Evaluation of Lipid Peroxidation and Antioxidants' Status in Metabolic Syndrome

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ABSTRACT

Background

Metabolic syndrome is a constellation of physical conditions and metabolic abnormalities, commonly occurring together, that increases an individual's risk for development of type 2 diabetes mellitus and cardiovascular diseases. Oxidative stress is associated with diabetes, hypertension and other cardiovascular diseases while the role of oxidative stress in pathogenesis of MS is not clearly defined.

Objectives

The study aims to find out the prevalence of metabolic syndrome in faculty and staff members at BP Koirala Institute of Health Sciences, Dharan, Nepal and to evaluate oxidative stress levels in subjects with metabolic syndrome.

Methods

118 healthy participants working at B. P. Koirala Institute of Health Sciences, Dharan, Nepal were selected at random for this cross-sectional study and blood samples were collected for the estimation of the following biochemical analytes; fasting glucose; triglycerides; total cholesterol; high density lipoprotein cholesterol; Albumin; uric acid; Bilirubin; Malondialdehyde; Catalase; Glutathione peroxidase; Superoxide Dismutase; Glutathione; vitamin C; and lastly vitamin E.

Results

In this cross-sectional study, 39% subjects were diagnosed with metabolic syndrome , particularly in sedentary subjects. There was no difference in oxidative stress except significant rises in serum uric acid levels and catalase activity in subjects diagnosed with metabolic syndrome .

Conclusion

The prevalence of metabolic syndrome is higher without oxidative stress in this study, which suggests that oxidative stress does not contribute to the pathogenesis of MS in otherwise healthy subjects.

Key Words

antioxidants, lipid peroxidation, metabolic syndrome, oxidative stress

INTRODUCTION

Metabolic syndrome (MS) is a constellation of physical conditions and metabolic abnormalities which increase an individual's risk of developing type 2 diabetes mellitus and cardiovascular diseases. Using the criteria of the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATPIII), the prevalence of the MS in U.S. adults' ≥20 years of age was recently estimated to be 23.7%.[1] Little is known about the prevalence of the MS in south-east Asians particularly in Nepal. [2, 3]

Oxidative stress is an imbalance between tissue, free radicals, reactive oxygen species (ROS), and antioxidants system. The imbalance which causes oxidative stress is caused by e highly reactive molecules with unpaired electrons, that bind with nearby molecules leading to oxidative damage. Antioxidants prevent the series of reactions that generate free radicals or neutralises them.[4] Oxidative stress is associated with obesity-related conditions such as diabetes, hypertension and other cardiovascular diseases. These diseases have been proven to have a direct association with MS.

This study aims to reveal the status of MS in faculty and staff members of B. P. Koirala Institute of Health Sciences, to enable the prevention of the disease; to promote overall health by changing people's lifestyles; and through pharmacological intervention if and when required. The study also aims to investigate lipid peroxidation and various non-enzymatic and the enzymatic antioxidants status in MS cases.

METHODS

With the approval of the B.P. Koirala Institute of Health Sciences Research Committee, 118 healthy participants (98 male and 20 female) were randomly selected. The participants were not receiving antioxidant vitamin supplementation. Anthropometric measurements, blood pressure, ethnicity and personal habits were recorded in a pre-designed pro forma from each respondent. Blood samples were taken in the morning after 12-hour overnight fasting. Serum, plasma and Erythrocyte lysate were prepared and stored at -20^{0}C until use. Plasma red cells were washed with normal saline three times, then red blood cells were lysed with four times its volume with ice-chilled, distilled water. As a result, Erythrocyte lysate formed, and was separated by centrifuging at 10,000 rpm for 15 minutes at 4^{o}C.

Definition of metabolic syndrome

Metabolic syndrome was diagnosed according to the NCEP ATP III criteria.[1] According to this criteria, the diagnosis of metabolic syndrome was established when three or more of the following risk factors were present; waist circumference >102 cm in men and >88 cm in women; fasting glucose ≥110 mg/dl and triglycerides ≥150 mg/dl in either sex: HDL cholesterol <50 mg/dl in women and <40 mg/dl in men: blood pressure - systolic blood pressure (SBP) ≥130 mm Hg, diastolic blood pressure (DBP) ≥85 mm Hg or use of antihypertensive medications.

Biochemical parameters

The biochemical parameters such as fasting glucose, total cholesterol, triglycerides, high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), uric acid, Albumin and Bilirubin were analysed from the serum on Vitalab Selectra-MERCK Clinical Chemistry Analyzer. Plasma concentration of lipid peroxidation product Malondialdehyde (MDA) was estimated by the measurement of thiobarbituric acid reactive substance by the method of Yagi et al. [5] Erythrocyte Catalase (CAT) was assayed colorimetrically as micromoles of hydrogen peroxide consumed per minute per milligram haemoglobin as described by Sinha et al. [6] Erythrocyte Superoxide Dismutase (SOD) was assayed in erythrocyte lysate by the method of Kakkar et al. This was based on inhibition of the formation of Nicotinamide Adenine Dinucleotide, Phenazine Methosulfate and amino blue Tetrazolium Formazan. A single unit of enzyme was expressed as 50% inhibition of NBT (Nitroblue Tetrazolium) reduction/ min/gm Hb[7]. Erythrocyte Glutathione Peroxidase (GPx) activity was assayed in erythrocyte lysate by the method described by Rotruck et al.[8] as microgram of Gluthatione (GSH) consumed per minute per gram haemoglobin. [8] Glutathione (GSH) in whole blood was determined by the method of Beutler et al.[9] Plasma vitamin C was estimated by method depending on the reduction of ferric ion (1% Ferric chloride) to ferrous ion by ascorbic acid present in protein free filtrate. It was reacted with 0.5% α,α'–Dipyridyl to form a red-orange α,α'–Dipyridyl complex measured spectrophotometrically at 520 nm.[10] Plasma vitamin E was estimated by the method in which α-Tocopherol extracted in petroleum ether is oxidized to tocopheryl quinine by ferric chloride and resultant ferrous ion is complexed with ethanolic α,α'–Dipyridyl (0.2% in ethanol) to produce a red coloured compound which was measured spectrophotometrically at 520 nm and expressed as mg/dl.[11] Haemoglobin (Hb) in both whole blood and erythrocyte lysate was measured as described by Drabkin and Austin. [12]

Statistical analyses

Data was analysed using SPSS–11.5 statistical package. Student st'test and chi-square testing was used. Values were expressed in mean SD , and differences were considered significant at $p<0.05$.

RESULTS

In this cross-sectional study, the prevalence of MS was 39%. The distributions of MS and NMS (Non-metabolic syndrome) with sex, ethnicity, lifestyle, exercise, smoking, alcohol intake is shown in Table-1. The association of MS with lifestyle and exercise was statistically significant, which indicated that higher incidences of MS were found in those with a sedentary lifestyle and non exercising subjects.

The comparison of anthropometric measurements, components of MS and other biochemical parameters in NMS and MS are shown in Table-2. All the components of MS were highly significant ($p< 0.001$). In addition MS Body Mass Index (BMI) than NMS group which was statistically significant. Total cholesterol and LDL were higher in MS group as compared to NMS group. But difference in LDL was highly significant between the NMS and MS group ($p<0.001$).

The comparison of antioxidants and lipid peroxidation in MS group and NMS group are shown in Table-3. Plasma MDA, Erythrocyte GPx and CAT activity were slightly increased while SOD was decreased in MS group as compared to NMS but was statistically not significant. Uric acid ($p=0.014$) and Catalase activity ($p=0.018$) were significantly higher in MS group as compared to NMS.

DISCUSSION

In the past few years there has been growing interest in the phenomenon of risk factor clustering that increases the "global risk" for atherosclerotic cardiac vascular diseases. One pattern of this clustering is amplified by Metabolic Syndrome, labeled as such because cardiovascular diseases risk factors that make up this pattern appear to be of metabolic origin. Moreover during the past few years, a large decline in cardiovascular disease mortality has been experienced in the west and substantial increases have been experienced in the developing countries.[16] These trends are expected to continue, with the developing countries experiencing double burden of both pre and post transitional disease. At the same time prevalence of MS also substantially increases and varies according to the population considered ranging from 8.8% to 14.3% in Europe and 22.6 % to 23.7% in United States.[1,16] In present study the prevalence of metabolic syndrome (39%) is higher than developed countries like Europe and United States, indicating that it is a syndrome not restricted to affluent countries only.[1]

The present investigations have established that the antioxidant is subnormal in subject exhibiting MS. Of the five criteria of MS defined in NCEP/ATP III four (notably hypertriglyceridemia, hypertension, hyperglycemia and abdominal obesity) are independently characterized by elevated systemic oxidative stress.[13]

Malondialdehyde is produced by peroxidative decomposition of polyunsaturated lipid. It is used as marker of free radical tissued amage and oxidative stress. In the present study MDA level was not statistically significant. But in similar study by Romero FG et al, there was significantly increased MDA level in MS but MDA alone is not associated with MS.[14] Erythrocyte GPx and CAT activity was increased but slight decrease in SOD activity was found in MS as compared to NMS. Superoxide dismutase (SOD) destroys free radical superoxides by converting it to hydrogen peroxide that is further decomposed by CAT or GPx. CAT plays an important role in the acquisition of tolerance to oxidative stress in adaptive response of cells. Since active GPx activity is dependent on GSH concentration increased GPx activity causes decreased reduced glutathione. Two vitaminic antioxidants α-Tocopherol and ascorbate act in synergism in the membrane and cytosol of the cell. α-Tocopherol scavenges lipid peroxy free radicals and interrupts the chain reaction of lipid peroxidation becoming oxidized itself in the process. Ascorbate present in the aqueous compartments (e.g. cytosol, plasma and other body fluids) act as a water soluble chain-breaking antioxidant, convert the tocopheroxyl radical back to active α-Tocopherol, there by replenishing antioxidant activity of α-Tocopherol.[15] The healthy subjects chosen in this study taking adequate diet may be helping to maintain normal homeostasis in relation to oxidative stress posed but sedentary lifestyle contributing to metabolic syndrome. A larger population study is needed to find correlation of oxidative stress with individual components of MS.

CONCLUSION

In conclusion, although prevalence of MS is higher but oxidative stress is not significantly increased suggesting that oxidative stress does not contribute much in the pathogenesis of MS in otherwise healthy subjects.

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Table 1. Distribution of MS on the basis of sex, ethnicity and other personnel habits

		Non Metabolic syndrome (n=72)	Metabolic syndrome (n=46)	p value
Sex	Male	58 (49.15%)	40 (33.89%)	0.36
	Female	14 (11.86%)	6 (5.08%)	
Ethnicity	Aryan	68 (57.62%)	39 (33.05%)	0.07
	Mongolian	4 (3.38%)	7 (5.93%)	
Life Style	Sedentary	12 (10.16%)	16 (13.55%)	0.02
	Non Sedentary	60 (50.84%)	30(25.42%)	
Exercise	Yes	60(50.84%)	32 (27.11%)	0.07
	No	12 (10.16%)	14 (11.86%)	
Diet	Vegetarian	10 (8.47%)	3 (2.54%)	0.21
	Non-vegetarian	62 (52.54%)	43 (36.44%)	
Smoking	Smokers	8 (6.77%)	8 (6.77%)	0.33
	Non Smokers	64 (54.23%)	38 (32.20%)	
Alcohol	Alcoholics	26 (22.03%)	24 (20.33%)	0.08
	Non alcoholics	46 (38.98%)	22 (18.64%)	

Table 2. Comparison of components of MS and other biochemical parameter in NMS and MS

Parameters	Non-metabolic syndrome (Mean ± SD)	Metabolic Syndrome (Mean ± SD)	p value
Age (year)	38.11±6.24	38.28±6.31	0.885
Weight (kg)	67.14 ± 9.89	70.13 ± 7.12	0.078
Height (cm)	164.45 ± 7.18	162.65 ± 6.74	0.175
Waist (cm)	88.0 ± 8.86	95.43 ± 9.77	0.001
Hip (cm)	99.18 ± 8.18	95.80± 12.89	0.084
BMI	24.79 ± 3.11	26.59 ± 3.14	0.002
SBP (mmHg)	118.38 ± 8.21	127.89 ± 10.55	0.001
DBP (mmHg)	78.22 ± 6.51	85.97 ± 11.79	0.001
Serum Glucose (mg/dl)	78.01 ± 12.36	92.91 ± 19.57	0.001
Serum HDL (mg/dl)	41.76 ± 5.44	38.43 ± 2.79	0.001
Serum Triglycerides (mg/dl)	156.76 ± 113.04	223.17 ± 75.97	0.001
Serum Cholesterol (mg/dl)	168.40 ± 30.94	171.37 ± 41.39	0.657
Hb (gm/dl)	14.65 ± 1.49	13.09 ± 1.47	0.001

Table 3. Comparison of Antioxidants and oxidants in NMS and MS group

Parameters	Non metabolic syndrome (Mean ± SD)	Metabolic Syndrome (Mean ± SD)	p value
Plasma MDA (nmol/ml)	3.66 ± 0.59	3.86 ± 0.66	0.081
Serum Bilirubin (mg/dl)	0.20 ± 0.07	0.23 ± 0.09	0.116
Serum Albumin (gm/dl)	4.44 ± 0.41	4.47± 0.26	0.594
Blood GSH (mg/dl)	29.67 ± 4.81	28.38 ± 2.84	0.102
Plasma Vitamin C (mg/dl)	1.19 ± 0.24	1.17 ± 0.16	0.664
Plasma Vitamin E (mg/dl)	1.00 ± 0.11	1.00 ± 0.15	0.757
Plasma Uric Acid (mg/dl)	6.03 ± 1.03	6.51 ± 1.04	0.014
Erythrocyte GPx (IU/gmHb)	45.93 ± 7.31	47.02 ± 6.12	0.403
Erythrocyte CAT (Unit/mgHb)	43.74 ± 7.42	46.73 ± 5.18	0.018
Erythrocyte SOD(IU/gmHb)	889.46 ± 152.04	882.14 ± 131.69	0.788

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