

Correlation between total antioxidant status and lipid peroxidation in hypercholesterolemia

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The aim of this study is to elucidate a link, if any, between lipid peroxidation and the total antioxidant status in serum of hypercholesterolemic patients. The lipid peroxidation was significantly elevated and the total antioxidant level was significantly reduced in the serum of hypercholesterolemic patients when compared to the age- and sex-matched healthy controls, thereby showing a negative correlation between these two parameters. Antioxidant therapy to the identified patients may be beneficial to avoid the damage that can be caused by free-radicals.

OXYGEN free radicals (OFR) play a significant role in the pathogenesis of many diseases like atherosclerosis, cancer, neurodegeneration and inflammation. Free radicals are produced endogenously during cellular metabolism. Their production may be greatly enhanced by exogenous factors like environment pollutants, drugs, radiation and pathogens¹. An increased free-radical generation has also been reported in several disease states. Hypercholesterolemia, one of the major risk factors of atherosclerosis²⁻⁷, enhances the free-radical generation in various ways⁸. Prime targets of OFR attack are the polyunsaturated fatty acids in the membrane lipids causing lipid peroxidation which may lead to disorganization of cell structure and function⁹. Decomposition of peroxidized lipids yields a wide variety of end products. To circumvent the damage caused by OFR, multiple defence systems collectively called antioxidants are present in human serum. This system consists of substances that provide the much-needed stability to the free-radical by allowing the pairing of electrons. The protective efficiency of antioxidants in hypercholesterolemic patients would depend on the balance between OFR and the availability of antioxidants themselves. Hence the present study was undertaken to evaluate a correlation between malondialdehyde (MDA) which is a marker of lipid peroxidation and total antioxidant status in serum of hypercholesterolemic patients.

Blood was drawn from normolipidemic controls ($n = 30$, average age: 40–60 years) and hypercholesterolemic patients ($n = 30$, age range: 38–64 years) after an overnight fast. The plasma cholesterol levels of

hypercholesterolemic patients ranged between 265 and 384 mg/dl. MDA was estimated according to the method of Satoh¹⁰ which is based on the coupling of MDA with thiobarbituric acid. Measuring individual antioxidant components is time-consuming and expensive. Hence an alternative approach for measurement of total antioxidants of serum using a commercial kit from Randox Laboratories (Antrim, Ireland) was used¹¹. In this assay metmyoglobin reacts with H_2O_2 to form ferrylmyoglobin, a free-radical species. A chromogen 2,2'-amino-di-[3-ethylbenzthiazole sulfonate] is incubated with ferryl myoglobin to produce a radical cation which has a blue-green colour and can be measured at 600 nm. Antioxidants in the added serum cause suppression of this colour production proportional to their concentration. The assay is calibrated using 6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid (trolox). The results are expressed as mmol/L of trolox equivalent. The statistical significance of the mean differences between groups was assessed by the Student's *t*-test. A *P* value ≤ 0.05 was considered significant. Values are expressed as mean \pm SD.

The MDA concentration (Table 1) was significantly elevated ($P < 0.001$) in the serum of hypercholesterolemic patients in comparison to the controls. The total antioxidant capacity of serum was significantly ($P < 0.001$) depleted in hypercholesterolemic patients (Table 1). A negative correlation ($r = -0.57$) between lipid peroxidation and total antioxidant capacity of the serum in hypercholesterolemic patients was observed (Figure 1).

Hypercholesterolemia, a major cause for atherosclerosis is gradually becoming a major health problem in India. Our previous studies^{12,13} with hypercholesterolemic rabbits and guinea pigs suggest that hypercholesterolemia increases the level of lipid peroxidation in the serum. Szczeklik *et al.*¹⁴ observed an increase in plasma MDA levels in rabbits that consumed a high cholesterol diet. Prasad and Kalra¹⁵ have also reported an increase in the blood MDA concentration with a high cholesterol diet. There have been reports of increased lipid oxidation products in the plasma of patients with higher risk of myocardial infarction^{16,17}. Lipid peroxidation results from release of free-radicals which can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes. Free-radicals can also cause oxidation of low density lipoprotein (LDL) cholesterol¹⁸ in the absence of protection provided by the antioxidants¹⁹. Mosca *et al.*²⁰ have reported a significant reduction in susceptibility of LDL to oxidation in patients with coronary artery disease supplemented with a combination of antioxidant vitamins. In the present study we have observed a significant increase in the MDA level of hypercholesterolemic patients which suggests the presence of increased oxidant stress. The total antioxidant status in these patients was significantly

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Table 1. MDA and total antioxidant status in serum samples of hypercholesterolemic patients

	Controls (n = 30)	Patient (n = 30)
MDA (nmol/ml)	3.6 ± 0.6	5.1 ± 0.8*
Total antioxidant status (mmol/L trolox equivalent)	1.68 ± 0.3	1.06 ± 0.15**

Values are given as mean ± SD; *Significantly greater than the control; **Significantly less than the control.

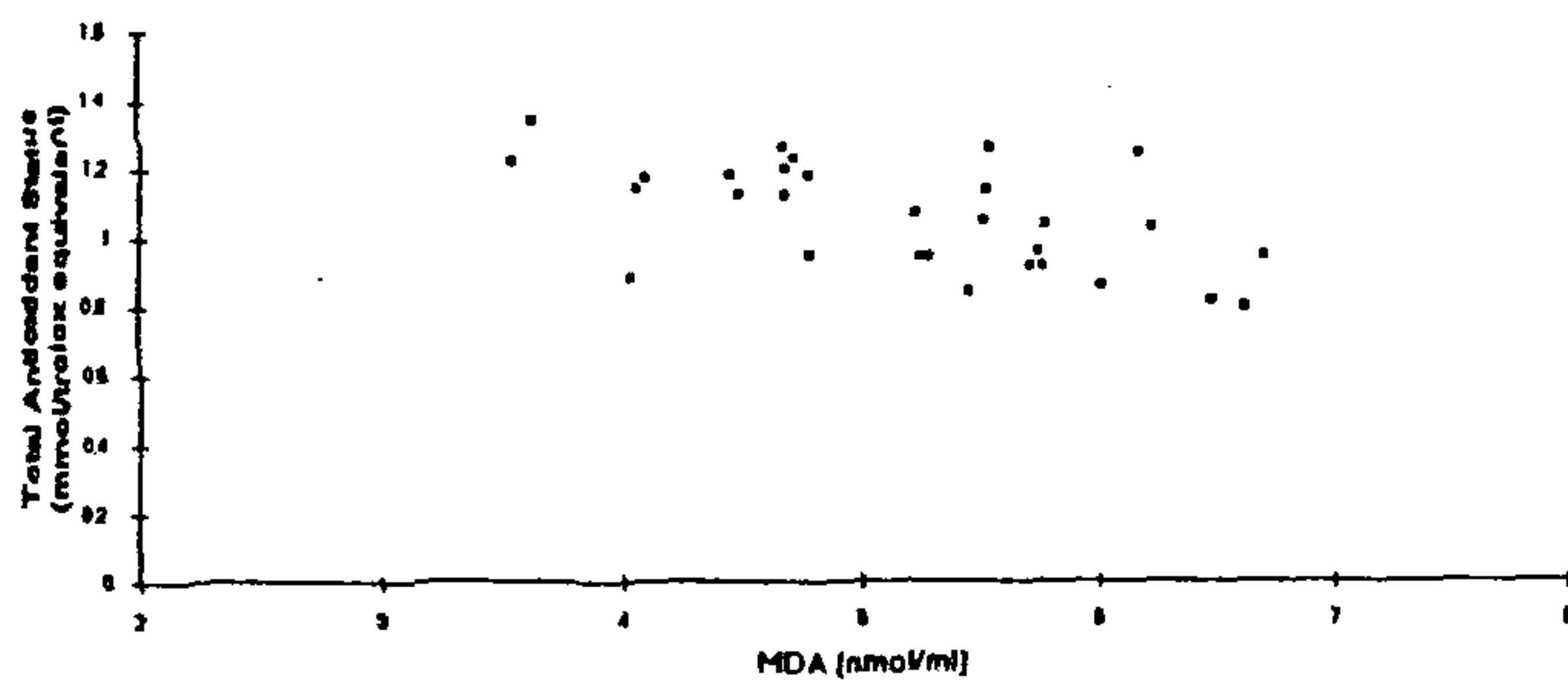


Figure 1. Negative correlation between MDA and total antioxidant status in serum samples of hypercholesterolemic patients ($r = -0.57$, $n = 30$).

depleted in comparison to the controls, thereby showing a negative correlation between the total antioxidant status and lipid peroxidation in the serum of hypercholesterolemic patients. In healthy conditions a balance exists between free-radical generation and antioxidant defence system which prevents occurrence of disease. However this study implies that hypercholesterolemia shifts the balance in favour of free-radical generation which leads to oxidative tissue damage. This in turn increases permeability and loss of membrane integrity^{21,22} and thus sets the stage for atherosclerosis.

Since hypercholesterolemia and lipid peroxidation both directly relate to the severity of atherosclerosis, elimination of free-radicals in the plasma before they can be taken up by the peripheral tissues might improve the prognosis for patients. In view of our present findings, a management strategy aimed at simultaneous control of lipid peroxidation and total antioxidant status in the serum of hypercholesterolemic patients may be effective and an antioxidant therapy may be beneficial to avoid the damage that can be caused by free-radicals.

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Fluorescence resonance energy transfer: A diagnostic tool in oligonucleotide therapy

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Synthetic oligonucleotides of variable lengths and sequences are being used for treatment of viral diseases at the genetic level. Specific methods are available for monitoring these oligonucleotides *in vitro* and *in vivo*, viz. radioisotopic labelling or non-covalent/covalent attachment of fluorescent reporter groups, spectroscopic assays and of late fluorescence resonance energy transfer (FRET). This paper integrates general features of FRET and its application as a diagnostic tool in oligonucleotide therapy.

RECENTLY the use of synthetic oligonucleotides in molecular biology has gained unprecedented progress,

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