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ORIGINAL ARTICLE

BIOCHEMICAL AND GENETIC STUDIES ON CARDIOMETABOLIC SYNDROME

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ABSTRACT

Cardiometabolic syndrome is one of the major public health issues of this century which describes a cluster of clinical characteristics. Seventy two patients with coronary artery disease (CAD) and cardiometabolic syndrome and forty healthy age and sex matched normal controls were selected for this study. Detailed clinical epidemiological and anthropometric characteristics were noted. Lipid profile and Cytokinesis-block micronuclei (CBMN) assay using cytochalasin B were carried out in all the subjects. Serum total cholesterol, triglyceride and LDL-cholesterol was significantly higher and HDL cholesterol was significantly lower in patients compared to their normal counter-parts (P<0.05). CBMN frequency of the patients was significantly higher at all ages compared to their normal counter parts (P<0.05). Various risk factors like diabetes, hypertension, dyslipidemia, abdominal obesity, smoking and alcoholism were found influenced the CBMN frequency; but the changes were not significant. From this study it can be concluded that DNA damage was found to be higher in patients with cardiometabolic syndrome which may be attributed to the generation of free radicals associated with alcohol consumption, tobacco use, dyslipidemia and glucose intolerance and the accumulation of free radicals with increase in age.

KEY WORDS

Cardiometabolic syndrome, Metabolic syndrome, Coronary artery disease, DNA damage, Lipid profile, Cytokinesis-block micronuclei assay.

INTRODUCTION

Cardiometabolic syndrome is one of the major public health issues of this century which describes a cluster of clinical characteristics whose components vary considerably among different individuals and different racial and ethnic groups (1). Cardiometabolic 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 disease (2, 3).

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In 2001, the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) provided a new definition for the metabolic syndrome, according to which a person must have three of the following five abnormalities: abdominal adiposity, hypertension, hypertriglyceridemia, low high-density lipoprotein cholesterol, and elevated fasting glucose (4).

The pathogenesis of the metabolic syndrome is multifactorial, but insulin resistance, obesity and sedentary lifestyle, and unknown genetic factors interact in its occurrence leading to type 2 diabetes, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease (5). There is wide variation in both the occurrence of disease and age of onset, even in individuals who display similar risk profiles. Interplay between genetic determinants and environmental factors (still largely unknown) are the reason for this large inter-individual variation in disease susceptibility (6).

It has been proved that oxidative stress can provoke extensive oxidative DNA damage, DNA strand breaks and chromosomal aberrations. DNA damage has been found as an emerging risk factor which plays an important role in atherosclerosis and coronary artery disease (7). DNA damage is caused by multiple endogenous and exogenous factors such as oxidative stress, age, smoking, hypertension, hyperlipidemia and diabetes mellitus (8). DNA damage is increased in patients with metabolic syndrome. This may be due to the increase in the imbalance between the production of oxidants and antioxidant defenses in subjects with metabolic syndrome (9).

Most of the previous studies in CAD associated with metabolic syndrome were conducted either on biochemical risk factors or genetic damages. Very few attempts were made to evaluate the DNA damages and biochemical alterations associated with cardiometabolic syndrome. Hence the present study was under taken to asses the epidemiological and biochemical risk factors and to correlate the changes with DNA damages, if any, in cardiometabolic syndrome.

MATERIALS AND METHODS

Seventy two patients with coronary artery disease (CAD) and cardiometabolic syndrome having various risk factors classified by ATP III criteria referred from Hridayalaya, Institute for Preventive Cardiology and General Hospital, Trivandrum were selected for this study. Forty healthy, age and sex-matched subjects without cardiometabolic syndrome and CAD formed the control group. Detailed clinical, epidemiological and anthropometric characteristics were recorded using proforma. Eight ml of fasting venous blood was collected aseptically from all the subjects by venipuncture. Five ml of blood was allowed to clot, serum separated and total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol were estimated in automated clinical chemistry analyzer.

Total cholesterol was estimated by cholesterol oxidase - peroxidase (CHOD- PAP) method (10), Triglyceride by glycerol phosphate oxidase - peroxidase (GPO - PAP) method (11), HDL cholesterol by homogenous enzymatic colorimetric test (12) and LDL cholesterol by homogenous enzymatic colorimetric test (13). Lipid profiles were estimated twice in all subjects at one weak interval and if the value were with in the allowable limit of variation the mean values were taken.

Table 1: Distribution of Lipid profile of subjects

Test	Control subjects (n=40) mean±SD	Study subjects (n=72) mean±SD
Total Cholesterol (mg/dl)	183.9±17.3	259.3±27.1
Triglycerides (mg/dl)	136.2±14.2	179.6±41.6
HDL Cholesterol (mg/dl)	45.1±4.3	34.1±4.6
LDL Cholesterol (mg/dl)	128.8±11.5	181.2±28.1

The remaining three ml of blood was transferred aseptically in heparinized vacuutainers and used for lymphocyte separation and CBMN assay. Peripheral lymphocyte culture was performed as described by Moorhead et al (14). The CBMN test was done using the cytochalasin B technique described by Fenech (15). Lymphocyte cultures were prepared for each subject. Each culture contained 2.0 x 10^6 cells in 5 mL RPMI 1640 supplemented with 100 units/mL penicillin, 100 µg/mL streptomycin, 10% fetal bovine serum and 1% phytohemagglutinin. At 44 hours after initiation, cells were blocked in cytokinesis by adding cytochalasin B (Sigma, St. Louis, MO; final concentration, 4 µg/mL). The total incubation time for all cultures was 72 hours. After incubation, the cells were fixed in 3:1 methanol/glacial acetic acid, dropped onto clean microscopic slides, air-dried, and stained with Giemsa stain. For each sample, 1,000 binucleated cells were scored blindly at 100X magnification. The numbers of micronuclei per 1,000 binucleated cells were recorded. 't' test was performed using SPSS for analyzing the differences.

RESULTS

The age of the patients ranged from 34 to 60 years with a mean age of 46.5 years. There were forty five (62.5%) males and twenty seven (37.5%) females in the study groups. The age of the control subjects ranged from 27 to 55 years with a mean age of 40.7 years. Majority (96%) of the study subjects were consuming mixed type of food and only 4% (n=3) were on pure vegetarian diet. Coconut oil was preferred by 85% of the study subjects whereas the remaining 15% preferred sunflower oil.

The result of the Lipid profile assay is given in Table 1. Serum triglyceride, total cholesterol and LDL cholesterol were significantly higher in patients compared to their normal counter parts ($P<0.05$). The HDL cholesterol was significantly lower in patients in comparison to the control group ($P< 0.05$).

The distribution of CBMN frequency with progression of age is given in Table 2. There was a progressive elevation in CBMN frequency with increase in age in the study subjects but the elevation in CBMN frequency was not significant. The CBMN frequency of the patients was significantly higher at all ages compared to their normal counter parts ($P<0.05$).

Table 2: Distribution of CBMN frequency with age

Subjects	All Ages	Age range				
		≤ 40	41-45	46-50	51-55	56-60
CBMN Frequency of Study subjects (n=72)	15.4±2.26	14.27± 2.12	15.23±2.06	15.88 ±2.26	16 ± 2.04	16.4 ±2.2
CBMN Frequency of Controls (n=40)	10.8±1.16	10.0± 1.5	10.2 ±1.3	10.8 ±1.1	11.22± 0.9	-

Values are Mean±SD. Compared to the control group the CBMN frequency was higher in the patients at all age groups (P<0.05). In the age group of 56-60 years no normal subjects were available without CAD and metabolic syndrome.

Association of CBMN frequency with varying risk/life style factors is given in Table 3. The subjects belonging to urban centers showed higher CBMN frequency (15.7±2.12) than the subjects belong to rural area (14.6±1.403). Regarding the physical activity of subjects with sedentary lifestyle showed a CBMN frequency of 15.5±2.32 whereas subjects with non-sedentary lifestyle showed a mean frequency of 15±2.01. Pure vegetarians showed a CBMN frequency of 15±0.957 and the non vegetarian subjects showed a CBMN frequency of 15.4±2.33. Various risk factors like diabetes, hypertension, dyslipidemia, abdominal obesity, smoking and alcoholism in both the patients and control subjects were evaluated and compared with CBMN frequency. Marginal elevation in CBMN frequency was found to be associated with risk/lifestyle factors, but it was not significant. Smoking and dyslipidemia resulted in an increased CBMN frequency in all the subjects compared to other risk factors.

DISCUSSION

The prevalence of metabolic syndrome varies by the definition used and population studied (16). The NCEP ATP III definition of the metabolic syndrome is based on simple clinical and biochemical parameters, which could be easily measured in a clinic and or in a clinical laboratory. According to a recent study on south Indians, the prevalence of the metabolic syndrome was found to be 23.2, 18.3 and 25.8 according to the WHO, ATP III and IDF (International Diabetes Federation) definitions respectively. Prevalence of metabolic syndrome was higher in women using ATP III criteria (men 17.1%, women

Table 3: Distribution of CBMN frequencies with risk/life-style factors

Risk/Life style factors		Study subjects (n=72)		Control (n=40)	
		Number	CBMN Frequency	Number	CBMN Frequency
Alcohol	Yes	19	16.0±1.744	3	13.0±1.73
	No	53	15.2±2.32	37	10.81±1.01
Smoking	Yes	26	16.8±2.18	5	12.6±1.21
	No	46	14.9±2.52	35	10.74±1.03
Diabetes	Yes	48	15.7±2.367	4	12.0±0.95
	No	24	14.8±1.96	36	10.86±1.07
Hypertension	Yes	64	15.6±2.272	5	11.6±1.14
	No	8	14.6±1.56	35	10.89±1.08
Dyslipidemia	Yes	67	15.5±2.221	5	12.0±0.81
	No	5	13.3±1.682	35	10.83±1.08
Abdominal Obesity	High	55	15.4±2.364	4	11.25±0.57
	Normal	17	14.8±1.78	36	10.94±1.12
Physical activity	Sedentary	60	15.5±2.32	38	12.5±0.7
	Non-sedentary	12	15.0±2.01	2	10.89±1.05
Diet	Vegetarian	3	15.0±0.957	3	10.0±0.8
	Non-vegetarian	69	15.4±2.33	37	11.05±1.11
Socio-economic status	High	46	15.6±2.13	10	11.1±1.04
	Average/Low	26	15.0±1.29	30	10.6±1.26
Area of residence	Urban	45	15.7±2.12	16	11.18±1.06
	Rural	27	14.6±1.403	24	10.83±1.18

Values are Mean±SD

19.4%) and IDF criteria (men 23.1%, women 28.2%), but it was higher in men by WHO criteria (men 27.3%, women 19.7%) (17). In the present study, the prevalence of the disease was higher in males (62.5%) compared to females (37.5%). The high prevalence of CAD in males in the present study may be due to other risk factors such as smoking, alcohol consumption etc. The increase in CAD with progression of age observed in present study is in agreement with that of the previous studies (18).

Ghosh et al (19) reported that lipid profile i.e. low HDL-cholesterol, high LDL cholesterol, high total cholesterol, high triglycerides playing important role in its causation. Lahdenpera et al (20) observed that hypertriglyceridemia is an independent risk factor for coronary artery disease (CAD) in type 2 diabetes. In the present study elevated LDL cholesterol and triglyceride and decreased HDL cholesterol was observed in patients. This is well in agreement with all the above studies.

Several studies have showed that the prevalence of metabolic syndrome was significantly higher in smokers compared with those who never smoked (21, 22). In this study the subjects with smoking habit had a high mean CBMN frequency which is a high risk for the development of cardiometabolic syndrome.

Ramachandran et al (23) indicated that central obesity was the most important cardiovascular risk factor in urban south Indians. Balkau et al (24) observed that metabolic syndrome and all cardiometabolic risk factors had a positive correlation with increasing waist. Wang et al (25) reported that the overall abdominal adiposity is a strong and independent risk factor for type II diabetes and metabolic syndrome. In the present study abdominal obesity was found in majority of the subjects.

Epidemiological studies (26-29) had shown that the features of metabolic syndrome can be traced even up to 5–8 years before the diagnosis of the type 2 diabetes. Once the diabetes develops, it would further increase the risk of developing CAD and the mortality due to CAD. In this study the majority of the metabolic syndrome subjects with CAD were diabetic.

Mahadik et al (30) observed that the percentage prevalence of abdominal obesity and hypertriglyceridemia was significantly higher in the urban population compared to the rural population. In the present study the subjects from the urban area had a high risk of developing the cadriometabolic syndrome. So it can be suggested that urbanization and change in life style can mediate the progress of cardiometabolic syndrome.

Fan et al (31) observed a positive correlation between lifetime average drinking intensity and the prevalence of metabolic syndrome. In this study also there is a positive correlation between alcoholic consumption and the cardiometabolic syndrome. The prevalence of metabolic syndrome in hypertensive patients of this study was high which is in agreement with that of Wei-ju et al (32).

DNA damage is the initiation step of diseases of genetic origin. Nishtha et al (33) observed that extent of DNA damage is more in diabetic rabbits as compared to the non-diabetic or antioxidant supplemented group. Abnormal metabolic parameters and their correlation with DNA damage, suggest the risk of development of metabolic syndrome in diabetic group. A possibility of repression of this risk by antioxidants and their ability to counteract oxidative stress may prevent DNA damage. According to Demirbag et al (9) DNA damage is increased in patients with metabolic syndrome. The increase in DNA damage might be occurring because of the increase in the imbalance between the production of oxidants and antioxidant defenses in subjects with metabolic syndrome. Satoh et al (34) observed that oxidative DNA damage in CAD patients with metabolic syndrome was higher than in those without metabolic syndrome. Metabolic syndrome induces an increase in oxidative stress and may be an important contributory factor for coronary artery disease (CAD).

This study clearly indicates that there is an increase in DNA damage in cardiometabolic syndrome which leads to CAD. This may be attributed to the generation and accumulation of free radicals due to risk factors with increase in age. Marginal elevation in DNA damage, as assessed by CBMN frequency with risk/life style factors observed in the present study was not significant. Life style modification with diet and exercise, maintaining blood pressure at normal level, lowering serum lipids and blood sugar and avoiding tobacco and alcohol will reduce the risk of metabolic syndrome and CAD.

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REFERENCES

1. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005; 112: 2735-52.

2. Govindarajan G, Whaley-Connell A, Mugo M, Stump C, Sowers JR. The cardiometabolic syndrome as a cardiovascular risk factor. Am J Med Sci 2005; 330(6): 311-18
3. Pandey S, Baral N, Majhi S, Acharya P, Karki P, Shrestha S, et al. Prevalence of the metabolic syndrome in acute myocardial infarction and its impact on hospital outcomes. Int J Diab Dev Ctries 2009; 29: 52-55
4. Frisard MI, Rood JC, Fang X, Su J, Welsh DA, Jazwinski SM, Ravussin E Metabolic syndrome and risk factors for cardiovascular disease: are nonagenarians protected? Age (Dordr) 2009; 31(1): 67-75.
5. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005; 365:1415-28.
6. Andreassi MG. Metabolic syndrome, diabetes and atherosclerosis: influence of gene-environment interaction.Mutat Res 2009; 667 (1-2):35-43.
7. Botto N, Mssetti S, Petrozzi L. Elevated levels of oxidative DNA damage in patients with coronary artery disease. Coron Artery Dis 2002; 13:269-74.
8. Andreassi MG. Coronary atherosclerosis and somatic mutations: an overview of the contributive factors for oxidative DNA damage. Mutat Res 2003; 543: 67-86.
9. Demirbag R, Yilmaz R, Gur M, Celik H , Guzel S , Selek S et al. DNA damage in metabolic syndrome and its association with antioxidative and oxidative measurements. Int J Clin Pract 2006; 60: 1187-93.
10. Rifai N, Bachorik PS, Albers JJ. Lipids,lipoprotein and apolipoprotein. In Burtis CA,Ashwood R.,editors. Tietz textbook of clinical chemistry 3rd ed. Philadelphia. W.B. Saunders company. 1999: 806-61.
11. Mc Gowan MW, Artiss JD, Standbergh DR, Zark B. A peroxidase coupled method for the colorimeteric determination of serum triglycerides. Clin Chem 1983; 29 (3): 538-42.
12. Sugiuchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara N, et al. Direct measurement of High- Density Lipoprotein Cholesterol in serum with polyethylene glycol- modified enzymes and sulphated alpha- cyclodextrin. Clin Chem 1995; 41:717-23.
13. Armstrong V, Seidel D. Evaluation of a commercial kit for the determination of LDL- cholesterol in serum based on precipitation of LDL with dextran sulphate. Arztl Lab 1985; 31: 325-30.
14. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res1960; 20: 613-6.
15. Fenech M. The cytokinesis-block micronucleus technique and its application to genotoxicity studies in human populations. Environmental Health Perspectives 1993; 101(3):101-07.
16. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome in U.S. adults. JAMA 2002; 287: 356-59.
17. Deepa M, Farooq S, Datta M, Deepa R, Mohan V. Prevalence of metabolic syndrome using WHO, ATP III, and IDF definitions in Asian Indians: the Chennai Urban Rural Epidemiology Study (CURES-34). Diabetes Metab Res Rev 2007; 23: 127-34.
18. Abbott RD, Curb JD, Rodriguez BL, Masaki KH, Yano K, Schatz IJ, et al. Age-related changes in risk factor effects on the incidence of coronary heart disease. Ann Epidemiol 2002; 12: 173-81.
19. Ghosh J, Mishra T K, Rao Y N, Aggarwal S K. Oxidised LDL, HDL cholesterol, LDL cholesterol levels in patients of coronary artery disease. Ind J Clin Biochem 2006; 21: 181-4.
20. Lahdenpera S, Syvanne M, Kahri J, Taskinen MR. Regulation of low-density lipoprotein particle size distribution in NIDDM and coronary disease: importance of serum triglycerides. Diabetologia 1996; 39(4):453-61.
21. Ishizaka N, Ishizaka Y, Toda E, Hashimoto H, Nagai R, Yamakado M. Association between cigarette smoking, metabolic syndrome, and carotid arteriosclerosis in Japanese individuals. Atherosclerosis 2005; 181: 381-88.
22. Nakanishi N, Takatorige T, Suzuki K. Cigarette smoking and the risk of the metabolic syndrome in middle-aged Japanese male office workers. Ind Health 2005; 43: 295-301.
23. Ramachandran A, Snehalatha C, Satyavani K, Weiss R, Dziura J, Burgert TS. Metabolic syndrome in urban Asian Indian adults: a population study using modified ATP III criteria. Diabetes Res Clin Pract 2003; 60: 199-204.
24. Balkau B, Picard P, Vol S, Fezeu L and Eschwège E. Consequences of Change in Waist Circumference.Diabetes Care 2007; 30 (7): 1901-03.
25. Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicing risk of Type 2 diabetes among man. Am J Clin Nutr 2005; 81:555-63.
26. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? JAMA 1990; 263: 2893-8.
27. McPhillips JB, Barrett-Connor E, Wingard DL. Cardiovascular disease risk factors prior to the diagnosis of impaired glucose tolerance and non-insulin-dependent diabetes mellitus in a community of older adults. Am J Epidemiol 1990; 131: 443-53.
28. Mykkanen L, Kuusisto J, Pyorala K, Laakso M. Cardiovascular disease risk factors as predictors of type 2 (non-insulin-dependent) diabetes mellitus in early subjects. Diabetologia 1993; 36: 553-59.
29. Zimmet PZ, Alberti KGMM. The changing face of macrovascular disease in non-insulin-dependent diabetes mellitus: an epidemic in progress. Lancet 1997;350 (1):1-4.
30. Mahadik SR, Deo SS, Mehtalia SD. Increased prevalence of metabolic syndrome in non-obese Asian Indian-an urban-rural comparison. Metab Syndr Relat Disord 2007; 5(2): 142-52
31. Fan AZ, Russell M, Dorn J, Freudenheim JL, Nochajski T, Hovey K, et al. Lifetime alcohol drinking pattern is related to the prevalence of metabolic syndrome. The Western New York Health Study (WNYHS). Eur J Epidemiol 2006; 21:129-38.
32. Wei-ju L, Hao X, Kai S, Xiao-dong S, Yi-bo W, Yi-song Z, et al Cardiovascular risk and prevalence of metabolic syndrome by differing criteria. Chin Med J 2008; 121: 1532-36.
33. Nishtha J, Imrana N, Jamal A. Evaluation of DNA damage and metabolic syndrome parameters in diabetic rabbits supplemented with antioxidants. Fundamental Clin Pharmacol 2009; 23 : 197-205
34. Satoh M, Ishikawa Y, Takahashi Y, Itoh T, Minami Y, Nakamura M Association between oxidative DNA damage and telomere shortening in circulating endothelial progenitor cells obtained from metabolic syndrome patients with coronary artery disease. Atherosclerosis 2008; 198(2): 347-53.