LIPID PEROXIDATION AND GLUTATHIONE PEROXIDASE IN ISCHEMIC HEART DISEASE

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ABSTRACT

With the growing interest in the concept of free radicals in the pathogenesis of myocardial ischemia, it was thought worthwhile to study the changes in lipid peroxides and antioxidant enzyme glutathione peroxidase (GPx) in ischemic heart disease (IHD). The study was carried out on 76 patients of IHD - 38 of acute myocardial infarction (AMI) and 38 of stable ischemic heart disease (SIHD). They were age and sex matched with 38 normal healthy controls. A significant increase ($p < 0.001$) in lipid peroxides as malondialdehyde (MDA) (5.9 ± 0.7 mmol/L) and a decrease in GPx (24.6 ± 2.2 U/gmHb) was found in patients of AMI when compared with controls. There was no significant difference in these values in SIHD. Thus this study confirms the earlier findings that MDA and GPx are useful parameters in IHD and their magnitude is dependent on severity and /or duration of ischemia. We suggest that these tests would be of use in smaller institutions with limited facilities.

KEY WORDS: Lipid peroxidation, Glutathione peroxidase, IHD.

INTRODUCTION

Ischemic heart disease (IHD) is impairment of heart function due to inadequate blood flow to the heart compared to its need, caused by obstructive changes in the coronary circulation to the heart. It accounts for 25-30 % of deaths in most industrialized countries (1), though due to different manifestations its accurate incidence is difficult to compute. Evidence shows that oxygen derived free radicals are important mediators of oxidation of lipoproteins leading to endothelial damage and are said to play a role in myocardial tissue damage (2). Numerous studies (3-7) show that in IHD there is an increase in lipid peroxidation products expressed as malondialdehyde (MDA) and decrease in antioxidants like vitamin A, ascorbic acid, selenium and antioxidant enzymes like superoxide dismutase, glutathione peroxidase (GPx) and catalase. In smaller medical colleges and hospitals where autoanalysers and modern facilities are not yet available it is difficult to estimate many such

parameters, hence this present communication tries to evaluate MDA and GPx as parameters, which are less expensive, in the syndromes of IHD mainly acute myocardial infarction (AMI) and stable IHD (SIHD).

MATERIALS AND METHODS

Thirty-eight patients of AMI (Group B : 51.9 ± 7.6 years, 3.7M : 1F), admitted in intensive cardiac care unit and 38 patients of SIHD (Group C : 52.5 ± 6.5 years; 2.8M : 1F) attending out-patient department of Indira Gandhi Medical College and Mayo Hospital, Nagpur were selected for this study. The criteria for AMI were that patients were symptomatic for ischaemia, hospitalized in ICCU with history of chest pain 5 hours prior to admission, fresh ECG showed sign of ST-P changes and CPK-MB level significantly increased (>100 U/L). The criteria for SIHD were that patients were without symptoms of fresh ischaemia, had an episode of acute MI > 1 year back and CPK-MB level within normal limit (<20 U/L). These patients were age and sex matched with 38 normal controls (Group A : 51.2 ± 7.9 years, 1.7M : 1F). Patients with valvular heart disease, diabetes, renal, pulmonary, hepatic, neuro-muscular disease were

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excluded from this study. 5ml blood (in plain and heparinized bulb) was collected soon after the admission i.e. before treatment of infarction in AMI patients, while blood was collected on same day of visit from SIHD patients. Serum cholesterol was estimated by method of Tappel et. al. (8).

Malondialdehyde (MDA), a marker for lipid peroxidation was estimated using the kit of Randox Laboratories, U.K., the principle being that lipid peroxides condense with 1-methyl-2-phenylindole under acidic conditions resulting in formation of red chromophore. Tetramethoxypropane is used as a standard for this investigation. Glutathione peroxidase was assayed in whole blood (9), the principle being that GPx catalyses the oxidation of glutathione by cumene hydroperoxide in the presence of glutathione reductase and NADPH. The oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340 nm was measured.

RESULTS

Table 1. shows that smoking and hypertension were the risk factors involved in both AMI and SIHD. Only 5.3% patients with family history of IHD developed AMI. Table 2. depicts the level of plasma lipid peroxides which was significantly increased in patients of AMI ($p < 0.001$) as compared to controls. The level of antioxidant enzyme glutathione peroxidase also was significantly decreased in AMI as compared to control group. There was no significant change in these values

Table 1. Comparison of risk factors in patients of AMI and SIHD with controls.

Risk factors	Group A	Group B	Group C
Smoking	-	14 (36.8%)	17 (44.7%)
Family history of IHD	-	02 (5.3%)	-
Hypertension	-	13 (34.2%)	07 (18.4%)

N=38 in each group

in patients of SIHD. None of the patients had increased serum cholesterol.

DISCUSSION

Lipid peroxidation is the oxidative fragmentation

Table 2. Serum cholesterol, prooxidant and antioxidant enzyme in AMI and SIHD.

	Group A	Group B	Group C
Serum cholesterol (mg%)	160.1 $\pm$ 31.1	167.3 $\pm$ 25.3	165.8 $\pm$ 22.5
Plasma MDA) (μ mol/lit)	3.2 $\pm$ 0.3	5.9 $\pm$ 0.7*	3.4 $\pm$ 0.5
GPx (U/gmof Hb)	29.6 $\pm$ 5.3	24.6 $\pm$ 2.2 *	28.2 $\pm$ 1.8

*N=38 in each group * $P < 0.001$ when group B is compared to control*

of unsaturated lipids which can proceed by controlled enzymatic steps or as an uncontrolled radical chain reaction. Two important factors are involved in the genesis of atherosclerosis and cardiac disease. On one hand there is hyperlipidemia and specifically hypercholesterolemia which can produce an increase in lipid peroxidation. On the other hand there is an increase in lipid peroxidation when arterial lesions are accompanied by hypoxia, ischemia and reperfusion (10). Absence of hypercholesterolemia in our study does not support hyperlipidemia as a cause for these processes. Several reports (7, 11, 12) are in agreement with the second and important factor. Our study confirms this view i.e. lipid peroxidation is increased in myocardial ischemia with a parallel reduction in antioxidant enzyme GPx. These findings suggest that increase in lipid peroxidation in the blood may be secondary to ischemia.

With ischemia or hypoxia, there is burst in oxygen derived free radicals which are directly toxic to the myocardium causing myocardial stunning and arrhythmia with the production of lipid peroxides. Moreover, lipid peroxidation products stimulate the cyclooxygenase pathway and thereby prostaglandins and thromboxane synthesis (13).

Lipid peroxides also reduce the activity of antithrombin III and thus contribute to the acceleration of blood clotting forces (14). This will cause increase in platelet aggregation leading to vascular complications.

Our study is in agreement with those of most of the authors (10-12) who found decreased levels of antioxidant enzyme glutathione peroxidase. It is likely

that rapid production of free radicals by ischemic myocardium triggers later secondary events such as depletion of protective antioxidant enzymes. However, our study is in contrast to that of Hoffmann et. al. (15) who found increased levels of this enzyme in erythrocytes in patients of AMI. The fact that the values in SIHD are similar to those in control group

indicates that MDA and GPx values return to normal levels as the patients recover after treatment. Thus, it can be concluded that AMI exhibits disturbance in oxidative and antioxidant metabolism. Quantitative estimation of MDA and GPx being simple, feasible and economical may be used as parameters to judge the oxidative damage taking place in the body in AMI.

REFERENCES

1. Park, J.E. and Park, K. (1994) Epidemiology of chronic non communicable diseases and conditions. In : Parks Textbook of Preventive and social medicine. Ed. Park, K. 14th edn. Banarsidas Bhanot, Jabalpur, p. 248 - 254.
2. Jain, A.P., Mohan, A., Gupta, O.P., Jajoo, U., Kalantri, S.P. and Srivastava, L.M. (2000) Role of oxygen derived free radicals in causing endothelial damage in Acute Myocardial Ischemia. J. Assoc. Physicians Ind. 48(5), 478-480.
3. Singh, R.B. (1994) Randomized, double blind placebo control intervention trial of antioxidant vitamins in AMI. Ind. Heart. J. 46, 210-211.
4. Ramirez, J. and Flowers, N.C. (1980) Leucocyte ascorbic acid and its relationship to coronary artery disease in man. Amer. J. Clin. Nutr. 33, 2079-2087.
5. Chandra, M., Naveen, C., Agrawal, A., Kumar, A., Ghatak, A. and Pandey, V. C. (1994) The free radical system in ischemic heart disease. Int. J. Cardiol. 43, 121-125.
6. Pechan, I., Minarova, H., Babusikova, F., Rendekova, V., Mizera, S., Schramekova, E., Ursinyiova, M. and Fabian, J. (1996) Parameters of oxidative stress in patients with cardiopathies. Bratisl. lek. Listy, 97(6), 344-347.
7. Nand, N., Budhiraja, W., Singh, G.P., Sharma, M. and Agrawal, A. (1997) Lipid peroxidation and vitamin E in ischaemic heart disease J. Assoc. Physicians Ind. 45(11), 839-842.
8. Tappel, A.L. (1973) Lipid peroxidation damage to cell components. Fed. Proc. 32, 1870.
9. Paglia, E. and Valentine, N. (1967) Studies on quantitative and qualitative characteristics on erythrocytes glutathione peroxidase. J. Lab. Clin. Med. 70, 158-169.
10. Loeper, J., Goy, J., Rozensztain, L., Bedu, O. and Moisson, P. (1991) Lipid peroxidation and protective enzymes during myocardial infarction. Clinica Chimica. Acta. 196, 119-126.
11. Hill, M.F. and Singal, P.K. (1996) Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infarction in rat. Am. J. Pathol. 148(1), 291-300.
12. Konukoglu, D. and Akcay, T. (1998) Susceptibility of erythrocyte lipids to oxidation and erythrocyte antioxidant status in MI. Clin. Biochem. 31(8), 667-671.
13. Hearse, D.J. and Tosaki, A. (1987) Free radicals and reperfusion induced arrhythmias : Protection by spin trap agent PBN in the rat heart. Circ. Res. 60, 375-383.
14. Plachta, H., Bartnikowska, E., and Obara, A. (1992) Lipid peroxide in blood from patients with atherosclerosis and coronary and peripheral arteries. Clin. Chim. Acta. 83(3), 343-345.
15. Hoffmann, A., Kok, F.J., Wittenman, J.C., de Bruijn, A.M., Khysssen, O.H., de Bruin, M. and Valkenburg, H.A. (1989) Decreased selenium levels in acute myocardial infarction. J. Am. Med. Assoc. 261(8), 1161-1164.