Antioxidant Status in Patients with Metabolic Syndrome as Measured by the Stable Free Radical Diphenylpicrylhydrazyl Assay

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Abstract

Objective: Oxidative stress is involved in the pathophysiology of diabetes and cardiovascular complications of metabolic syndrome. The main objective of this study was to evaluate total antioxidant status by diphenylpicrylhydrazyl (DPPH)-scavenging activity in patients developed metabolic syndrome (MetS).

Materials and Methods: Forty-four patients with MetS diagnosed on the basis of adult treatment panel (ATPIII) criteria along with 46 age- and gender-matched healthy controls were studied. A blood sample was taken after a 12-hour fasting period, and blood glucose, lipid profile, and DPPH were determined.

Results: A significant decrease (p=0.03) in DPPH-scavenging activity levels in the study group was observed compared to the control group. Among the components of metabolic syndrome, hyperglycaemia, hypertriglyceridaemia and hypertension were negatively correlated with DPPH-scavenging activity levels.

Conclusions: The findings of the present study suggest the oxidative stress in patients with MetS which further increases the cardiovascular risk and diabetes mellitus in these patients. We posited that studying of the oxidative status is crucial in order to prevent type 2 diabetes development and cardiovascular disease and its complications because it is installed long before the disease actually appears.

Keywords: Oxidative stress, DPPH-Scavenging activity, Metabolic syndrome

Introduction

Metabolic syndrome comprises various disorders, such as hypertension, obesity, dyslipidaemia and hyperglycaemia (1,2). Metabolic syndrome has been demonstrated as a common precursor to the development of diabetes mellitus (DM) and cardiovascular disease (CVD). The risk of DM and CVD is five and two fold in patients affected by metabolic syndrome, respectively (3). It has also been connected with obesity and inactive lifestyle, both of which are modifiable (4). Oxidative stress is involved in the pathophysiology of diabetes and cardiovascular complications of

MetS. Four out of five criteria of MetS defined in ATPIII, namely, hypertriglyceridaemia hypertension, hyperglycaemia, and abdominal obesity are independently characterized by elevated systemic oxidative stress (5). Oxidative stress results from disturbed balance between prooxidants and antioxidants and plays a role in pathophysiology of DM and CVD. A number of factors of MetS, such as hyperglycemia may cause increased production of reactive oxygen species (ROS). There are very few publications (6-8) which have studied the total antioxidant capacity as an index of antioxidant defense in patients with MetS with incompatible results. The present study was conducted to compare the oxidative stress, including total antioxidant status in patients with MetS and healthy ones.

Materials and Methods

A case-control study was conducted in Abarkooh, Iran in 2013. In this study, ninety 30-50 years old subjects were selected. Forty-four patients with MetS were compared with forty-six age- and gender-matched healthy controls. MetS patients were chosen from Abarkooh Khatamal Anbia hospital. As inclusion criteria, we used MetS guidelines provided by the adult treatment panel (ATPIII) (9): Waist circumference (>102cm for men and >88cm for women), triglycerides (TG) (>150mg/dl), high-density lipoprotein cholesterol (HDL-c) (<40mg/dl for men and <50mg/dl for women), blood pressure (>130/85 mmHg, at least 3 times at rest), and fasting blood glucose (FBS) concentrations (>110mg/dl). Using vitamin supplements, history of smoking, alcoholism, chronic kidney/liver disease, and malignancy were used as exclusion criteria. Written informed consent was obtained from all and anthropometric measurements of all participants, including height, weight, waist circumference, and systolic and diastolic blood pressures were recorded. After overnight fasting, 5ml of peripheral venous blood sample was collected. The samples were then centrifuged at 3000rpm for 10 minutes. TG, HDL-c and FBS immediately were measured, but the remaining separated serum was stored at -20^0C until DPPH-scavenging activity measurement. FBS (oxidase-peroxidase method, Pars Azmoon Co., Tehran, Iran), TG (glycerol phosphate oxidase-peroxidase method, Pars Azmoon Co., Tehran, Iran) and HDL-c (immunoinhibition method, Pars Azmoon Co., Tehran, Iran) were analyzed using on fully automated analyzer (Sanjesh Tajhiz, Isfahan, Iran).

Serum DPPH radical-scavenging activity was measured by DPPH reduction assay as follows (10): Briefly, 0.1 mL of deproteinized serum (with adding 100µl acetonitrile solution to serum and centrifuging for 5min) in acetate buffered solution (10µM, pH=7.8) was incubated in the methanolic solution of DPPH (0.1mM). After 30 minutes in room temperature, the absorbance at 517nm was measured (Epoch, England). Measurements were tetraplicated finally the free radical DPPH-scavenging activity calculated by following formula: Activity [% of DPPH reduction] = [(A−Ax)/A] ×100%, where A and Ax stand for the absorbance of DPPH solution with methanol, and the absorbance of a DPPH solution with serum, respectively.

Statistical Analysis

Data were checked by the Kolmogorov-Smirnov test for normal distribution. For men and women, a student t-test or Mann-Whitney nonparametric test was used to compare between control and MetS participants for normal or non-normal distribution, respectively. P-value less than 0.05 was considered statistically significant. Correlation of DPPH-scavenging activity with components of MetS was assessed by Spearman's rank correlation analysis. Statistical analyses were done by using SPSS for windows, version 16.

Results

The mean±standard deviation (SD) of the age of the patients with MetS and control group were 41.45±6.27 and 39.67±5.94 years (p=0.170), respectively. There were no

differences in age between groups. Table 1 shows comparison of metabolic syndrome components in MetS patients and control group. DPPH-scavenging activity and blood pressure had not normal distribution. So, Mann-Whitney analysis conducted to compare related data between the groups. This test shows significant difference in terms of DPPH-scavenging activity levels (p=0.03), systolic blood pressure (SBP) (p<0.001) and diastolic blood pressure (DBP) (p<0.001) between the group (Table 2). Spearman's rank correlation analysis showed significant correlations between DPPH-scavenging activity levels and FBS (r=-0.245, p=0.020), TG (r=-0.327, p=0.002), SBP (r=-0.289, p=0.006) and DBP (r=-0.248, p= 0.018) (Table 3).

Discussion

Recent evidences have shown the association between oxidative stress and development of metabolic syndrome (11,12). The main objective of this study was to evaluate total antioxidant status by DPPH-scavenging activity in subjects developed MetS.

In the present study, DPPH-scavenging activity levels were found to be significantly lower in the MetS individuals when compared to the control group (p=0.03). DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol (13). This free radical,

Table 1. Comparison of the components of metabolic syndrome except blood pressure in the study and control groups

Parameters	MetS group n=44	Control group n=46	p-value
Waist circumference (cm)	105.91 ± 11.56	93.24 ± 11.97	< 0.001
FBS (mg/dl)	111.86 ± 29.1	84.74 ± 10.70	< 0.001
HDL-c (mg/dl)	46.82 ± 13.26	58.02 ± 11.73	< 0.001
TG (mg/dl)	203.09 ± 92.6	103.02 ± 40.5	<0.001

All data are expressed as mean ± standard deviation.
MetS: Metabolic syndrome; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; HDL-c: high-density lipoprotein cholesterol; TG: triglycerides.

Table 2. Percentile of systolic and diastolic blood pressure, and DPPH-scavenging activity in metabolic syndrome patients and control group.

Groups	SBP (mmHg)			DBP (mmHg)			DPPH-scavenging activity (%)		
	Percentile 25	Percentile 50	Percentile 75	Percentile 25	Percentile 50	Percentile 75	Percentile 25	Percentile 50	Percentile 75
MetS	120	130	135	80	85	90	-10.441	2.500	16.323
Control	110	115	120	70	80	80	0.221	12.206	21.985
p-value*		<0.001			<0.001			0.03	

MetS: Metabolic Syndrome ; SBP: systolic blood pressure; DBP: diastolic blood pressure
*Mann–Whitney test

Table 3. Correlation coefficient between DPPH-scavenging activity with components of metabolic syndrome.

Parameters	DPPH-Scavenging Activity Correlation coefficient (r*)	p-value
Systolic Blood Pressure (mmHg)	-0.289	0.006
Diastolic Blood Pressure (mmHg)	-0.248	0.018
WC (cm)	-0.026	0.809
FBS (mg/dl)	-0.245	0.020
TG (mg/dl)	-0.327	0.002
HDL-c (mg/dl)	0.078	0.466

*Spearman's Correlation
SBP: systolic blood pressure; DBP: diastolic blood pressure; WC: waist circumference.
FBS: fasting blood sugar; TG: triglycerides HDL-c: high-density lipoprotein cholesterol.

constant at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. Findings from the Third National Health and Nutrition Examination review show a significant decrease in the serum levels of antioxidants in patients with metabolic syndrome (14,15).

In the present study, we found a negative correlation between FBS levels with DPPH-scavenging activity levels (p=0.02) (Table 3). There are several studies showing accumulation of protein and lipid oxidative products in diabetic patients, increased levels of circulating oxidative stress markers and reduced antioxidant defenses (16,17). The increased intracellular metabolism of glucose in hyperglycemic state induces overproduction of NADH and FAD, results in ATP production by the electron transport chain. Increase of NADH enhances the mitochondrial proton gradient, and as a result, electrons are transferred to oxygen, generating superoxide in some complexes of electron transport chain (18).

Also, dyslipidaemia component of metabolic syndrome further contributes to this oxidative stress. Hypertriglyceridaemia seen in these patients, acts as a basis of free-fatty acids which are the substrate for ROS. Our findings of a significant negative correlation between TG and DPPH-scavenging activity **(**p=0.002) supports this view. Our results are in accordance with Van Guilder et al. study which showed significant increase in triglyceride concentration in patients with metabolic syndrome (19).

Hypertension is a cause as well as effect of oxidative stress (20). Hypertension as a result of reduced bioavailability of nitric oxide, causes converting of nitric oxide to peroxynitrite. Also, endothelial nitric oxide synthase (eNOS) can undergo uncoupling in the presence of peroxynitrite which is diverted towards lipid peroxidation (21). In the present study, we found a negative correlation between systolic and diastolic blood pressure with DPPH-scavenging activity **(**p=0.006 and p=0.018 respectively; Table 3).

The findings of the present study show that the individual components of MetS, especially hyperglycemia, hypertriglyceridaemia and hypertension are related to oxidative stress. This is in agreement with previous studies (15,22,23). However, some studies (24) showed the minimal contribution of components of metabolic syndrome in oxidative stress. As the aim of the present study was to realize the utility of measurement of the antioxidant capacity, we did not estimate the interactions of the influencing components in producing oxidative stress.

The findings of the present study amplified the role of oxidative stress in the MetS as indicated by decreased DPPH-scavenging activity levels.

However, this study has some limitations. The first, its cross-sectional design and the inherent potential that genetic and lifestyle factors may have affected the results of our comparisons. However, we attempted to minimize the influence of lifestyle behaviors by studying subjects of similar age who were non-smokers, not currently taking medication that could influence inflammatory and oxidative markers, not having underlying cardiovascular and metabolic diseases, and were similar in habitual physical activity. Due to its cross-sectional design without longitudinal follow-up, we could not define a causal relationship between DPPH-scavenging activity and the components of metabolic syndrome. Second, the study was performed in younger white subjects, and the association between DPPH-scavenging activity and the variables of MetS may be different in elder individuals and other races.

In conclusion, we observed that decreased serum concentration of total antioxidant levels as measured by DPPH-scavenging activity is associated with some components of MetS, such as hyperglycemia, hypertriglyceridaemia, and hypertension. Future studies are needed to clarify the mechanism responsible for the oxidative stress in the risk of metabolic syndrome.

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References

1. Ford ES. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. Diabetes Care 2005;28:2745-9.
2. Laaksonen DE, Lakka HM, Niskanen LK, Kaplan GA, Salonen JT, Lakka TA. Metabolic syndrome and development of diabetes mellitus: ap - plication and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study. Am J Epidemiol 2002;156:1070-7.
3. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735-52.
4. Sarrafzadegan N, Kelishadi R, Baghaei A, Hussein Sadri G, Malekafzali H, Mohammadifard N, et al. Metabolic syndrome: an emerging public health problem in Iranian women: Isfahan Healthy Heart Program. Int J Cardiol 2008;131:90-6.
5. Grattagliano I, Palmieri VO, Portincasa P, Moschetta A, Palasciano G. Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. J Nutr Biochem 2008;19:491-504.
6. Leiva E, Mujica V, Sepúlveda P. High Levels of Iron Status and Oxidative Stress in Patients with Metabolic Syndrome. Biol Trace Elem Res 2013;151:1–8
7. Kwak HK, Lee MS, Lim SY,Yoon S. Relationship between Ferric Reducing Antioxidant Power and Metabolic Risk Factors in Korean Women Living in Seoul. Korean J Community Nutr 2008;13:91-9.
8. Veigas NM, Dharmalingam M, Marcus SR. Oxidative stress in obesity and metabolic syndrome in Asian Indians. J Med Biochem 2011;30:115-20.
9. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005;365:1415-28.
10. Janaszewska A, Bartosz G. Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma. Scand J Clin Lab Invest 2002;62:231–6.
11. Holvoet P, Lee DH, Steffes M, Gross M, Jacobs DR. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. JAMA 2008;299:2287–22.
12. Furukawa S, Fujita T, Shimabukuro T, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I . Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–1761.
13. Huang DJ, Ou BX, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005;53:1841-1856.
14. Ford ES, Mokdad AH, Giles WH, Brown DW. The metabolic syndrome and antioxidant concentrations: findings from the Third National Health and Nutrition Examination Survey. Diabetes 2003;52:2346-52.
15. Beydoun MA, Shroff MR, Chen X, Beydoun HA, Wang Y, Zonderman AB. Serum antioxidant status is associated with metabolic syndrome among US adults in recent national surveys. J Nutr 2011;141:903-13.
16. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol, 2004;24:816-23.
17. Jay D, Hitomi H, Griendling K.K. Oxidative stress and diabetic cardiovascular complications. Free Radic Biol Med 2006;40:183-92.
18. Grattagliano I, Palmieri V.O, Portincasa P, Moschetta A, Palasciano G. Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. J Nutr Biochem 2008;19:491-504.
19. Van Guilder G.P, Hoetzer G.L, Greiner J.J, Stauffer B.L, Desouza Ch.A. Influence of Metabolic Syndrome on Biomarkers of Oxidative Stress and Inflammation in Obese Adults. Obesity 2006;14: 2127-31.
20. Alexander RW. Theodore Cooper Memorial Lecture. Hypertension and the pathogenesis of atherosclerosis. Oxidative stress and the mediation of arterial inflammatory response: a new perspective. Hypertension 1995;25:155-61.
21. Kalinowski L, Malinski T. Endothelial NADH/NADPH-dependent enzymatic sources of superoxide production: relationship to endothelial dysfunction. Acta Biochim Pol 2004;51:459-69.
22. Vander Zwan LP, Scheffer PG, Dekker JM, Stehouwer CD, Heine RJ, Teerlink T. Hyperglycemia and oxidative stress strengthen the

association between myeloperoxidase and blood pressure. Hypertension 2010;55:1366-72.
23. Aparna R, Bitla N. Manasa Kumari N. Antioxidant status in patients with metabolic syndrome as measured by ferric reducing ability of plasma (FRAP) assay. J Clin Sci Res 2012;3:114-20.
24. Abdilla N, Tormo MC, Fabia MJ, Chaves FJ, Saez G, Redon J. Impact of the components of metabolic syndrome on oxidative sress and enzymatic antioxidant activity in essential hypertension. J Hum Hypertens 2007;21:68-75.