Oxidative stress, antioxidant status and lipid profile in ischemic heart disease patients from western region of Nepal

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ABSTRACT

Disturbed lipid profile is one of the most important and potent risk factors in ischemic heart disease (IHD). In recent years, it has been demonstrated that raised oxidative stress promotes several undesirable pathways including the formation of oxidised LDL (O-LDL) and oxidized cholesterol which encourages cholesterol accumulation in arterial tissues. We, therefore, aimed to ascertain the redox balance by measuring oxidative stress (OS) and total antioxidant activity (TAA) along with lipid profile to determine their possible association with IHD. Our study group comprised of 28 confirmed cases of IHD. The inclusion criterion was history of chest pain, ischemic changes in the ECG and good left ventricular (LV) function. Patients with diabetes mellitus, poor LV function, previous infarct and valvular heart disease were excluded. Lipid profile, plasma thiobarbituric acid reactive substances (TBARS), plasma total antioxidant activity (TAA) and urinary TBARS were estimated in these patients by standard procedures and the values were compared with 30 age, sex and socioeconomically matched normal healthy control subjects. Body mass index (BMI) and waist/hip ratio (W/H ratio) was also noted in both the groups. Lipid profile and OS (TBARS levels) were significantly raised in IHD patients. Though statistically not significant but TAA tended to be lower and urinary TBARS levels tended to be higher in patients. BMI, W/H ratio, smoking and alcohol did not show discernible association with lipid profile, OS or TAA. OS is significantly raised in majority of IHD patients. The non association of BMI, W/H ratio, smoking and alcohol with lipid profile, OS and TAA suggest that there are other risk factors which primarily contribute to the initiation and progression of IHD.

Keywords: Oxidative stress, ischemic heart disease, lipid profile, antioxidants, Nepal.

INTRODUCTION

Since we are not designed and destined to live forever, the "Aging" process is a natural phenomenon not only in human but in all organisms. During its prospective journey, the genetic ribbon energetically moves onward with crescendo till youth and then deterioratingly slows down progressively. This inevitable downward drift and decline of physiological and biochemical processes is referred to as "Aging" and is contemplated to be due to genetic dictation, entwined with turbulent environmental interactions which may lead to age related diseases such as ischemic heart disease (IHD), cancer, diabetes mellitus, neurological disorders and many others and set faster under inclement settings.[1-5] Till date there is no single theory encompassing the full spectrum of changes in aging, senescence and age related diseases as myriad of reasons have been reported from time to time. Among them, one of the prominent emerging hypothesis is that inherent as well as accelerated aging symptoms and diseases thereof, are due to genetic defects in genome maintenance and stability driven by oxidative damage. Numerous factors are intricately interwoven in maintaining, translocating and transposing the genetic tape. In this self deteriorating and receding tape, high caloric intake leading to incendiary lipid profile and obesity, raised oxidative stress and weak antioxidant defense are presently labeled as prime risk factors in IHD. It has also been demonstrated in some series of patients and strongly pleaded that oxidative stress exerts conjugal influence in concert with unfavorable lipid profile in initiating or exacerbating these diseases.[6-8] But the hypothesis is neither fully proven nor universally applicable nor guarantees against them even if antioxidant network is optimally installed or reinstalled. This maiden study from Nepal address is to see the possible involvement of OS, TAA and lipid profile in IHD patients.

MATERIALS AND METHODS

This study was undertaken in the Department of Biochemistry, Manipal Teaching Hospital, Pokhara. The study group consisted of 28 patients with confirmed ischemic heart disease (20 males and 8 females) with a mean age of 57.35±15.65 years. The control group consisted of 30 healthy individuals (17 males and 13 females) with a mean age of 50.80±14.84 years.

Detailed present and past history of the patients was

Table-1: Lipid profile, oxidative stress and total antioxidant activity in normal subjects and IHD patients

Parameters	Control Mean±SD	IHD Mean±SD
Age (years)	50.80±14.84	57.35±15.65
BMI (Kg/m^2)	23.65±3.94	23.82±5.07
W/H ratio	0.94±0.09	0.96±0.08
TC (mg/dl)	155±25	177±37 [b]
TG (mg/dl)	123±36	146±44 [a]
HDL (mg/dl)	45±8	40±7 [a]
VLDL (mg/dl)	25±7	30±10 [a]
LDL (mg/dl)	85±20	106±28 [b]
TAA (nmol/ml)	726±200	698±175
TBARS (µmol/l)	2.41±0.61	3.38±0.87 [c]
UTBARS (µmol/l)	3.84±1.6	4.69±2.36

Independent't' test: 'p' value a = <0.05 b = <0.01 c = <0.001

collected with the help of pre-tested proforma. The proforma included name, age, sex, dietary habit (vegetarian/non-vegetarian), family history of disease, smoking and alcohol consumption, socioeconomic status, community and occupation. In addition to this we also measured physical parameters like waist and hip (W/H) ratio and BMI (kg/m^2). Prior informed consent was taken from all the subjects participating in the study.

After 12 hours overnight fast, 6ml of blood was collected from each subject by venipuncture with standard blood collection technique. The plasma separated was used for the analyses of total antioxidant activity (TAA)[9] plasma thiobarbituric acid reactive substances (TBARS)[10] and lipid profile total cholesterol (TC)[11], triglycerides (TG),[12] HDL-cholesterol, LDL- cholesterol and VLDL-cholesterol).[13] About 30ml of urine was collected without preservative for the analysis of urinary TBARS in clean dry container.

Analytical reagents for estimation of TAA and TBARS were purchased from Central Drug House Pvt Ltd., Bombay-Delhi India and fresh reagents were prepared in laboratory prior to estimation. For lipid profile, we purchased kits from Randox Laboratories Ltd, United Kingdom and processed through semi-autoanalyzer, Microlab-300, Netherland.

Computer software program SPSS version 10.0 was used for the statistical analysis of different biochemical parameters for the interpretation of the result. Data were processed for obtaining 'p' value of Independent Sample t-test, one way ANOVA test and Correlation. 'p' value <0.05 was considered for all statistical analysis and were categorized a, b, c, with the 'p' value <0.05, <0.01 and 0.001 respectively.

RESULTS

The average age of the normal subjects and IHD patients were 50.80±14.84 years and 57.35±15.65 years respectively. Their lipid profile, plasma TBARS, urinary

Table-2: Percentage of controls and IHD patients in the safe, desirable and high risk zone of cholesterol, HDL-C and LDL-C

Description	Controls			IHD Patients		
	%	BMI	W/H ratio	%	BMI	W/H ratio
Cholesterol						
<200mg/dl	96.7%	23.5±0.9	0.93±0.09	71.4%	23.9±5.2	0.96±0.07
>240mg/dl	-	-	-	3.57%	19.3	0.97
HDL-C						
<40mg/dl	30%	24.3±4.6	0.97±0.11	53.6%	24.3±5.5	0.96±0.08
40-59mg/dl	63.3%	23.3±3.8	0.91±0.08	46.5%	23.2±4.6	0.97±0.08
>60mg/dl	6.7%	23.8±1.6	0.98±0.04	-	-	-
LDL-C						
<130mg/dl	93.3%	23.8±3.9	0.94±0.09	78.5%	23.8±5.1	0.97±0.07
130-159mg/dl	6.6%	18.6	0.85	17.8%	24.6±5.4	0.99±0.10
160-189mg/dl	-		-	-	3.6%	19.2 0.78

Table-3: Correlation of different parameters in normal controls and IHD patients

Parameters	Control		IHD	
	r-value	p-value	r-value	p-value
TBARS Vs TAA	-0.339	0.008 [b]	0.012	0.954
TBARS Vs TC	0.030	0.821	0.035	0.859
TBARS Vs TG	-0.075	0.571	0.133	0.501
TG Vs TC	0.383	0.003 [b]	0.243	0.213
TG Vs HDL	-0.240	0.650	-0.275	0.157
TG Vs LDL	0.123	0.351	0.003	0.988

TBARS and TAA are given in table 1. Most 96.7% of the normal subjects had total cholesterol <200mg/dl whereas 71.4% of the IHD patients had TC in this level. Similarly, 93.3% of the normal subjects and 78.5% of the IHD patients had LDL-cholesterol level <130mg/dl (safe level). Noticeably 30.0% of normal subjects and 53.6% of the IHD patients had HDL-cholesterol level <40 mg/dl, whereas none of the IHD patients had HDL-cholesterol levels >60mg/dl (Table-2). Triglyceride level was significantly raised ($p<0.05$) in IHD patients (146±44mg/dl) when compared to normal subjects (123±36 mg/dl) (Table-1). We observed positive correlation between TG and TC in normal subjects (r= 0.383, p= 0.003) but not in IHD. No significant correlation was observed between TG and LDL cholesterol in normal subject as well as in IHD patients (Table-3).

We observed no significant difference in lipid profile among smokers and alcohol consumers in normal subjects and IHD patients. Lipid profile considered on the basis of BMI, Waist-Hip ratio, socioeconomic status and dietary habits did not provide any conclusive trend.

The plasma TBARS levels in IHD patients were significantly higher than normal subjects ($p<0.001$) but TAA levels were comparable between two groups (Table-1). Strikingly, there was an inverse relationship between TAA and plasma TBARS in normal subjects (r= -0.033, p= 0.008), but not in IHD patients. Both in normal subject and IHD patients there was no significant correlation between plasma TBARS and TC and also between plasma TBARS and TG (Table-3).

No significant difference in OS and TAA was noted between smokers vs. non-smokers and alcohol consumer vs. alcohol non-consumer in both normal subjects and IHD patients. No definitive pattern was observed in the OS and TAA in relation to BMI (Table-4), W/H ratio, socioeconomic status and dietary habits (Table not enclosed).

DISCUSSION

The proportionate distribution of cholesterol in HDL and LDL fractions is necessarily important for health equilibrium and that disturbed lipid profile with low HDL-cholesterol and high LDL-cholesterol is an established cogent risk factor in initiation, progression and also in precipitation of IHD.[14,15] Lately, the distribution of cholesterol in different fractions of LDL has also been emphasized. Accordingly their levels have been defined into three categories viz. desirable, borderline high and high risk levels.[16,17] In our study group, the total cholesterol level in normal subjects was within desirable limits in 96.7%, within borderline level

Table- 4: Lipid profile, oxidative stress and total antioxidant activity in normal subjects and IHD patients based on their BMI

Parameter	Controls			IHD Patients		
BMI	<18.5 (2)	18.5-25 (5)	>25 (11)	<18.5 (4)	18.5-25 (13)	>25 (11)
TC (mg/dl)	154±24	150±20	160±32	149±17	186±43	176±30
TG (mg/dl)	131±4	113±37	135±29	146±66	150±41	140±42
HDL (mg/dl)	46±9	45±8	43±8	39±8	39±8	41±6
VLDL (mg/dl)	26±8	22±7	27±6	29±13	32±10	27±8
LDL (mg/dl)	82±22	84±19	87±21	80±17	113±30	106±25
TAA (nmol/ml)	588±110	685±161	814±239	550±133	682±172	769±150
TBARS (µmol/l)	2.92±0.28	2.50±0.60	2.18±0.61	3.73±1.08	3.23±0.84	3.43±0.89
UTBARS (µmol/l)	3.37±0.37	3.94±1.82	3.77±1.53	4.12±2.68	4.88±2.80	4.67±1.82

One way ANOVA test done in controls and type 2-DM separately: p<0.05=a, p<0.01=b and p<0.001=c

in 3.3% and none in the high risk level (>240mg/dl). In IHD patients the respective figures were 71.4%, 25% and 3.6%. These data indicated that although the total cholesterol increased in IHD patients, the magnitude of rise was not so severe as to implicate it as major determinant of IHD risk.

Presently, the level of HDL-C and LDL-C is considered to be superior index of CVD risk than total cholesterol. The defined desired, borderline high and high risk levels for LDL-C are <130mg/dl, 130-160mg/dl and >160mg/dl respectively. Among normal subjects 80%, 13.3% and 6.6% were in respective category. In patient group, the figures in respective groups were 78.5%, 17.8% and 3.6%. Thus LDL-C also did not seem to be a major risk factor in IHD patients. On the contrary, most striking feature was low HDL-C in 30% normal subjects and 53.6% IHD patients. The LDL-C increases the risk of atherosclerosis by its longer resident time in blood whereas the low HDL-C increases the risk by slower removal of cholesterol from blood to liver for the synthesis of various sterol compounds and also for excretion. As such, the lower level of HDL-C in the local population seems to a significant factor.

The triglyceride levels were significantly raised in IHD patients. The data of individual patients revealed that 64.3% were in safe limits (<150mg/dl) and only 35.7% patients had borderline high level. However, the recent recommendations suggest that clinically it makes sense to treat for mild hypertriglyceridemia (>500mg/dl) and if the patients had HDL-C low level.[16,17] Accordingly, we did not find a rationale to treat any of the IHD patients for hypertriglyceridemia.

Simultaneously, we also examined lipid peroxidation by measuring serum and urinary TBARS levels and TAA, because raised OS, either due to enhanced reactive oxygen species (ROS) generation or weaker antioxidant defense are postulated to atherogenesis for several reasons.[18, 19] ROS depending on the intensity of their production cause: a) oxidation of LDL to form oxidized-LDL (varying from mildly oxidized LDL to highly oxidized LDL). The oxidized-LDL loses its protean character for its movement between blood and vascular cells and gets accumulated in the cells forming foam cells which ultimately are converted into fatty streaks, forming integral part of atheromatous plaques; b) disturbed endothelial dysfunction; c) exaggerated platelet aggregation and adhesion and d) dyslipidemia and hypertension. Interestingly, recent studies indicate that oxidized HDL further adds to IHD risk, Kris-Etherton and West[20] critically assessed the new observations about oxidants and antioxidants in vascular system and pointed out that the primary constituent of HDL, apolipoprotien A-1, is the target for myeloperoxidase catalysed nitration and chlorination in vivo. This altered HDL-C with oxidized protein inhibits cholesterol efflux from macrophage. Lastly, the oxidative adducts of fatty acids exert additional toxicity on vascular system.[21]

The observations indicate that the IHD patients had significantly raised serum OS. The urinary TBARS levels though did not attain statistical significant difference but distinctly tended to be higher (Control-3.84±1.60µmol/L, IHD- 4.69±2.36µmol/L). Two deductions can be drawn from these two observations: First, free radical activity was provoked in IHD patients and second, kidneys have capacity, though limited, to eliminate lipid peroxidative adducts.

The antioxidant defense system functions as a well knit team consisting of endogenous antioxidants and dietary antioxidants comprising of both nutrient and non-nutrient antioxidants. The endogenous antioxidants have circumscribed capacity and are appropriately augmented by dietary antioxidants. FRAP procedure for TAA does not measure reduced glutathione (GSH) and other thiol compound which are quantitatively important endogenous antioxidants nor it measures enzymatic antioxidant activity. Hence practically, it represents the dietary antioxidant activity. In normal persons, it ranges between 600-1600µmol/L. In the local population, TAA was within normal limits in both the groups; though statistically not significant it tended to be lower in patients. Interestingly, correlation coefficient (r) revealed that serum TBARS and TAA were inversely related ($p<0.008$).

There is now overwhelming evidence that overweight and obesity are harmful to health; that higher the body weight the greater is the risk of heart disease; and that higher body mass engenders ROS production. Both Body Mass Index (BMI) and Waist/Hip ratio (W/H ratio) are currently used as anthropometric predictors of chronic diseases and lipid profile including cardiovascular diseases but with variable conclusions in different populations.[22-24] We have, therefore, examined the relationship of BMI and W/H ratio with lipid profile, oxidative stress and total antioxidant activity in both normal subjects and IHD patients. We did not observe any conclusive trend in both patients and normal subjects indicating that neither BMI nor W/H ratio are predictors of lipid profile or oxidative stress or TAA.

Mounting evidence indicate that alcohol in moderate quantities is beneficial for health whereas,[25] tobacco in any form is not only dangerous but also a strong risk factor for IHD.[26] In our study groups smoking and drinking did not show any effect on lipid profile, OS

and TAA. However, it would be germane to comment on it only after a long series is completed and adjustments for other relevant factors are taken into account.

Accounting all the data together, we can conclude from our observations that, in IHD, lipid profile unfavorably shifts, that OS is frequently raised and kidneys have only limited capacity to excrete lipid peroxidation adducts. Most importantly diet contributes good amount of antioxidants but disappointingly many of them do not seem to be able to exert their antioxidant activity in vivo to scavenge free radicals. Finally, BMI, W/H ratios do not seem to be indicators of examined biochemical indices in this population.

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