

inhabitants at high altitude. (vi) Individuals raised at high-altitude villages can be utilized for revegetating the wild population, consumption and trade.

1. Franz, C., *Plant Res. Dev.*, 1993, **37**, 101–111.
2. Gupta, A., Vats, S. K. and Lal, B., *Curr. Sci.*, 1998, **74**, 555–556.
3. Lange, D., *Med. Plant Conserv. Newslett.*, 1997, **3**, 16–17.
4. Sastry, A. R. K. and Chatterjee, S., *Setting Biodiversity Conservation Priorities for India* (eds Singh, S. et al.), WWF-India, 2000, vol. II, pp. 467–473.
5. Srivastava, A., Shukla, Y. N. and Sushil, K., *J. Med. Aromat. Plant Sci.*, 1999, **21**, 1131–1138.
6. Kirtikar, K. R. and Basu, B. D., *Indian Medicinal Plants*, Bishen Singh Mahendra Pal Singh, Dehradun, 1984, vol. 3, p. 697.
7. Harborne, J. B. and Baxter, H., *Dictionary of Plant Toxins*, John Wiley, New York, 1996.
8. Kashiwada, Y. et al., *J. Nat. Prod.*, 1995, **58**, 392–400.
9. Jain, S. K. and Sastry, A. R. K., *The Indian Plant Red Data Book*, Department of Environment, Botanical Survey of India, Government of India, 1984, vol. 1.
10. Chauhan, N. S., in *Natural Resources and Development in Himalaya* (ed. Verma, L. R.), Malhotra Publishing House, New Delhi, 2000, pp. 319–370.

11. Rundel, W. P. and Witter, M. S., *Tropical Alpine Environments Plant form and Function* (eds Rundel, W. P., Smith, A. P. and Meinzer, F. C.), Cambridge University Press, Cambridge, 1994, pp. 295–306.
12. Bahuguna, R., Purohit, M. C., Rawat, M. S. M. and Purohit, A. N., *J. Plant Biol.*, 2000, **27**, 179–183.
13. Halliwell, B., *The Propagation of Alpine Plants and Dwarf Bulbs*, Pasten Press, London, 1992, p. 79.
14. Nautiyal, M. C. and Purohit, A. N., *Curr. Sci.*, 2000, **78**, 1062–1063.
15. Prasad, P., *Plant Genet. Res. Newslett.*, 2000, **124**, 1–8.
16. Ved, D. K. and Tandon, V., *CAMP Report for High-Altitude Medicinal Plants of Jammu-Kashmir and Himachal Pradesh*, FRLHT, Bangalore, 1988.
17. Frankel, O. H., Brown, A. H. D. and Burdon, J. J., *The Conservation of Plant Biodiversity*, Cambridge University Press, Cambridge, 1995, p. 299.

ACKNOWLEDGEMENTS. We thank the Director, GBPIHED for encouragement and facilities. S.M. thanks Drs R. S. Rawal, Subodh Airi and Indra Dutt Bhatt, Shri Raghuvir Singh Rana and colleagues at the CBD lab for their help and support. The Department of Biotechnology, Govt. of India is thanked for financial assistance.

Received 9 January 2002; revised accepted 13 June 2002

Interrelationship between lipid peroxidation, ascorbic acid and superoxide dismutase in coronary artery disease

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With the current understanding on the mechanism of LDL oxidation and oxidized LDL in coronary artery disease (CAD), a pilot study was conducted to evaluate the interrelationship between lipid peroxidation, ascorbic acid and superoxide dismutase in patients suffering from CAD. Plasma malondialdehyde – a marker of lipid peroxidation was significantly elevated and the levels of ascorbic acid and superoxide dismutase were significantly reduced in patients. This scenario suggests that higher oxidant stress and reduced antioxidant status along with hypercholesterolemia and hypertriglyceridemia are the key factors for progression of atherosclerosis and hence, a management strategy aiming at simultaneous reduction of cholesterol and control of lipid peroxidation in CAD is envisaged.

CORONARY artery disease (CAD) is the single most important disease entity in terms of both mortality and morbidity in the entire world population. Both men and

women between the age group 40 and 60 are susceptible to it. Despite all-round efforts in the prevention and management of this disease, it remains a major challenge to the health managers and scientists. It is predicted that by the year 2020 this disease would persist as the major and the most common threat to human life¹. In developing countries, the incidence of CAD is increasing alarmingly. India is on the verge of a cardiovascular epidemic! By the year 2015, cardiovascular mortality is likely to rise to the order of 103% in males and 90% in females. The circulatory system disorders are going to be the greatest killer in India by the end of the year 2015 (ref. 2). The underlying cause of CAD is atherosclerosis – a disease involving a complex array of circulating blood proteins, lipoproteins and cells, and their interaction with the cells and matrix proteins of the arterial wall. It is well established that high circulatory serum cholesterol, low density lipoprotein cholesterol (LDL-C) and low levels of circulating high density lipoprotein cholesterol (HDL-C) are the main causatives of this disease. Basic research has provided strong evidence that oxidation of LDL also plays an important role in the pathogenesis of atherosclerosis. Oxidative modification of LDL is brought about by free radicals, which cause degradation of polyunsaturated fatty acids and the formation of lysolecithin, oxysterols and aldehyde modification of lysine residues on Apo B (ref. 3). Lipid peroxidation is the most studied biologically relevant, free-radical chain reaction. Cells have a comprehensive array of antioxidant defence mechanisms to reduce free radical formation or limit their damaging effects. These include enzymes such as superoxide dismutase (SOD) and catalase to degrade superoxide and peroxides respectively, and essential radical scavengers

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like ascorbic acid, alpha tocopherol and carotenoids. A potential role of the antioxidant micronutrients in modifying the risk, which results from oxidative stress and CAD, has stimulated intense research and has increased interest in micronutrient supplement. Antioxidants tend to reduce the risk and severity of atherosclerosis by inhibiting lipid peroxidation. This scenario suggests that together with the estimation of plasma lipids and lipoproteins, evaluation of oxidant-antioxidant profile in an individual will contribute significantly to the risk assessment, prophylaxis and management of CAD.

Ascorbic acid or vitamin C is an important antioxidant in plasma, where it consumes oxygen free radicals and helps to preserve alpha tocopherol in lipoproteins⁴. Extracellular SOD (EC-SOD) is a secretory glycoprotein⁵, which is found in blood-vessel walls at a high level to suppress oxidative stress under normal conditions. EC-SOD is the major SOD isoenzyme in plasma.

The role of free radicals and free-radical scavenging mechanisms in the progression and prevention of CAD in humans remains more of a speculation. Data on the state of free radicals and their scavengers under *in vivo* conditions in humans are lacking. Hence we have attempted a pilot study on the state of SOD, lipid peroxidation and ascorbate, and their interrelationship in the normal controls and patients suffering from CAD, to elucidate the role of the above factors in the progression and prevention of CAD.

A total number of 48 angiographically-proven CAD patients from the Department of Cardiology, All India Institute of Medical Sciences, New Delhi, between age group 33 and 71 years were studied. Blood samples from 27 normal persons served as controls. Plasma total cholesterol, HDL-cholesterol and triglyceride were assayed using an enzymatic estimation kit (Randox Laboratories Limited, Crumlin, UK). Plasma LDL-cholesterol levels were determined from the values of total cholesterol and HDL cholesterol using the following formula:

$$\text{LDL-cholesterol} = \text{Total cholesterol} - \frac{\text{Triglyceride}}{5} - \text{HDL-cholesterol (mg/dl)}$$

Plasma ascorbic acid was estimated following the methodology of Jacob⁶. Ascorbic acid in serum was oxidized by Cu(II) to form dehydroascorbic acid, which reacts with acidic 2,4-dinitrophenyl hydrazine (2,4-DNPH) to form red *bis*-hydrazone. The colour developed was measured at 520 nm in a spectrophotometer. SOD was assayed in the plasma following the methodology of Nandi and Chatterjee⁷. This method utilizes the inhibition of auto-oxidation of pyrogallol by SOD enzyme in the sample. Malondialdehyde (MDA) is estimated as a marker of lipid peroxidation following the methodology of Satoh⁸, which is based on the coupling of MDA with thiobarbituric acid. For statistical analysis two-sample *t*-test was performed and the results were expressed as mean \pm SD. $P \leq 0.05$ was considered significant.

The mean value of plasma total cholesterol was significantly high by 15% ($P < 0.001$) in patients in comparison to normal controls (Figure 1). Similarly the levels of LDL-C, VLDL-C and triglyceride were also significantly high by 22% ($P < 0.001$), 18% ($P < 0.05$) and 24% ($P < 0.01$) respectively, in patients when compared to controls. On the contrary, the level of HDL-C was significantly less by 9% ($P < 0.005$) in patients in comparison to healthy controls (Figure 1). Plasma MDA was 1.73 nmol/ml in healthy controls and there was a 90% increase in its level in patients ($P < 0.0001$; Figure 2). A positive relationship was observed between total cholesterol and lipid peroxidation, and a negative relationship was observed between lipid peroxidation with ascorbic acid and SOD in patients. The mean plasma SOD level in normal controls was 2.73 U/ml and there was a 49% decrease ($P < 0.0001$) in its level in patients compared to controls (Figure 3). An inverse relationship was observed between plasma SOD and serum total cholesterol. The

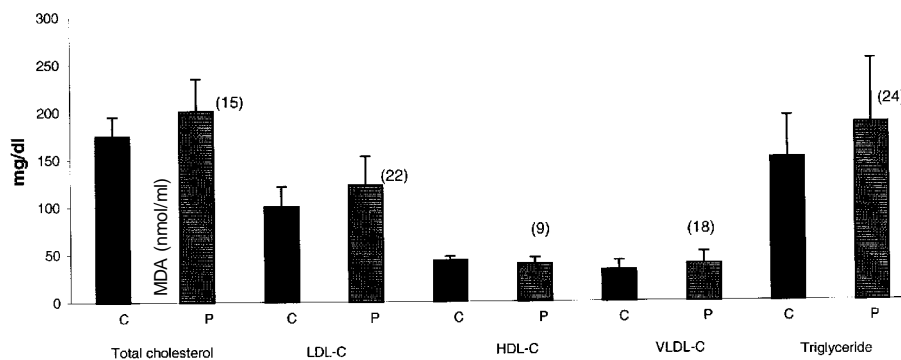


Figure 1. Lipid profile of controls (C, $n = 27$) and patients (P, $n = 48$). Values are expressed as mean \pm SD. Figures in parenthesis represent the per cent change in comparison to the respective control.

mean plasma ascorbic acid level of healthy controls was 0.6 mg/dl and the level decreased significantly ($P < 0.0001$) by 40% in patients in comparison to controls (Figure 4). An inverse relationship was observed between serum total cholesterol and serum ascorbic acid.

CAD is a fatal disease with no known cure. Fortunately, continued scientific probe into the understanding of the pathophysiology of this disease and causative factors made this disease highly preventable and treatable.

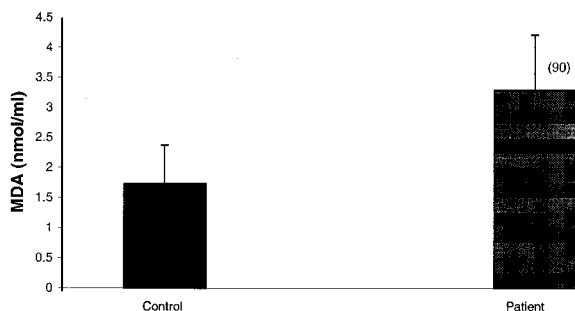


Figure 2. Plasma lipid peroxidation expressed as MDA in controls ($n = 27$) and patients ($n = 48$). Values are expressed as mean \pm SD. Figures in parenthesis represent the per cent change in comparison to controls.

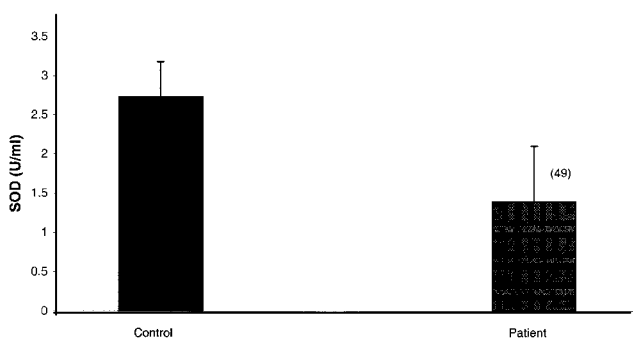


Figure 3. Plasma SOD in controls ($n = 27$) and patients ($n = 48$). Values are expressed as mean \pm SD. Figures in parenthesis represent the per cent change in comparison to controls.

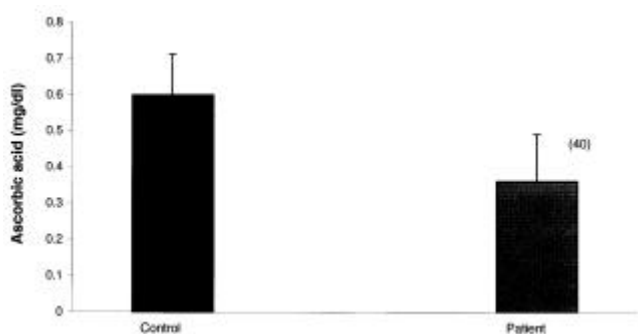


Figure 4. Plasma ascorbic acid in controls ($n = 27$) and patients ($n = 48$). Values are expressed as mean \pm SD. Figures in parenthesis represent the per cent change in comparison to controls.

Hypercholesterolemia has been known to be an important cause for the development of CAD. A positive relationship between plasma total cholesterol levels and risk of CAD has also been earlier demonstrated in many studies⁹⁻¹¹. Later, the role of HDL as a scavenger of cholesterol was recognized¹². In the Indian context, high level of plasma triglycerides, especially elevated levels of VLDL had been found associated with CAD. With these developments, conventionally, high plasma levels of total cholesterol, LDL and triglyceride, are considered as risk factors for CAD and high HDL levels are considered to be protective. In our study, lipid profile remained in consonance with the previous reports. The levels of risk-associated lipids, i.e. total cholesterol, triglycerides and LDL were high and levels of HDL were low in patients.

It is currently believed that lipid peroxidation is involved in the oxidative modification of LDL. Yalcin *et al.*¹³ observed an increase in the lipid peroxidation level in hyperlipidemic patients. Similarly, Mehmetcik *et al.*¹⁴ have also observed an increased level of lipid peroxidation in hypercholesterolemic subjects. In rabbits, Das *et al.*¹⁵ and Prasad and Kalra¹⁶ have reported a similar increase in serum and blood MDA levels respectively, on cholesterol feeding. The level of lipid peroxidation or MDA content indicates the state of free radicals and oxidative stress. In this study we have observed an increase in the MDA content in CAD patients compared to normals.

The level of ascorbate was low in patients compared to healthy controls. Ascorbic acid is a water-soluble antioxidant that acts as the body's primary defence against peroxy radicals formed in the aqueous phase¹⁷. It is the only antioxidant in plasma capable of completely inhibiting oxidative modification of LDL by aqueous peroxy radicals^{17,18}. Deficiency of ascorbic acid has a direct association with increased atherosclerosis in guinea pigs and its intake was shown to have an inverse relationship to atherosclerosis in quail, rabbits, rats and humans¹⁹. Ascorbate administration exerts an antioxidant effect in hypercholesterolemic rats; it also exerts a protective role against the peroxidative damage of lipids²⁰. It has also been reported that ascorbic acid increases the number of LDL receptors on arterial smooth cells, thereby facilitating lipoprotein cholesterol clearance²¹. Gokce *et al.*²², in their study with CAD patients, have proved that long-term ascorbic acid treatment has a sustained beneficial effect on endothelium-derived nitric oxide action. Low levels of ascorbic acid in CAD patients observed in the present study may be linked with increased consumption of ascorbic acid due to increased oxidant stress, as evident from higher MDA levels. In such patients ascorbic acid supplementation might help in providing the necessary protection needed for balancing the deleterious effects of free radicals.

EC-SOD is the major isozyme in plasma⁵ and removes superoxide radical²³. The level of SOD was low in pat-

ients compared to healthy controls in the present study. A negative association of EC-SOD with MDA, total cholesterol and triglyceride and a positive association with HDL in patients observed in this study are in consonance with earlier findings²⁴⁻²⁶. Landmesser *et al.*²⁷ have found a substantially reduced vascular EC-SOD activity and have suggested that reduced EC-SOD activity contributes to endothelial dysfunction in patients with CAD. Wang *et al.*²⁸, in their study of SOD levels in the Australian population, have established that circulatory EC-SOD is lower in men than in women and in smokers of each sex, and that low levels are independently associated with a history of MI. The reduced levels of SOD in patients observed in our study could result from reduced production of SOD levels, thereby rendering an individual susceptible to oxidative damage due to non-clearance of free radicals and their further propagation through chain reactions. It has also been suggested that low plasma SOD levels may directly be related to an increased production of superoxide, which interacts either directly with EC-SOD or with EC-SOD in vascular wall, which is in equilibrium with plasma levels. Thus plasma levels of SOD may be envisaged as a potential marker for the risk assessment of CAD in addition to the lipid profile. In the Indian context it may have an added advantage, as many of the CAD patients do show only a marginal increase or even normal levels of plasma cholesterol.

The study brings to focus the significance of free-radical homeostasis in CAD. In conclusion, it appears that though the number of control subjects was less than the patients, there is a distinct trend which suggests that higher oxidant stress and diminished antioxidant status along with hypercholesterolemia, hypertriglyceridemia and low HDL levels, constitute the key factors in the progression of atherosclerosis. Hence, a management strategy aiming at simultaneous reduction of cholesterol and control of lipid peroxidation in CAD is envisaged.

1. Yusuf, S., Ounpnu, S. and Anand, S., in *Coronary Artery Disease in Indians – A Global Perspective* (ed. Sethi, K. K.), 1998, pp. 11–25.
2. Kaul, U., Sapra, R. and Ghose, T., *ibid*, pp. 83–91.

3. Dobreanu, M. and Mody, E., *Rom. J. Intern. Med.*, 1997, **35**, 55–62.
4. May, J. M., *Front. Biosci.*, 1998, **3**, D1–D10.
5. Adachi, T., Yamazaki, N., Tasaki, H., Toyokawa, T., Yamashita, K. and Hirano, K., *Biol. Pharm. Bull.*, 1998, **21**, 1090–1093.
6. Jacob, R. A., *J. Nutr.*, 1990, **120**, 1480–1485.
7. Nandi, A. and Chatterjee, I. B., *J. Biosci.*, 1988, **13**, 305–315.
8. Satoh, K., *Clin. Chem. Acta*, 1970, **90**, 37–43.
9. Gordon, T., Kannel, W. B., Castelli, W. P. and Dawber, T. R., *Arch. Intern. Med.*, 1981, **141**, 1128–1131.
10. Stamler, J., Wentworth, D. and Neaton, J., *JAMA*, 1986, **256**, 2823–2828.
11. Anderson, K. M., Castelli, W. P. and Levy, D., *ibid*, 1987, **257**, 2176–2180.
12. Carew, T. E., Koschinsky, T. and Hayes, S. B., *Lancet*, 1976, **1**, 1315–1317.
13. Yalcin, A. S., Sabuncu, N. and Kilinc, A., *Atherosclerosis*, 1989, **80**, 169–170.
14. Mehmetcik, G., Toker, G. and Uysal, M., *Horm. Metab. Res.*, 1997, **29**, 63–65.
15. Das, Sabari, Snehlata and Srivastava, L. M., *Nutr. Res.*, 1997, **17**, 231–241.
16. Prasad, K. and Kalra, J., *Am. Heart J.*, 1993, **125**, 958–973.
17. Frei, B., *Am. J. Clin. Nutr.*, 1991, **54**, 1113S–1118S.
18. Frei, B., Stocker, R. and Ames, B. N., *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 9748–9752.
19. Duell, P. B., *J. Nutr.*, 1996, **126**, 1067S–1071S.
20. Santillo, M., Mondola, P. and Milone, A., *Life Sci.*, 1996, **58**, 1101–1108.
21. Aulinskas, T. H., Westhuvzen Deneys R. Van der and Coetzee, A. G., *Atherosclerosis*, 1983, **47**, 159–171.
22. Noyan, Gokce, Keaney, John F. and Balz, Frei, *Circulation*, 1999, **99**, 3234–3240.
23. Huang, P., Feng, L., Oldham, E. A., Keating, M. J. and Plunkett, W., *Nature*, 2000, **407**, 390–395.
24. Marklund, S. L., Nilsson, P., Israelsson, K., Schampi, I., Peltonen, M. and Asplund, K., *J. Intern. Med.*, 1997, **242**, 5–14.
25. Sjoquist, P. O. and Marklund, S. L., *Cardiovasc. Res.*, 1992, **26**, 347–350.
26. Tomodo, H., Morimoto, K. and Aoki, N., *Am. Heart J.*, 1996, **131**, 849–856.
27. Landmesser, U., Merten, R., Spiekermann, S., Buttner, K., Drexler, H. and Horning, B., *Circulation*, 2000, **101**, 2264–2270.
28. Wang, X. L., Adachi, T., Sim, A. S. and Wilcken, D. E., *Arterioscler. Thromb. Vasc. Biol.*, 1998, **18**, 1915–1921.

ACKNOWLEDGEMENTS. The support rendered by the Head, Department of Biochemistry, AIIMS, New Delhi, is gratefully acknowledged. We are also grateful to Dr L. M. Srivastava for his inspiration and support.

Received 27 October 2000; revised accepted 27 June 2002