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Risk assessment of cardiovascular diseases among bank employees — a biochemical approach

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In different epidemiological studies, an association between sedentary life style and incidence of cardiovascular diseases has been demonstrated. This study was carried out among 50 sedentary bank employees working at different nationalized banks within Calcutta City, India and 50 individuals with non-sedentary jobs matched for age group and sex for the control group. Body mass index (BMI), blood pressure (BP), plasma level of glucose, total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides (Tgs) were found to be significantly higher whereas high density lipoprotein (HDL) and reduced glutathione were found to be significantly lower among the bank employees as compared with the control subjects. Further, significant alteration in the activity of different enzymes (*e.g.*, glutathione reductase, glutathione S-transferase, glucose 6 phosphate dehydrogenase, catalase and superoxide dismutase) involved in glutathione-mediated antioxidant scavenging systems and increases in lipid peroxidation were also observed in the bank employees. From the study, it appears that the relative risk for cardiovascular disease is increased among bank employees due to the sedentary nature of their jobs.

Key words: Antioxidant defence; cardiovascular disease; lipid profiles; sedentary jobs.

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INTRODUCTION

Cardiovascular diseases are among the most common causes of illness and death in the working population in industrialized as well as in developing countries.[1] India is witnessing an epidemic of atherosclerotic coronary heart disease.[2,3]

The significant role of free radicals in the pathogenesis of certain human diseases, such as cardiovascular disease, cancer, rheumatoid arthritis, drug associated toxicity and post-ischaemic reoxygenation injury is becoming increasingly recognized.[4,5] There is growing interest in the mechanisms of antioxidants as well as in the development of adequate indices of both oxidative stress and damage caused by aggressive oxygen species (superoxide anion radical, hydroxyl radicals, hydrogen peroxide and singlet oxygen) and thiyl radicals. The main contribution of antioxidants appears to be in scavenging these free radicals and converting them to inactive substances. As a result antioxidants have been postulated to be potential protective agents which help to guard against atherosclerosis and its clinical manifestation.[6]

Antioxidants are therefore crucial to the body's multilevel defence against free radicals. Lines of defence include enzymes (*e.g.*, glutathione reductase, glutathione peroxidase, glutathione S-transferase, catalase, superoxide dismutase) non-essential endogenous antioxidants (glutathione, proteins, uric acid, *etc.*) and essential radical scavengers, *i.e.*, vitamin C, vitamin E and caroteinoids.[5,7–14]

A consistent association between sedentary life style and coronary heart disease (CHD) has been demonstrated in epidemiological studies, thereby explaining the higher prevalence of CHD reported in sedentary workers.[15–17] Conversely, epidemiological and other evidence suggest that regular exercise habits protect against the development of cardiovascular disease, and may also improve sense of well-being and protect against

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some complications of diabetes, lung disease and osteoporosis.[18]

People with diabetes appear to have an excess of complicated atherosclerotic lesion and the frequency of myocardial infraction in diabetics is double that in non-diabetics.[19,20] It has long been recognized that high blood pressure is an independent risk factor for cardiovascular disease and the association between systolic hypertension and CHD in diabetics is more consistent than that which has been observed for diastolic pressure.[21] Further, increases in body mass index are strongly associated with an increased risk of non-insulin dependent diabetes, especially when the obesity is centrally distributed.[22]

Plasma total cholesterol is an indicator of lipid status and important aetiological factors are also involved in CHD.[23] It has been established that high density lipoprotein (HDL) cholesterol is inversely correlated with risk of ischaemic heart disease (IHD);[24,25] physical activity[26,27] is inversely correlated with raised HDL cholesterol levels and cigarette smoking[28,29] and obesity is inversely correlated with lower HDL cholesterol levels. There is now considerable evidence in support of oxidative modification of low density lipoprotein (LDL) playing an important causative role in atherosclerosis.[30] LDL in oxidized form, present in blood and the arterial walls has been found to be more atherogenic than in native LDL.[30] The evidence for triglycerides as a risk factor for CHD is less convincing; however, triglycerides are positively correlated with cholesterol, obesity, glucose intolerance and are inversely correlated with HDL-cholesterol levels.[31]

In view of the above we wished to ascertain whether the risk for cardiovascular disease increases among bank employees with sedentary life styles by assessing their personal data and different blood biochemical indices, *viz.*, glucose, different lipid fractions, lipid peroxidation, glutathione and enzymes related to glutathione-mediated antioxidant defence systems.

MATERIALS AND METHODS

Subjects

Study group. The study was carried out among 50 bank employees drawn at random from the 400 bank employees working more than seven years in different nationalized banks in Calcutta City, India. All employees were male and within the age group of 35–50 years. The nature of their jobs were clerical, managerial and administrative jobs which mainly involved handwriting, dictating, typing, ledger keeping, cash payment–receipt and mental activities. These are usually classified as sedentary activities.[32] An extensive questionnaire documenting age, height, weight, blood pressure, dietary habits, smoking habits, exercise habits, monthly income, number of dependants, position held and occupational history was completed by each employee.

Control group. Fifty subjects selected at random from the 372 males in the age group 35–50 years who were working in non-sedentary jobs (*e.g.*, sales representatives, technical workers and field workers) as ascertained by a sedentary co-efficient were chosen as controls. The same questionnaire was completed by both groups.

Biological measurements

Whole blood was collected by venepuncture in heparinized vials from both study and control groups. The plasma was collected by centrifugation of whole blood at 2,500 ***g*** for 15 min at 5°C, for the estimation of enzymes, lipid status, lipid peroxidation, glucose and reduced glutathione content. The packed erythrocytes were washed three times with four volumes of isotonic saline and buffy coat was removed by gentle aspiration. Washed packed erythrocyte was lysed by adding distilled water for 1 hour and lysate was centrifuged at 13,000 ***g*** for 1 hour at 5°C. The supernatant was used to estimate the enzymes and reduced glutathione content.

Reduced glutathione was measured by the method of Beutler *et al.*[33] Glutathione peroxidase was assayed by the method of Paglia and Valentine.[34] Glutathione reductase was assayed by the method of McKenna *et al.*,[35] and glucose-6-phosphate dehydrogenase was measured by the method of Löhr and Waller.[36] Superoxide dismutase and catalase were assayed following the method of Paoletti *et al.*[37] and Cohen *et al.*[38] respectively. Glutathione S-transferase was measured according to the method of Levender *et al.*,[39] using 1-chloro 2, 4 dinitrobenzene as substrate. Lipid peroxidation was determined by the method of Takayama *et al.*[40]

Lipid status of the blood plasma was measured in terms of total cholesterol,[41] HDL, LDL, VLDL and triglyceride as described by Varley *et al.*[42] Glucose was estimated by the oxidase–peroxidase method.[43] The protein content was determined according to the method of Lowry *et al.*[44]

RESULTS

The following observations were obtained from an analysis of the questionnaires completed by both study and control groups: (1) all participants of both groups were of more or less the same economic status as ascertained by monthly income *vs.* number of dependants analysis; (2) there was no marked difference in the nature of jobs among the bank employees irrespective of their positions; (3) no individuals from either group engaged in any form of physical exercise habits (*i.e.*, aerobic, isotonic, isometric or passive); (4) all individuals of both groups were non-smoking and did not consume alcohol; (5) dietary habits (total calorie-intake, calories from fat, *etc.*) of the participants in both groups were similar.

The above criteria has therefore been excluded from the comparative analysis in our present study. However, body mass index (BMI) and blood pressure (BP) both systolic and diastolic, were significantly higher ($p < 0.001$) in bank employees as compared with control subjects (Table 1).

A significant increase ($p < 0.001$) in plasma glucose, cholesterol, low density lipoprotein (LDL), very low

Table 1. Body mass index, blood pressure, glucose level, cholesterol status and lipid peroxidation of bank employees and control group

Group	Body mass index (BMI) Kg/m^2	Blood pressure mm/Hg		Plasma glucose mg/dl	Plasma cholesterol mg/dl	Plasma HDL-cholesterol mg/dl	Plasma LDL-cholesterol mg/dl	Plasma VLDL cholesterol mg/dl	Plasma trigycerides mg/dl	Plasma lipid peroxidation (μ moles of malondialdehyde formed/mg protein)
		Systolic	Diastolic							
Control	21.26 ±1.32	117.12 ±9.82	72.93 ±8.11	78.24 ±13.29	178.05 ±24.92	33.34 ±6.37	121.03 ±20.45	26.81 ±5.98	112.18 ±26.92	0.00821 ±0.00161
Bank employee	24.39 ±1.21**	134.56 ±12.57**	87.32 ±5.93**	96.69 ±14.84**	202.47 ±26.73**	27.31 ±4.64**	147.94 ±23.83**	32.27 ±7.86**	146.25 ±18.42**	0.0105 ±0.00516*

Values are mean, SD from 50 experiments in each case.
* $p < 0.05$; ** $p < .001$.

Table 2. Glutathione status and specific activity of different enzymes of glutathione-mediated antioxidant defence system in plasma & RBC of bank employees and control group

Group	Glutathione[a] (reduced)		Glutathione[b] reductase		Glutathione[c] S-transferase		Glucose-6[d] phosphate dehydrogenase		Glutathione[b] peroxidase		Catalase[d]		Superoxide[e] dismutase	
	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC
Control	3.1596 ±0.4802	4.2347 ±0.6493	0.4357 ±0.0751	1.6352 ±0.2958	0.08378 ±0.0137	1.0142 ±0.2058	0.1485 ±0.0281	1.5352 ±0.2239	3.1246 ±0.2630	8.6088 ±1.0569	0.00102 ±0.000122	0.1236 ±0.0235	0.5629 ±0.1231	1.238 ±0.2907
Bank employee	2.3641 ±0.3877**	5.8932 ±0.9432**	0.6214 ±0.0817**	1.782 ±0.3742*	0.06956 ±0.0097**	0.9737 ±0.1334	0.09732 ±0.0145**	1.6951 ±0.2737*	2.7631 ±0.2417**	7.5314 ±1.220**	0.00147 ±0.00243	0.1473 ±0.0309**	0.6482 ±0.1316*	1.537 ±0.3612**

[a] μ mole/mg protein.
[b] n mole of NADPH oxidized/min/mg protein.
[c] m mole of product formed/min/mg protein.
[d] moles of H_2O_2 decomposed/min/mg protein.
[e] units/mg protein.

Values are mean SD from 50 experiments in each case.
* $p < 0.05$; ** $p < 0.001$.

density lipoprotein (VLDL) and triglycerides (Tgs) and a significant decrease in high density lipoprotein (HDL) ($p < 0.001$) were observed among bank employees. Moreover, lipid peroxidation was found to be increased ($p < 0.05$) significantly in bank employees as compared with the control subjects (Table 1).

Significantly lower levels of glutathione content in plasma ($p < 0.001$) and higher level of glutathione content in RBC ($p < 0.001$) were observed among bank employees as compared with the control subjects. The activity of glutathione reductase (GR) in bank employees increased significantly both in plasma ($p < 0.001$) and RBC ($p < 0.05$). The activity of glutathione S-transferase (GST) in plasma decreased significantly ($p < 0.001$) whereas the decrease was statistically insignificant in the case of RBC. In plasma the activity of glucose 6 phosphate dehydrogenase (G6PD) decreased significantly ($p < 0.001$), while in RBC it increased significantly ($p < 0.05$) among bank employees. Again glutathione peroxidase (GP_X) activity was found to decrease significantly both in plasma and RBC ($p < 0.001$) in bank employees as compared with the control subjects. The activity of catalase was found to be very low in plasma for both the groups. A significant increase in catalase activity in RBC ($p < 0.001$) was, however, observed in bank employees as compared with control subjects. Further, superoxide dismutase (SOD) activity was found to be increased both in plasma ($p < 0.05$) and RBC ($p < 0.001$) in bank employees as compared with control subjects (Table 2).

DISCUSSION

In different studies, obesity, hypertension, raised level of cholesterol and glucose were identified as important risk factors for CHD.[45–49] In our study, the BMI, BP (both systolic and diastolic), plasma cholesterol and glucose level were significantly higher in bank employees than in the control group which may suggest that bank employees are at higher risk for CHD. It was established that total cholesterol and LDL-cholesterol are major independent risk factors for CHD, while on the contrary there is an inverse relationship between HDL-cholesterol concentration and CHD incidence; triglycerides, though less convincing, are also a risk factor for CHD.[50] Hence the increased levels of LDL-cholesterol, VLDL-cholesterol and Tgs and the decreased level of HDL-cholesterol in bank employees as compared with control subjects also confirms increased relative risk of cardiovascular disease for bank employees who are performing sedentary jobs.

Lipid peroxidation was found to be increased in bank employees. Higher rates of lipid peroxidation in bank employees may lead to increased formation of the peroxide modified component of LDL. Uptake of this component by macrophage and monocyte may be enhanced, which may lead to atherogenic foam cell formation, the initiating step in the development of atherosclerosis.

The tripetide glutathione (GSH) is a widely distributed compound in animal tissue, and a potent endogenous antioxidant that helps to protect cells from oxidative injury. Besides its role in the maintenance of redox potential within the cells, it is also a key component of the enzymatic antioxidant system. Since GSH is the substrate for gluthathione peroxidase, an enzyme involved in detoxification of lipid peroxides and also the substrate for glutathione S-transferase, an enzyme involved in detoxification of xenobiotics and compounds formed from free radical reactions. GR produces reduced glutathione from oxidized glutathione at the expense of endogenous reducing equivalent NADPH and G6PH of pentose phosphate pathway produces NADPH from $NADP^+$ with the help of glucose 6 phosphate consumption.

The results of our study indicate that GR activity was found to be increased and GSH level was found to be decreased in the plasma of bank employees. This may be due to inhibition of G6PD, which can not supply necessary amounts of reducing equivalent required for normal functioning of GR. As a result even an increase in GR activity failed to maintain GSH level at normal level. Moreover, SOD activity was found to be increased in bank employees which may be involved in scavenging superoxide radical (O_2^-), thereby producing more hydrogen peroxide. Increase in catalase activity may be considered as a secondary response to counteract oxidative stress as it may be involved in the removal of hydrogen peroxide produced by increased SOD activity. Moreover, the detoxification mechanism against oxidative damage may be hampered in bank employees due to the significant decrease in their GPX and GST activity.

Hence a significant alteration of certain identified predisposing factors, *i.e.*, BMI, BP, blood glucose level and lipid fractions, may result in an elevated risk for cardiovascular disease among bank employees due to their sedentary job pattern. At the same time it is also evident that the inhibition of glutathione-mediated free radical scavenging enzyme systems resulting in increased lipid peroxidation may enhance atherogenesis among subjects.

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