

OXIDATIVE STRESS IN OBESITY AND METABOLIC SYNDROME IN ASIAN INDIANS

OKSIDATIVNI STRES U GOJAZNOSTI I METABOLIČKOM SINDROMU KOD INDIJACA

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Summary: Oxidative stress is associated with the individual components of metabolic syndrome and has been implicated in the development of complications of these metabolic disorders. In this study oxidative stress levels have been compared in obese Indians (a high-risk population for diabetes and cardiovascular disorders) with and without metabolic syndrome. 30 adult normotensive, normoglycemic obese subjects and 35 adults with metabolic syndrome of either sex with BMI >23 kg/m² were compared with 30 adult, healthy volunteers with BMI <23 kg/m². Anthropometric parameters, blood pressure, biochemical parameters, hydroperoxides levels and total antioxidant capacity were estimated. The obese groups with and without metabolic syndrome had significantly increased anthropometric parameters like waist circumference and index of central obesity and aqueous phase hydroperoxides when compared with normal controls. The metabolic syndrome group also had significantly increased blood sugar levels, lipid profile and hydroperoxide levels when compared to obese or control groups. There was no alteration in the total antioxidant capacity in any of the groups. The Triglyceride/HDL-Cholesterol ratio (>3), a surrogate marker of insulin resistance, indicates insulin resistance in the metabolic syndrome group. The anthropometric profile, insulin resistance and oxidative stress seen in obesity are further elaborated in metabolic syndrome. Thus, the early identification of high-risk individuals based on anthropometric parameters, lipid profile, insulin resistance and indices of oxidative stress may help to prevent the development of complications of metabolic syndrome.

Keywords: obesity, metabolic syndrome, oxidative stress, insulin resistance, hydroperoxides, cardiovascular disorders

Kratak sadržaj: Oksidativni stres dovodi se u vezu sa pojedinačnim komponentama metaboličkog sindroma i povezan je sa razvojem komplikacija u metaboličkim poremećajima. U ovoj studiji upoređeni su nivoi oksidativnog stresa kod gojaznih Indijaca (populaciji sa visokim rizikom za razvoj dijabetesa i kardiovaskularnih poremećaja) sa i bez metaboličkog sindroma. Trideset odraslih normotenzivnih, normoglikemičnih gojaznih ispitanika i 35 odraslih osoba sa metaboličkim sindromom oba pola sa ITM >23 kg/m² poređeno je sa 30 odraslih zdravih dobrovoljaca sa ITM <23 kg/m². Ispitivani su antropometrijski parametri, krvni pritisak, biohemijski parametri, nivoi hidroperoksida i ukupni antioksidantni kapacitet. U gojaznim grupama sa i bez metaboličkog sindroma antropometrijski parametri kao što su obim struka i indeks centralne gojaznosti i aqueous phase hidroperoksidi bili su značajno povišeni u poređenju sa kontrolnim subjektima. Grupa sa metaboličkim sindromom takođe je imala značajno povišene nivoe šećera u krvi, lipidni profil i nivoe hidroperoksida u poređenju sa gojaznom ili kontrolnom grupom. Ni u jednoj grupi nije bilo promena u ukupnom antioksidantnom kapacitetu. Odnos trigliceridi/HDL holesterol (>3), kao surogat marker insulinske rezistencije, ukazuje na rezistenciju na insulin u grupi sa metaboličkim sindromom. Antropometrijski profil, insulinska rezistencija i oksidativni stres prisutni u gojaznosti dalje se razvijaju u metaboličkom sindromu. Otud rana identifikacija osoba sa visokim rizikom na osnovu antropometrijskih parametara, lipidnog profila, insulinske rezistencije i indeksa oksidativnog stresa može doprineti sprečavanju razvoja komplikacija metaboličkog sindroma.

Ključne reči: gojaznost, metabolički sindrom, oksidativni stres, insulinska rezistencija, hidroperoksidi, kardiovaskularni poremećaji

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Introduction

Indians are a population with a greater predisposition towards diabetes and cardiovascular disorders (CVD) at a younger age, probably due to insulin resistance (IR) and its consequences (1). Although the

cause of IR and endothelial dysfunction is not clear, several genetic and environmental factors have been implicated in their development (2).

Metabolic syndrome, a constellation of glucose intolerance, IR, dyslipidemia, hypertension together with visceral obesity as the central and/or causal component, is a marker of increased cardiovascular risk (2). Obesity, characterised by energy imbalance, arises from excessive calorie intake, lifestyle modifications and genetic factors affecting the susceptibility to environmental changes (3). The accumulation of fat (visceral fat) in the body alters both the endocrine and inflammatory responses of the adipose tissue resulting in changes in the systemic physiology (3).

Oxidative stress and chronic low-grade inflammation associated with obesity and metabolic syndrome probably contribute to their progression to obesity-induced metabolic disorders (4). IR is generally considered to be the main pathogenic mechanism underlying metabolic syndrome; however, evidence for the role of oxidative stress as a link between the components of metabolic syndrome and its prognosis have been reported (5, 6). Furukawa et al. (7) have observed a correlation between oxidative stress and fat accumulation and proposed the role of oxidative stress in the development of obesity-associated metabolic syndrome. Further, oxidative stress has been observed in all the individual components of metabolic syndrome (all independent risk factors for CVD) and also with the onset of CV manifestations of metabolic syndrome (8).

Several markers of oxidative stress including products of oxidative damage and antioxidant levels have been studied (8). In the present study, hydroperoxide levels (both water-soluble and lipid soluble) have been estimated using ferrous oxidation-xylenol orange (FOX) methods (9) and total antioxidant capacity (TAC) by the ferric reducing ability of plasma (FRAP) assay (10) in obese patients with and without metabolic syndrome and healthy controls, to assess the levels of oxidative stress in the three groups of Indians.

Materials and Methods

Adult subjects attending the Endocrine Clinic of a Tertiary Care Hospital in Bangalore, South India, were recruited after informed consent. The study protocol was approved by the Ethics Review Board of the Institution.

The subjects of either sex aged between 25 and 50 years were divided into three groups: Group I (controls): 30 adult non-obese healthy volunteers with BMI <23 kg/m². Group II (obesity): 30 adult normotensive, normoglycemic obese subjects with BMI >23 kg/m² as cut off for obesity as per WHO standards for Asians (12). Group III (metabolic syndrome): 35 adults with

BMI > 23 kg/m² and metabolic syndrome (IDF criteria – central obesity, hyperglycemia and hypertension/dyslipidemia) (13).

The inclusion criteria were: subjects between 25 and 50 years of age of either sex diagnosed as simple obesity without metabolic syndrome (Group II) and with obesity, hyperglycemia and hypertension/dyslipidemia (Group III).

The exclusion criteria included: subjects with diabetes mellitus and other endocrine disorders, systemic disorders like hypertension, ischemic heart disease, asthma (Group II); subjects with secondary endocrine disorders (Group III). Smokers, tobacco users, alcoholics, those on other medication like vitamins, steroids and antioxidants and subjects with acute illness or chronic inflammatory conditions were excluded from the study.

A detailed clinical examination and family history was taken of all the subjects. Blood Pressure (BP) was measured by standard methods. Anthropometric measurements including height, weight, waist (WC) and hip circumferences (HC) were measured as per standard procedures. BMI, waist: hip ratio (WHR) and index of central obesity (ICO) (WC/height) (14) were calculated.

Analytical Methods

Blood samples were drawn, after a 12-hour overnight fast, for the determination of fasting blood glucose (FBS) (Enzymatic kit, Vital Diagnostics Pvt. Ltd, Mumbai, India), triglycerides (TG) (Enzymatic kit, Vital Diagnostics Pvt. Ltd., Mumbai), total cholesterol (TC) (Enzymatic kit, Biosystems, S.A. Barcelona, Spain) and HDL-cholesterol (HDL-C) (Bayer Diagnostics, Vadodara). VLDL-cholesterol (VLDL-C) and LDL-Cholesterol (LDL-C) were calculated using Friedwald's equation. The hydroperoxide levels (water and lipid-soluble hydroperoxides) were estimated by ferric-xylenol orange (FOX) methods (10) and total antioxidant capacity (TAC) by the ferric reducing ability of plasma (FRAP) assay (11).

Statistical Analysis

The data are presented as mean \pm SD. Statistical analysis was done using the SPSS version 11 software. Analysis of variance (ANOVA) was used for the comparison of the three groups. Multiple comparisons by LSD and Chi-square test were done. Pearson's coefficient was used for the correlation and regression analysis. $P < 0.05$ was taken as significant.

Results

The anthropometric parameters such as height, weight, WC, HC, BMI, WHR and ICO and BP of the

3 groups are shown in *Table I*. While the BMI was significantly increased ($P < 0.001$) in both groups II and III when compared with the control group I, the WHR was significantly increased ($P < 0.01$) in group III when compared with the control group I. The significant increase ($P < 0.001$) in WC in both groups II and III is representative of visceral obesity. Another marker of visceral obesity, ICO, is also significantly elevated both in groups II and III when compared to group I.

The biochemical parameters – FBS, lipid profile, hydroperoxide levels and total antioxidant capacity

are presented in *Tables II* and *III*. There was a significant increase in FBS ($P < 0.001$), TG ($P < 0.001$), VLDL-C ($P < 0.001$), TC ($P < 0.05$) and water-soluble hydroperoxide levels ($P < 0.001$) in group III when compared to group I. In group II, a significant decrease in FBS ($P < 0.001$), TG ($P < 0.001$) and VLDL-C ($P < 0.001$) levels in comparison to group III was observed. The water-soluble hydroperoxides were significantly elevated in the obese group II (when compared to group I controls) and in the metabolic syndrome group III (when compared to the obese group II) (*Table III*). There was no alteration in the

Table I Anthropometric characteristics of Group I (controls), Group II (obesity) and Group III (Metabolic syndrome) (Mean \pm SD).

Parameter	Group I, n=30	p-value (I vs II)	Group II, n=30	p-value (II vs III)	Group III, n = 35	p-value (I vs III)
Age, yrs	32.33 \pm 7.56	–	37.23 \pm 7.4	–	45.57 \pm 5.93	
Sex, M/F	9/21	–	5/25	–	15/20	
Weight, kg	53.18 \pm 10.24	0.000	76.44 \pm 16.24	–	74.15 \pm 11.78	0.000
Height, cm	160 \pm 12.0	–	159 \pm 9.0	–	159 \pm 8.5	
BMI, kg/m ²	20.68 \pm 1.67	0.000	30.43 \pm 7.04	–	29.6 \pm 4.54	0.000
BP Systolic (mm of Hg)	113.07 \pm 7.83	–	117 \pm 7.18	0.000	139.09 \pm 17.55	0.000
BP Diastolic (mm of Hg)	75.67 \pm 7.63	–	78.8 \pm 7.82	0.000	89.3 \pm 9.10	0.000
Waist circumference, cm	71.15 \pm 8.64	0.000	93.57 \pm 11.68	–	97.56 \pm 10.67	0.000
Hip circumference, cm	84.33 \pm 7.11	0.000	108.76 \pm 15.24	–	107.72 \pm 9.40	0.000
WHR	0.84 \pm 0.08	–	0.86 \pm 0.73	0.040	0.91 \pm 10	0.003
ICO	0.45 \pm 0.04	0.000	0.59 \pm 0.08	–	0.61 \pm 0.07	0.000

Statistically significant P values < 0.05 are indicated

Table II Biochemical parameters of Groups I, II and III (Mean \pm SD).

Parameter	Group I	p-value (I vs II)	Group II	p-value (II vs III)	Group III	p-value (I vs III)
FBS mmol/L	4.75 \pm 0.51	–	4.67 \pm 0.41	0.000	7.58 \pm 2.25	0.000
Total cholesterol, mmol/L	4.37 \pm 0.56	–	4.50 \pm 0.40	–	4.77 \pm 0.81	0.011
Triglycerides, mmol/L	1.18 \pm 0.37	–	1.36 \pm 0.39	0.000	1.91 \pm 0.67	0.000
HDL-C, mmol/L	1.10 \pm 0.15	–	1.09 \pm 0.11	–	1.08 \pm 0.12	–
VLDL-C, mmol/L	0.54 \pm 0.17	–	0.62 \pm 0.18	0.000	0.88 \pm 0.31	0.000
LDL-C, mmol/L	2.72 \pm 0.50	–	2.79 \pm 0.34	–	2.81 \pm 0.73	–
TG/HDL-C ratio	2.52 \pm 0.89	–	2.91 \pm 0.85	0.000	4.16 \pm 1.88	0.000

Statistically significant P values < 0.05 are indicated

Table III Hydroperoxide levels and total antioxidant capacity in Groups I, II, III (Mean \pm SD).

Parameter	Group I	p-value (I vs II)	Group II	p-value (II vs III)	Group III	p-value (I vs III)
Water soluble hydroperoxides, μ moles/L	20.74 \pm 16.21	0.009	44.18 \pm 17.90	0.000	128.1 \pm 51.74	0.000
Lipid soluble hydroperoxides, μ moles/L	79.15 \pm 4.64	–	75.98 \pm 3.35	–	79.93 \pm 4.78	–
Total antioxidant capacity μ moles/L	168.67 \pm 63.92	–	179.47 \pm 72.93	–	188.6 \pm 85.3	–

Statistically significant P values < 0.05 are indicated

other parameters including lipid-soluble hydroperoxides and TAC among the 3 groups.

The TG/HDL-C ratio of >3 , a surrogate marker of insulin resistance (14) was observed in group III. However, the TG/HDL-C ratio of almost 3 in the obese group II also suggested the tendency towards insulin resistance.

In group I, there was a positive correlation between BMI and water-soluble hydroperoxides, and in group III between FBS and TAC. Hence, the increase in oxidative stress was independent of alterations in parameters of obesity like WC, BMI, ICO and WHR.

Discussion

The prevalence of obesity is rising among Indians, a high-risk population for type 2 diabetes and CVD. The anthropometric profiles (BMI and WC) of the obese and metabolic syndrome groups were similar and characteristic of obesity as per Asian Standards (12). While BMI does not define body fat distribution, parameters like WC (15) and Index of central obesity (ICO) (14) are better indices of visceral obesity, a risk factor for the development of metabolic disorders. Visceral obesity is observed in both the obese and metabolic syndrome groups using the parameters of WC and ICO. Further, South Asians have been shown to have a higher percent of body fat and visceral adipose tissue than Europeans (16), and in Indians there was a strong correlation between visceral fat and IR (17, 18).

The obese and control groups had similar lipid profiles; but, the metabolic syndrome group exhibited hypertriglyceridemia, hypercholesterolemia and increased VLDL-cholesterol levels. The defective metabolism of stored triglycerides in the adipose tissue increases the flux of free fatty acids to the liver resulting in the excessive production of VLDL and hypertriglyceridemia (19). Hypertriglyceridemia of metabolic syndrome could also be due to decreased lipoprotein lipase activity caused by the increase in TNF- α concentrations in the adipose tissue (20). The altered lipid profile could contribute to endothelial dysfunction (21).

Obesity is associated with IR and IR with oxidative stress (21). The surrogate measure of IR, TG/HDL-cholesterol ratio (23), indicates the presence of IR in the metabolic syndrome group and a tendency towards IR in the obese group. Disturbances in the normal functioning of the adipose tissue as observed in obesity, leads to the development and/or progression of IR (24).

In humans, besides hyperglycemia, fat accumulation also can increase systemic oxidative stress and is responsible for the dysregulation of the adipocytokines. An increased production of H_2O_2 in the adipose tissue and consequently in systemic oxidative stress together with elevated expression of NOX4 (a

member of the NADPH oxidase family which generates H_2O_2) and decreased expression of antioxidant enzymes has been reported in obese mice (6). Insulin itself generates transient amounts of H_2O_2 in fat cells to mediate its signalling action (25), and TNF- α , a cytokine which induces IR, mediates its effect through H_2O_2 (26). Hence, obesity is a state of heightened oxidative stress as also observed by increased aqueous hydroperoxides in both the obese and metabolic syndrome groups.

The lipid hydroperoxide levels in both the obese and metabolic syndrome groups did not show any significant change. Gopaul et al. (27) also did not observe any alteration in lipid hydroperoxide levels in their study on Indian Mauritians at different stages of development of type 2 diabetes. However, they observed higher plasma total 8-epi-PGF₂ levels (indicator of oxidative stress) in the prediabetic stage (impaired glucose tolerance, IGT) in the absence of changes in endothelial function and insulin sensitivity and have suggested that oxidative stress precedes endothelial dysfunction and IR.

There are several reports of decreased antioxidant capacity and increased lipid peroxidation in patients with visceral obesity and metabolic syndrome (7, 28). Skalicky et al. (29) observed a significant increase in TAC in the obese group with metabolic syndrome when compared to the obese without metabolic syndrome, but not when compared to controls. They also reported an increase in the uric acid levels (an antioxidant) in both the obese groups (with and without metabolic syndrome); but there was no change in some other antioxidant levels like Vitamin E and albumin in the three groups. In this study, the TAC remained unchanged in both the obese and metabolic syndrome groups, probably due to the hyperuricemia associated with obesity and metabolic syndrome. The major contribution of uric acid towards TAC in the FRAP assay (11) would probably mask the alteration in other antioxidants in both obese and metabolic syndrome groups.

Conclusions

The observed increase in aqueous hydroperoxides without alterations in lipid hydroperoxides and TAC suggests the presence of oxidative stress in both the obese and metabolic syndrome groups; the metabolic syndrome group showing greater oxidative stress. The visceral obesity, IR, disturbances in adipose tissue function with dysregulation of adipokines and inflammation are sources of oxidative stress in the obese (3); the individual components of metabolic syndrome being independent contributors to increase the oxidative stress. Hence, oxidative stress, IR, and visceral obesity are involved in the development of complications and when associated with dyslipidemia as observed in metabolic syndrome

enhance the risk for type 2 diabetes and CVD in Indians. Early identification of at-risk obese populations of Indians by markers like oxidative stress, IR, inflammation and dyslipidemia together with anthropometric parameters of WC and ICO may reduce the progression to metabolic syndrome and its complications.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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