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PROXIDANT AND ANTIOXIDANT STATUS IN PATIENTS OF TYPE II DIABETES MELLITUS WITH IHD

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ABSTRACT

Patients with type II Diabetes Mellitus (NIDDM) are more prone to Ischaemic Heart Disease (IHD). Although, oxygen free radicals are known to contribute to the development of IHD, conflicting reports are available regarding the antioxidant status in patients of NIDDM complicated with IHD. This study was undertaken to investigate the oxidative status in patients of NIDDM and to assess their correlation with plasma glucose, glycosylated haemoglobin and duration of diabetes. The levels of malondialdehyde were significantly increased where as levels of superoxide dismutase, Glutathione peroxidase and vitamin C were significantly decreased in diabetics without complications and non-diabetics with IHD when compared with the controls. The levels of malondialdehyde and Glutathione peroxidase were significantly increased where as levels of superoxide dismutase and vitamin C were significantly decreased in diabetics with IHD when compared with diabetics without complications and non-diabetics with IHD. The implications of the results are discussed.

KEY WORDS

Non insulin Dependent Diabetes Mellitus, Ischaemic Heart Disease, oxidative status.

INTRODUCTION

Oxygen free radicals contribute to the development of exacerbation of many mankinds' most common ills, including heart attacks. The high global burden of IHD is highlighted by the world health report which estimates that 30.9% of all deaths in 1998 as well as 10.3% of the total disease related burden were attributed to cardiovascular disease (1). IHD could be diagnosed as chest pain arising from the heart, usually under the sternum due to an inadequate supply of oxygen to the heart muscle. The commonest cause is coronary artery disease with partial blockage caused by a cholesterol plaque or atherosclerosis, which is accelerated in type II diabetes (2,3). It is hypothesized that this relationship is due to common genetic factors (pleiotropic genes that mediate the development of both type II diabetes and atherosclerosis), common environmental factors or a combination of both environmental and genetic factors (4). Recent experimental findings suggest that overproduction of reactive oxygen and nitrogen species may be involved in the initiation and development of vascular complications in diabetes (5).

Oxidation of low-density lipoprotein particles and cytotoxic effects of lipid peroxides enhance the formation of foam cells and atherosclerotic lesion. During the formation and development of atherosclerosis, the intensity of lipid peroxidation and activity of the antioxidant defense system significantly change (6).

Conflicting reports are available regarding the antioxidant status in patients of Type II Diabetes Mellitus complicated with IHD (7,8,9). Hence it was thought worthwhile to study the oxidative status in patients of Type II Diabetes Mellitus complicated with IHD and to assess their correlation with plasma glucose, glycosylated haemoglobin (HbA_{1c}) and duration of diabetes.

MATERIALS AND METHODS

The criteria for the diagnosis of diabetes was done by the National Diabetes Data Group of the National Institute of Health in 1979. 40 patients with diabetes (Group II) but without any micro or macro vascular complications were included in the study. Patients with IHD (n=26) (Group III) were symptomatic for ischaemia, hospitilized in ICCU with history

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of chest pain prior to admission; ECG showed sign of ST-P changes and had significantly increased (>1000 U/L) CPK-MB levels. Patients of IHD with DM (Group IV)(n=30) were also included in the study group. These patients were age and sex matched with 50 normal controls (group I).

All the diabetics were on hypoglycemic drugs and none of the study subjects were on antioxidant supplementation or lipid lowering drugs. Subjects suffering from renal, hepatic disease and any chronic or acute inflammatory illnesses were excluded from the study.

Informed consent was obtained from all the participants of the centre and the protocol was approved by the ethical committee.

Haemolysed samples were excluded from the study. The fasting blood samples were collected in an EDTA/heparin/ plain bulb vial for estimation. Plasma glucose, serum triglycerides, total cholesterol, HDL-cholesterol were measured using enzymatic kit methods. LDL-cholesterol and VLDL cholesterol were calculated using Friedewalds formulae (10). Malondialdehyde,(MDA) a marker of lipid peroxidation was estimated on the fact that lipid peroxides condense with 1 methyl 2 phenyl indole under acidic conditions resulting in the formation of a chromophore (Randox Laboratories, UK). Superoxide dismutase (SOD) estimation was based on the reaction between superoxide radicals and 2-4 iodophenyl-3-4 nitrophenol-5-phenyl tetrazolium chloride (11). Ascorbic acid (Vitamin C) was measured using phosphotungstic acid as colouring agent (12). Glutathione peroxidase (GPx) was measured by the method of Paglia and Valentine (13).

Statistical significance was analyzed by students 't' test and correlation between variables were studied by using Pearson's correlation coefficient test.

RESULTS

As seen in table (1) all the diabetic patients without complications and with IHD had significantly higher levels ($p<0.001$) of HbA_{1c} than in controls.

As in table (2) the levels of total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol were significantly altered ($p<0.001$) in patients of diabetes without complications and non diabetics with IHD when compared with the controls. The levels of total cholesterol ($p<0.001$; $p<0.001$), triglycerides ($p<0.001$;$p<0.05$), LDL-cholesterol ($p<0.001$, $p<0.001$) and VLDL-cholesterol ($p<0.01$; $p<0.001$) and HDL-cholesterol ($p<0.05$; NS) were significantly altered in patients of diabetes with IHD when compared with diabetics without complications and non diabetics with IHD respectively.

In table (3) the levels of MDA were significantly increased ($p<0.001$) where as levels of SOD, GPx and vitamin C were significantly decreased ($p<0.001$) in diabetics without complications and non-diabetics with IHD when compared with the controls. No significant difference was found in the levels of MDA, SOD, GPx and vitamin C levels when non-diabetic patients with IHD were compared with diabetics without complications.

The levels of MDA and GPx were significantly increased ($p<0.001$) where as levels of SOD and vitamin C ($p<0.001$) were significantly decreased in diabetics with IHD when compared with the diabetic group without complications and non diabetics with IHD.

DISCUSSION

The increase in the levels of MDA in our study indicates that lipid peroxidation is taking place in patients of DM, IHD, and

TABLE 1
General data of population in the various subgroups

	Group I (n=50)	Group II (n=40)	Group III (n=26)	Group IV (n=30)
Age in years	46 ± 8	40 ± 6	48 ± 5	49 ± 6
Weight in kg	58 ± 7	57 ± 10	59 ± 10	57 ± 12
Body mass index (kg/m^2)	23 ± 3.9	26 ± 4.2	28 ± 4.8	29 ± 4.7
Plasma glucose (mg/%)	79.89 ± 12.30	173.22 ± 37.86	85.28 ± 10.94	238.26 ± 46.47
HbA_{1c} (%)	7.18 ± 0.87	9.28 ± 1.88	8.16 ± 0.45	12.45 ± 1.15
Duration of diabetes in years	-	6.36 ± 2.33	-	12.17 ± 3.0

P<0.001 when group II and group IV compared with group.

DM complicated with IHD. This is in accordance with various other studies (8,9,14).

In our study dyslipidemia (Table 2) is seen in patients with IHD, diabetics without complications and in diabetics with IHD.

Correlation analysis reveals a significant correlation between values of MDA and total cholesterol [0.66,p<0.001; 0.74,p<0.001; 0.75, p<0.001], triglycerides [0.57, p<0.001;0.75, p<0.001 ; 0.78,p<0.001], LDL-cholesterol [0.58, p<0.001 ; 0.68,p<0.001 ; 0.69,p<0.001] and VLDL cholesterol[0.57,p<0.001 ; 0.75,p<0.001 ; 0.78, p<0.001] in patients with diabetes without complications, non diabetics with IHD and in diabetics with IHD respectively.

As indicated in (Table 4) MDA values increase with an increase in the levels of plasma glucose, HbA_{1c} and duration of diabetes in patients with diabetes without complications and in diabetics with IHD. This is in accordance with the study of Kesavulu MM (8) and Sundaram RK (15).

Numerous theories have been attributed to this increase. Hyperglycemia is acknowledged as a mediator of vessel damage. It involves a number of different parameters (acute, chronic and post prandial hyperglycemia), all of which contribute to vascular damage. High levels of glucose are associated with non-enzymatic glycation of both extra and intra cellular proteins, the accumulation of sorbitol via the aldose reductase pathway, the activation of protein kinase C isoforms, and the reduced bioavailability of nitric oxide (NO) (16). The endothelium derived nitric oxide is a potent endogenous nitrovasodilator and plays an important role in the modulation of vascular tone. The impaired nitric oxide release in experimentally induced diabetes may be prevented by a number of antioxidants. It has been hypothesized that oxygen derived free radicals generated during both glucose autooxidation and formation of advanced glycosylation end products may interfere with NO action and attentuate its vasodilatory activity. An imbalance due to reduced production of NO or increased production of oxygen free radical may facilitate in the development of an arterial functional spasm (17).

Thus the generation of reactive oxygen species may be a common downstream mechanism by means of which the multiple by products of glucose exert their adverse effects

TABLE 2
Lipid profile of controls, patients of IHD and diabetes with &without IHD

	Group I (n=50)	Group II (n=40)	Group III (n=26)	Group IV (n=30)
Total Cholesterol	143 ± 19.9	230 ± 17.4*	220 ± 17.5*	259 ± 29*ad
Triglycerides	131.2 ± 16.45	225 ± 18.67*	219.6 ±16.55*	245 ± 21.44*ae
HDL	48.48 ± 4.37	40.17 ± 4.93*	39.53 ± 4.76*	36.86 ± 3.02*c
VLDL	26.24 ± 3.29	44.99 ± 3.73*	43.92 ± 3.3*	49 ± 4.28 *bd
LDL	67.84 ± 20.73	145.93 ± 17.12*	136.76 ± 16.32*	170.82 ± 28.53 *ad

*p<0.001 when group I compared with group II, III and IV
p<0.001=a, p<0.01=b, p<0.05=c; when group II compared with Group III and group IV
p<0.001=d, p<0.01=e, when group III compared with Group IV

TABLE 3
Prooxidant and antioxidant status in patients of diabetes, IHD and diabetic IHD

	Group I (n=50)	Group II (n=40)	Group III (n=26)	Group IV (n=30)
MDA (nmol/ml)	0.92 ± 0.24	1.72 ± 0.27*	1.78 ± 0.26*	3.32 ± 0.48 *ab
SOD (U/gmHb)	6.83 ± 0.7	5.35 ± 0.36*	5.31 ± 0.45 *	4.08 ± 0.7 ab
GPx (U/gmHb)	14.64 ± 1.43	13.37 ± 0.33*	13.36± 0.38*	16.9 ± 1.51 *ab
Vit C (mg%)	1.13 ± 0.33	0.71 ± 0.22*	0.64 ± 0.15*	0.31 ± 0.07 *ab

*p<0.001 when group I compared with group II, III and IV
p<0.001=a, when group II compared with Group III and group IV
p<0.001=b when group III compared with Group IV

TABLE 4
Correlation analysis of malondialdehyde levels in diabetic patients

	Group II	Group IV
MDA/SOD	- 0.53	-0.56
MDA/ GPx	-0.57	+0.52
MDA/ Vit C	-0.81	-0.45
MDA/ Plasma glucose levels	+0.72	+0.64
MDA/ HbA_{1c}	+0.68	+0.52
MDA/ Duration of Diabetes	+0.62	+0.55

on the blood vessels. A number of oxidative products of lipid and proteins have been demonstrated in atheroma (18,19,20).

Although the exact mechanism of endothelial dysfunction in atherosclerotic disease is unknown, there is evidence that homocysteine exerts its effect by promoting oxidative damage (21). Excess homocysteine can form homocysteine lactone, a highly reactive intermediate, which combines with LDL to form aggregates that are taken up by the intimal macrophages and incorporated into foam cells within nascent atheromatous plagues (22). The accumulation of homocysteine also produces ROS including superoxide and hydrogen peroxide which initiate lipid peroxidation and support the oxidation of LDL (23). This homocysteine induced disturbance in oxidative metabolites also leads to overproduction of oxidative radicals that induce internal injury, activate elastase and increased calcium deposition.

The role of antioxidants in diabetic patients is controversial (9,24,25). In our study the decrease in the levels of antioxidants SOD and vitamin C occurs with the progression of lipid peroxidation. The lowered values of vitamin C are because it functions as an important component of cellular defense against oxygen toxicity and lipid peroxidation caused by free radical mechanism.

The decrease in GPx in diabetics without complications is to counteract the oxidative stress. The increase in GPx in-patients of diabetes complicated with IHD is probably adaptive in nature. At physiological rates of hydrogen peroxide generation, the glutathione system is more important in catabolising hydrogen peroxide. GPx is a selenium dependent enzyme and any alteration in the tissue levels of selenium would alter GPx activity. Insulin deficiency promotes β oxidation of fatty acids with resulting increase in hydrogen peroxide formation. Thus with increase in the lipid peroxide levels the paradoxical increase in the levels of GPx is an interesting finding and could be a compensatory mechanism by the body to prevent tissue damage (26,27).

In conclusion, with progression of diabetes, ie development of complications increase in lipid peroxidation takes place and an imbalance occurs in the antioxidant status to compact the oxidant injury. Hence monitoring of antioxidant parameters in diabetic patients could be of vital importance in prevention of development of complications in diabetic patients.

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