as a potent antioxidant in patient with an increased level of cholesterol. Materials: After obtaining prior consent, blood (2 ml) was taken using aseptic precautions from hypercholesterolemic patients (n = 25) and age and sex matched healthy controls (n = 25) in plain vacutainers. Serum protein thiols were measured by spectrophotometric method using 5, 5′ dithio-bis (2-nitrobenzoic acid) (DTNB). Triglyceride levels were measured by Cobas 6000 using a GPO Trinder method and HDL levels by Cobas 6000 using a direct-homogenous method. LDL levels were calculated. Results: There was a significant decrease in the levels of protein thiols $p< 0.001$ in hypercholesterolemic patients when compared to healthy controls and a corresponding correlatable increase in the level of LDL cholesterol due to oxidative damage. Conclusion: There may be a role for protein thiols as a biomarker in pathophysiology of cardiovascular and cerebrovascular disorders in patients with hyperlipidemia. [Prasad A et al NJIRM 2013; 4(4) : 64-68]

Key Words: Hypercholesterolemia, Protein thiols, Reactive oxygen species, LDL cholesterol.

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Introduction: Hyperlipidemia is characterized by elevated levels of total cholesterol (TC), triglycerides (TG), LDL cholesterol or decreased HDL. Increase of cholesterol in the blood is associated with elevation of tissue cholesterol levels resulting in oxidative stress. Under these conditions, xanthine oxidase uses oxygen molecules as electron acceptors to produce superoxide radicals beside uric acid. Superoxide can convert Nitric Oxide (NO) to peroxynitrite that mediates nitration of sulphur and aromatic residues of amino acids in polypeptide chains. These radicals can react with either aliphatic, sulphur containing or aromatic amino acid, leading to activation or inhibition of the protein functions. The cellular defense against oxidative stress is carried out by enzyme systems such as superoxide dismutase, catalase and glutathione peroxidase and also by protein thiols, non-protein thiols and other non-enzymatic antioxidant systems.[1]

Oxidative stress is known to be a component of molecular and cellular tissue damage in a wide spectrum of human diseases.[2] Hypercholesterolemia can cause increase the oxidative stress by several mechanisms, Several studies indicated that, increase in the free radical activity was supposed to play an important role in the lipid peroxidation and protein oxidation of cellular structures causing cell injury. This is implicated in the pathogenesis of vascular disease in hypercholesterolemic patients. The –SH (reduced thiol) groups that exist both intracellularly and extracellularly either in free form (reduced glutathione) or bound to proteins (protein bound thiols) play a major role in maintaining the antioxidant status of the body. Thiols are a major source of antioxidants in body fluids which are known to reduce highly reactive free radicals thus protecting the biomolecules.[3] Such thiols have been studied and determined in different disease conditions and found to be

decreased in different diseases compared to healthy controls. [3, 4] Thiol status has also been determined in other disorders associated with oxidative stress and the levels were found to be decreased. [5, 6] It is been shown that oxidized LDL is no longer a ligand for the native LDL receptor but a ligand for the acetyl LDL receptor and its uptake by macrophages was therefore much more rapid, sufficient to cause cholesterol accumulation.

Materials & Methods: The study group consisted of twenty five diagnosed hypercholesterolemic patients not associated with any other systemic diseases and twenty five, age (35- 65 years) and sex (15 females and 10 males in each group) matched healthy controls. This study was carried out between January to June 2012 after obtaining informed consent from the participants at our university hospital. Patients with total cholesterol more than 220mg/dL diagnosed on routine check up were enrolled to participate in the study. Hypercholesterolemia having associated diabetes mellitus, hypothyroidism, and coronary artery disease were excluded from this study.

Serum protein thiols and cholesterol levels were estimated in fresh sample obtained from both controls and individuals with hypercholesterolemia and assays were performed immediately.

Special chemical 5 5' dithio-bis (2-nitrobenzoic acid) (DTNB), was obtained from Sigma Chemicals, St Louis, MO, USA. All other reagents were of analytical grade. Serum thiols were measured by a spectrophotometric method using DTNB.[7,8] Briefly, 900 µL of 0.2 M Na_2HPO_4 containing 2 mM Na_2EDTA, 100 µL serum and 20µL of 10 mM DTNB in 0.2M Na_2HPO_4 were taken in an Eppendorf tube. The solution was mixed in a vertex mixer and transferred to a cuvette, and the absorbance was measured at the end of 5 min at 412 nm in Genesys™ 10 UV spectrophotometer. Appropriate sample and reagent blanks were prepared and the corrected absorbance values [absorbance of T– (absorbance of standard blank + absorbance of reagent blank)] were used to calculate the concentration of thiols using calibration curve. Values were expressed in µmole/L for serum thiols.

Cholesterol level was measured by Cobas™ 6000 using a CE-COD-POD (cholesterol ester-cholesterol oxidase-per oxidase) method. Triglyceride level was measured by Cobas™ 6000 using a triglyceride GPO Tender method. HDL levels were measured by Cobas™ using a direct-homogenous method and the LDL levels were calculated.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS V 16). Values were expressed as mean ± SD, and p value < 0.01 was considered statistically significant. Independent sample's t test was used to compare mean values.

Results: As shown in Table 1, serum protein thiol levels were significantly decreased and total cholesterol was increased in cases compared to healthy controls. (P < 0.001)

Serum triglycerides, HDL and LDL levels of these patients in the hypercholesterolemic (case) group were as follows (mean ± SD) - triglycerides (133.98 ± 48.91 mg/ dL), HDL (46.75 ± 5.8 mg/ dL), LDL (132.82 ± 35.11mg/ dL). There was a direct correlation between increased level of LDL and decreased level of protein thiols, which is an indication of oxidative stress in the patient group. There was no significant correlation between with triglyceride and HDL Levels.

Table 1: **Serum levels of cholesterol and protein thiols in cases and controls**

Variable	Control (n=25)		Case (n= 25)	
	Mean	SD	Mean	SD
Protein thiols (Normal range- 400- 600 µ mol/L	441.9	51.49	206	49.45
Cholesterol (Normal range- 120- 200 mg/dL)	178.5	29.97	342.38	138.8*

*p<0.001, Significant, (Independent Sample's t test)

Discussion: Essentially, all the plasma sulfhydryl groups are protein associated, and behave as powerful extracellular antioxidants. Proteins containing reactive sulfhydryl groups under oxidative stress are converted to mixed disulfides with attached glutathione (S-thiolation), leading to a fall in their levels. These thiolated proteins are very early products of protein oxidation during oxidative stress, occurring within seconds after generation of oxygen radicals.[9]

Decreased –SH levels may be due to enhanced free radical generation in hypercholesterolemia. These reduced thiol groups were oxidized by electron deficient free radicals, in the process of oxidation of –SH groups present over plasma proteins. Since –SH groups are the major antioxidants that contribute to antioxidant pool of the body fluids, oxidation of such –SH groups can significantly contribute to the oxidative damage to biomolecules of hypercholesterolemic patients.
We speculate that the decreased thiols in serum of hypercholesterolemic patients could be because of increased oxidation of –SH groups in serum due to already existing oxidative stress.

The -SH groups present on the protein are the major antioxidants *in vivo*. The serum levels of protein-SH in the body indicate the antioxidant status and low levels of protein-SH correlated with the increased levels of advanced oxidation protein products.[10]

It has been stressed recently that oxidants and oxidative modifications do indeed play a major role in permanent tissue damage.[11] The detrimental effects of reactive oxygen species (ROS) have also been documented in renal parenchyma, mesangial cells in culture, and on matrix components. [12- 15]

Much recent interest has focused on the role of an excessive inflammatory response in atherosclerosis. Although the association between atherosclerosis and inflammation has been well-documented in CRF,[16] the initiating inflammatory factors remains largely unknown. Reactive oxygen species generated in oxidative stress has been demonstrated to be a signal for the activation of nuclear factor-κB (NF-κB), a major inflammatory transcription factor that triggers the transcription of several inflammation mediators.[17] These inflammatory mediators can act in concert thereby promoting atherogenesis, particularly through oxidation of LDL, leukocyte recruitment and SMC proliferation. [18]

Our findings show a significant relationship between LDL cholesterol levels and protein thiol as a marker of oxidative stress in patients with hypercholesterolemia. Hypercholesterolemia has been shown to attenuate endothelium-dependent vaso-relaxation in numerous vascular beds, including human coronary arteries and forearm resistance vessels.[19, 20] In animal models of hypercholesterolemia, attenuated endothelial function associated with increased superoxide production is observed. [21] Our current study does not provide a mechanistic explanation for the relationship between LDL cholesterol and superoxide generation. However, LDL cholesterol and oxidized LDL cholesterol have been shown to affect the trafficking of eNOS to caveolae. [22] Both native and oxidized LDL cholesterol may cause an uncoupling of eNOS, resulting in superoxide production in endothelial cells [23] In addition; components of oxidized LDL have been reported to stimulate superoxide production via NADPH oxidase. [24] Lipid-lowering therapy with statins has been demonstrated to improve endothelial function. [25, 26]

LDL cholesterol was also related to superoxide production via NADPH oxidase as measured by relaxation to apocynin, although at a lower level of significance. NADPH oxidase is the predominant source of superoxide in the human vasculature. Smoking, hypertension, hypercholesterolemia, and diabetes have all been reported to upregulate this enzyme [27- 29] In contrast, LDL cholesterol had no influence on the production of superoxide from xanthine oxidase, but superoxide production via this enzyme was significantly enhanced in diabetic subjects.

The present study demonstrated that elevation of cholesterol and LDL resulted in a reduction of

protein thiol concentration and increase in oxidative stress that might be evaluated as a marker for development of cardiovascular/ cerebrovascular disease in such patients.

Conclusion: From the above study, we may conclude that hypercholesterolemia and increased LDL levels are associated with a decrease in protein thiol activity which is a reflection of increased oxidative stress.

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