EFFECT OF SUPPLEMENTATION OF VITAMIN E, VITAMIN C AND REDUCED GLUTATHIONE ON COPPER ION INDUCED LIPOPROTEIN OXIDATION IN RENAL DISEASED PATIENTS – AN *IN VITRO* STUDY

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ABSTRACT

The oxidative modification of lipoprotein especially low density lipoprotein (LDL) plays a key role in the initiation and progression of atherosclerosis. Serum apolipoproteinB (apoB) level is found to be an important marker for atherosclerosis. The present paper focuses on the measurement of serum apoB levels and the effect of Vitamin E, Vitamin C and reduced glutathione on the copper ion induced oxidation of LDL + VLDL (VLDL-Very Low Density Lipoprotein) lipoprotein fraction isolated from the serum of chronic renal failure (CRF) and renal transplanted patients. The level of apoB is significantly higher in the serum of transplanted patients when compared to CRF patients and normal subjects. The level of TBARS formed in oxidized LDL + VLDL lipoprotein fraction of CRF and renal transplanted patients are significantly increased than normal even in the presence of antioxidants but the level of TBARS produced with antioxidants is comparatively lesser than that produced by oxidized lipoprotein fraction without antioxidants. It may be concluded that oxidation of LDL can be prevented at an earlier stage by Vitamin E supplementation. The supplementation with Glutathione serves as the best method of preventing the lipoprotein oxidation among the renal diseased patients.

KEYWORDS

Lipoprotein oxidation, antioxidants, renal transplantation, lipid peroxidation and apolipoprotein B

INTRODUCTION

Cardiovascular disease (CVD) is one of the important causes for the higher mortality rate among the CRF and the renal transplant patients (1) CVD and cerebrovascular complications account for 15-30% of deaths in patients suffering from CRF (2-4). The renal transplantation offers a survival advantage when compared with dialysis for CRF patients, CVD is the major complication and main cause of death after transplantation (5) and cardiovascular disease occurs within 15 years of surgery in upto 20% of organ transplant recipients (6). The oxidation of LDL is of particular interest due to its potential role in the unregulated uptake of lipids and cholesterol by macrophages which contribute to the initial stage of

foam cell formation in atherosclerosis (7). The most common method for the initiation of LDL oxidation in invitro condition involves incubation with copper ions which follows a mechanism associated to specific sites of LDL and copper is present in human athermatous plaques as prooxidant (8). It is postulated that there is involvement of apoB-100 in the initiation of LDL oxidation both by providing electrons for the reduction of copper ions and binding it in a specific, saturable manner(9). The oxidative modification of LDL lipids and apoB - 100 by the reactive oxygen species is widely regarded as a crucial event in atherogenesis (10, 11, 12). Antioxidants are the first line defense against free radical damage and are critical for maintaining optimum health and well being. The resistance of LDL to oxidation depends on the equilibrium between prooxidants and antioxidants characteristic of the lipoprotein. Hence the effect of various concentration of antioxidants like Vitamin E, Vitamin C and reduced glutathione on the Cu²⁺ ion (copper ion) induced oxidation of lipoprotein fraction is studied.

This study will help to design a strategy to prevent the oxidation of LDL + VLDL so that mortality due to

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atherosclerosis among CRF patients and renal transplant patients can be reduced.

MATERIALS AND METHODS

The blood samples for the present study were collected from the Nephrology department, Stanley Medical College Hospital, Chennai. The healthy volunteers served as control, Group I. The study was carried out in 20 CRF patients having creatinine level > 5mg/dl of blood and were undergoing hemodialysis using cuprophane membrane and bicarbonate buffered dialysate for two or more sessions of 4 hours of dialysis per week. They served as Group II. Twenty patients who have undergone live related renal transplantation 1-2 years before at the Nephrology department, Stanley Medical College Hospital, Chennai served as Group III. They were under treatment with Cyclosporine A at a dosage of 6 mg / kg body weight for 1 year and steroid 1 mg / kg body weight with Azathioprine of 2.5 mg / kg body weight as life long treatment. None of the patients had clinical or laboratory evidence of fever, diabetes mellitus, elevated liver enzyme levels, infective disorders, smoking and having antilipidemic drug treatment. The patients and the healthy volunteers were male in the age group of between 20 and 45 years.

Informed consent was obtained from each patient before the sample collection. The study was approved by the committee of Ethics at Stanley Medical College Hospital, Chennai. The patients were requested to be in overnight fasting. From each patient, 10 ml of blood was collected following 12 hours of fasting in sterile tubes without any additives and then centrifuged. The resulting serum was used for the studies.

The serum apoB level was estimated by the Rate Immunonephelometric Assay method of Maciejko *et al.* (13). The LDL + VLDL lipoprotein fraction was isolated from the serum by the method of Burstein and Scholnick (14) using sucrose, heparin and magnesium chloride. The protein in LDL + VLDL was estimated by the method of Markwell *et al.* using bovine serum albumin as the standard (15). The isolated LDL + VLDL lipoprotein fraction was oxidized invitro by the method of Wallin *et al.* with some modifications (16). The extent of lipid peroxidation before and after the induction of oxidation was measured by routine thiobarbituric acid reacting substances (TBARS) assay in terms of Malondialdehyde (MDA). The amount of MDA formed was calculated from a standard curve prepared using 1, 1', 3, 3'-tetra-methoxypropane.

The effect of various antioxidants on copper ion induced invitro oxidation of the LDL + VLDL fractions were studied by adding 50 l of 5 M Copper sulphate solution in various tubes containing different concentration of antioxidants at the level of 100 g Of Vitamin E, and 100 g of Vitamin C, 50 g of Vitamin

E+50 g of Vitamin C 5 moles of Reduced Glutathione and 10 moles of reduced Glutathione and the TBARS was estimated as mentioned above (16).

Numerical data were presented as mean values $\pm$ standard deviation. The values were subjected to analysis of normal tests of significance. The statistical significance was evaluated by One Way-ANOVA (Analysis of Variance).

RESULTS

The level of serum apoB is presented in Figure I. The level of apoB is significantly higher in the serum of Group III, when compared to Group I and Group II.

Table 1. The levels of TBARS in the native and Cu²⁺ ion induced invitro oxidized forms of LDL + VLDL fractions from the serum of Group I, Group II and Group III

Each Group consists of 15 subjects

Subjects	TBARS	
	Native	Oxidised
Group I	23.6 $\pm$ 0.63	43.5 $\pm$ 0.76
Group II	***A 29.19 $\pm$ 0.65	***A 72.75 $\pm$ 1.44
Group III	***B ***C 81.4 $\pm$ 1.71	***B ***C 116.15 $\pm$ 2.89

*** F < 0.001 - highly significant by ANOVA.

The levels of TBARS are expressed as nmoles of MDA formed /mg of LDL+ VLDL protein.

The values are mean $\pm$ SD

A - Comparison of Group I and Group II

B - Comparison of Group I and Group III

C - Comparison of Group II and Group III.

Table 1 shows the level of TBARS in the native and oxidized forms of LDL + VLDL fractions of serum. When compared to Group I and Group II, Group III patients show a significant increase in TBARS level. The level of TBARS measured in the copper ion induced oxidized forms of LDL + VLDL fractions and oxidized forms of lipoprotein fraction in the presence of various antioxidants like Vitamin E, Vitamin C and reduced Glutathione are presented in Figure 2, 3 and 4. The levels of TBARS in the oxidized LDL + VLDL fractions in the presence of antioxidants are significantly reduced in all the groups when compared with oxidized forms of LDL + VLDL without any antioxidants.

Fig. 1. The levels of apolipoprotein B in the serum of Group I, Group II and Group III

Fig. 2. The levels of TBARS in Native LDL + VLDL, Copper ion induced *in vitro* oxidised LDL + VLDL and *in vitro* oxidised LDL + VLDL in the presence of vitamin E and vitamin C

The levels of TBARS formed in oxidized LDL + VLDL fractions in Group II and Group III are significantly increased than Group I even in the presence of antioxidants but the level of TBARS produced is comparatively lesser in oxidized LDL + VLDL fractions without antioxidants.

DISCUSSION

There is an association between increased levels of apoB containing lipoproteins and the development of atherosclerosis (17). It has been reported that apolipoprotein, a glycoprotein is the more sensitive marker than lipids in detecting discrete lipid transport

abnormalities and for the evaluation of atherogenic risk in CRF and renal transplant patients (18,19). The dyslipoproteinemia of CRF is characterized by discrete apoB containing lipoprotein particles which contributes to atherosclerotic vascular disease and to glomerulosclerosis and progression of renal insufficiency (20). In our study, the levels of apoB in the serum of CRF and renal transplant patients are higher than the control and among the diseased patients, the renal transplants are found to have higher values.

The oxidative modification of lipoprotein yield the marker of lipid peroxidation such as MDA. So the

Fig. 3. The levels of TBARS in Native LDL + VLDL, *in vitro* oxidised LDL + VLDL and *in vitro* oxidised LDL + VLDL in the presence of vitamin E + C

Fig. 4. The levels of TBARS in Native LDL + VLDL, *in vitro* oxidised LDL + VLDL and *in vitro* oxidised LDL + VLDL in the presence of 5 and 10 micromoles of Glutathione

TBARS is assessed in LDL + VLDL fractions of CRF and renal transplant patient which serve as the good marker of lipid peroxidation. It has been reported that the oxidation of VLDL in *in vitro* condition increases macrophage uptake and promotes foam cell formation in CRF patients (21).

The oxidative modification of the LDL plays a significant role in atherogenesis and if it is inhibited

by an appropriate antioxidant, it slows down the progression of disease. Hence, a study of preventing *in vitro* oxidation of LDL + VLDL fraction by antioxidants is made using various concentration of antioxidants like Vitamin E, Vitamin C and glutathione. The antioxidant effect is more pronounced with 10 μ M of Glutathione than with other antioxidants. The reason for the increased susceptibility of LDL to *in vitro* oxidation and reduced concentration of LDL Vitamin

E in uremic patients may be due to hyperproduction of free radicals with the subsequent formation of lipid peroxidation products in CRF (22). Some studies have reported that oxidative stress is greater in hemodialysis patients with prevalent CVD and if supplemented with 800 IU/day Vitamin E reduces composite cardiovascular disease endpoints and myocardial infarction (23, 24). Ascorbate is a potent inhibitor of LDL oxidation and is known to recycle α -tocopherol from its oxidized to native form (25). Vitamin C prevents apoB-100 fragmentation and inhibit conjugated diene formation during LDL oxidation (26). These results are consistent with the hypothesis that increased ascorbic acid intake may decrease the risk of coronary artery disease. Vitamin C prevents the incidence of mortality of CVD more likely derived from its antioxidant activity (27). The supplementation with antioxidants like Vitamin C and Vitamin E retards early progression of transplant associated coronary arteriosclerosis (28).

Hence the α -tocopherol supplementation in diets of patients may lead to enrichment of LDL with antioxidant and decrease its susceptibility to oxidation at an earlier stage. The change in thiol content of apoB is the major contributing factor for lipid-protein interaction of lipoprotein and implies the role of glutathione in lipoprotein oxidation. The glutathione supplementation may be a contributing factor in maintaining apoB thiol content which prevents fragmentation of apoB 100 and LDL oxidation may be further reduced. The use of reduced glutathione will decrease the mortality due to CVD and prevent the chronic rejection of kidney in renal transplanted patients.

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