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Oxidative, inflammatory and cardiometabolic biomarkers of clinical relevance in patients with metabolic syndrome

Biomarcadores oxidativos, inflamatórios e cardiometabólicos de relevância clínica em pacientes com síndrome metabólica

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ABSTRACT

Introduction: Obesity is characterized by excessive deposition of fat in adipose tissue and is associated with the development of pathological damage in several metabolic processes that are associated with oxidative stress and inflammation. **Objective:** To evaluate the levels of adiponectin, inflammatory markers and oxidative markers, with the objective of determining a biomarkers profile in adults that influences the metabolic risk of developing the metabolic syndrome (MetS). **Methods:** The groups studied included 84 adults (48 Without MetS and 36 With MetS). General and biochemical parameters were determined. Adiponectin levels, inflammatory markers [C-reactive protein (CRP)], interleukin 6 (IL-6), adenosine deaminase (ADA), dipeptidyl peptidase-IV (DPP-IV) and oxidative markers [thiobarbituric acid reactive substances (TBARS), sulfhydryl groups (SH), total ferric antioxidant power (FRAP) and vitamin C] were also measured. **Results:** The MetS group presented a significant increase in insulin, triglycerides, cholesterol, low-density lipoprotein cholesterol (LDL-C), glutamic-pyruvic transaminase (GPT) and uric acid, as well as gamma-glutamyl transferase (GGT), glutamic-oxaloacetic transaminase (GOT), and vitamin C. **Conclusion:** The combination of IL-6, ultra-sensitive C-reactive protein (us-CRP), ADA, DPP-IV and the increase of TBARS, with the reduction of vitamin C, SH groups and adiponectin, promote inflammation and compromise insulin sensitivity, thus presenting an active role in the pathogenesis of MetS. These findings are significant because they may assist in monitoring clinical changes, in the prevention of future cardiometabolic events in individuals with MetS, and in the identification of inflammatory and oxidative markers that assist in the monitoring and prevention of MetS.

Key words: insulin resistance; obesity; diabetes *mellitus*.

INTRODUCTION

Once obesity is present in the metabolic syndrome (MetS) the release and production of pro-inflammatory adipokines and free fatty acids (FFA) occurs. This action is associated with chronic cases of low-grade inflammation and infiltration of additional products of macrophages in the tissue, providing a decrease of insulin receptors[1]. When this happens, the extracellular concentration of adenosine increases progressively[2], interfering with the biosynthesis of proinflammatory cytokines and decreasing the action of neutrophils[3]. In a counterregulatory response to this process, adenosine deaminase (ADA) is released in greater amounts to catalyze adenosine into isonine, regulating the intracellular and extracellular concentrations of this nucleoside. Thus, ADA is suggested as a key enzyme for the modulation of insulin bioavailability, once it regulates several aspects of adipose tissue function, including lipolysis and increased insulin sensitivity (IS) in adipocytes[4].

Recent research suggests that dipeptidil peptidase-IV (DPP-IV) is also associated with the hability to induce insulin resistance in adipocytes and muscle cells in obese individuals[5, 6]. DPP-IV induces the modification and/or inactivation of N-terminal peptides and is, therefore, a strong inhibitor of the antilipolytic activity of the neuropeptide Y[7, 8]. It has been proposed that the

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association between ADA and DPP-IV on the surface of the T cell determines costimulation of the T cell receptor against the antigen[9], in this context, DPP-IV activity together with ADA may be necessary for the cascade of T cell activation, playing a key role in the development of immune responses[6, 10].

In addition to the abnormal production of adipocytokines and deregulated proinflammatory responses, oxidative stress is another mechanism associated with the onset of MetS[11-13]. The oxidative stress occurs when there is a significant increase of free radicals in the tissues, exceeding the neutralizing capacity of the antioxidants, observed especially by the increase of the thiobarbituric acid reactive substances (TBARS)[14]. The increase of oxidative stress is involved in the pathogenicity of hypertension, atherosclerosis and contributes to cardiometabolic disorders[5]. Therefore, a large number of body's cells have adequate defensive action to avoid harmful oxidative events. This action includes the presence of antioxidant enzymes, such as peroxides and non-enzymatic antioxidants, including uric acid, sulfhydryl (SH) groups and total ferric antioxidant power (FRAP) (for determination of total antioxidant capacity)[15]. It is also observed that the levels of TBARS increase progressively according to the increase of body weight, unlike SH groups and FRAP that decrease according to body weight[16].

OBJECTIVE

Since MetS is associated with a chronic inflammatory response, characterized by abnormal adipokine production and the activation of several proinflammatory and oxidative signaling pathways resulting in the increase of several biomarkers, the objective of this study was to evaluate the levels of adiponectin, inflammatory markers with ultra-sensitive C-reactive protein (us-CRP), interleukin-6 (IL-6), ADA, DPP-IV and oxidative markers (TBARS), SH groups, FRAP and vitamin C, and to clarify, in adults, a profile of biomarkers that influence the metabolic risk of development of MetS.

METHODS

Study design and population

This was a cross-sectional study in which measurements and analyzes were performed in a single moment. The participants were recruited from January to May 2017 in the city of São Miguel do Oeste, in the state of Santa Catarina, Brazil. The patients were from basic health units. The Ethics Committee of the Universidade do Oeste de Santa Catarina (UNOESC) approved the study protocol no. 219091, and a written informed consent document was provided to all participants. The study groups included 84 adults between 22 and 58 years of age.

The control group consisted of 48 subjects Without MetS, healthy volunteers of both sexes (28 women and 20 men) and a test group composed of 36 subjects clinically characterized by the laboratory With MetS (21 women and 15 men). Participants were non-smokers and did not use any medication continuously, and did not report metabolic diseases or events, such as coronary diseases, strokes, neoplasia and other diseases or disorders that could influence the biomarkers studied.

Indexes and ratings

MetS was verified from a careful definition, taking into account the presence of at least three of the following risk factors: 1) waist circumference ≥ 90 cm for men or ≥ 85 cm for women [using cuts established by the World Health Organization (WHO)]; 2) serum high-density lipoprotein cholesterol (HDL-C) level < 40 mg/dl for men or < 50 mg/dl for women; 3) serum triglyceride level ≥ 150 mg/dl; 4) systolic blood pressure ≥ 130 mmHg, or diastolic blood pressure ≥ 85 mmHg, or treatment with antihypertensive; and 5) fasting blood glucose level ≥ 100 mg/dl[17].

Anthropometric measurements

All measures were taken in the Anthropometry Laboratory at UNOESC. Standing height (H, cm) using a wall mounted stadiometer (Charder, model HM-210D). Weight (W, kg) was measured using a calibrated electronic scale (Toledo, model 2124). Body mass index (BMI) was calculated as W/H2 (kg/m^2). Waist circumference (WC), neck circumference (NC) and hip circumference (HC) were measured in centimeters with a flexible tape. For WC the tape was applied above the iliac crest with the subject standing upright with abdomen relaxed, arms at the sides and feet together (feet close in the same position and facing forward fully supported on the platform). For NC measurement, the participant remained in the same position and tape was placed around the half of the neck on the hyoid bone. The percentages of fat and fat weight were determined by bioimpedance (Biodynamics Model 450). All measurements were taken on the left side of the body, according to standardized procedures by Lourie and Weiner (1981)[18]. During the anthropometric measurements, all participants were barefoot and clothed appropriately.

Laboratory measurements

Blood samples containing ethylenediamine tetraacetic acid (EDTA) and serum samples were obtained from blood samples collected from participants after an overnight fast of at least 12 h. Total blood cholesterol, HDL-C, triglyceride, creatinine, urea, glucose, gamma-glutamyl transferase (GGT), glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), amylase, estimated average blood glucose (GMe), insulin sensitivity (IS) and uric acid were measured enzymatically using a commercial assay kit (Labtest Diagnostics® - Brazil). Low-density lipoprotein cholesterol (LDL-C) was subsequently calculated using the Friedewald formula[19].

The IS and the high-sensitive C-reactive protein (hs-CRP) were determined by electrochemiluminescence immunoassay using an Elecsys 2010 analyzer (Roche diagnostics®). Insulin resistance index was calculated by homeostasis model assessment of insulin resistance (HOMA-IR) as (fasting insulin mIU/l) × (fasting glucose mg/dl)/22.5, and evaluation of insulin sensitivity the quantitative insulin sensitivity check index (QUICKI) was used. Glycated haemoglobin (HbA1C) was measured by high-performance liquid chromatography and expressed as %.

The serum adiponectin concentration were measured in duplicate using an enzyme-linked immunosorbent assay (ELISA), according to manufacturer (EMD Millipore Corporation, Billerica, MA, EUA) in the Luminex 100 IS Analyzer System (Luminex Corp, Austin, TX, USA). Adiponectin showed a sensitivity of 1.5 ng/ml, accuracy of 92%-102%, inter-assay precision was 2.4%-8.4%, intra-assay 1%-7.4% and the curve range: 1.5-100 ng/ml. Serum IL-6 levels were determined by ELISA using commercial kits from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. The detection limits of the assays were: 0.09 pg/ml, the sensitivity of 2 pg/ml and the curve range: 23.3 to 2560 pg/ml.

DPP-IV activity was determined by spectroscopic quantification of glycyl-prolyl-p-nitroanilide hydrolysis[20]. Results were expressed as the specific activity (U/l). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). ADA activity was determined by commercial kit (Ebram Products Laboratory Ltda®, SP, Brazil) according to the enzymatic deamination of adenosine to inosine by kineticmanner. The values were expressed in U/l.

Lipid peroxidation was estimated by TBARS measuring according to the method of Lapenna, *et al.* (2001)[21]. The determination of sulfhydryl group levels was based on Boyne and Ellman (1972)[22]. FRAP levels was estimated according to Singh *et al.* (2012)[23]. All were determined by spectroscopic quantification. Vitamin C was measured by the Enzyme Linked Immunosorbent Assay (ABCAM - Ascorbic Acid Assay Kit® and Rac Beta - Tocopherol Assay Kit®), expressed as nmol/µl.

Statistical analysis

The data were analyzed using Statistica 6.0 software (StatSoft, Tulsa, OK, USA). Data are expressed as means ± standard deviation (SD) or median (interquartile ranges). The Kolmogorov-Smirnov test was used to examine the distribution of variables. Comparisons of baseline data between groups were performed using the unidirectional variance analysis (Anova) followed by the Tukey's test or the Kruskal-Wallis test followed by the Dunn Multiple Comparison Test to determine the statistical differences between groups. A $p < 0.05$ value was considered statistically significant.

RESULTS

General characteristics of the study population

The general characteristics of the study participants are described in **Table 1**. As expected, the weight, BMI, body fat percentage, diastolic and systolic blood pressure, and hip, waist and abdomen circumferences showed a significant increase in the With MetS group ($p < 0.001$) when compared to the Without MetS group.

TABLE 1 – Baseline characteristics of study participants

	Groups		
	Without MetS	With MetS	*p*
n	48	36	
Female/male	28/20	21/15	
Age (years)	30 ± 8	46 ± 12	
BMI (kg/m^2)	21.1 ± 2.1	40.1 ± 7.6*	0.001
Neck Cir. (cm)	31.3 ± 2.3	37.4 ± 3.4*	0.001
WC (cm)	70.8 ± 7.1	116 ± 13*	0.001
HC (cm)	95 ± 4.3	120 ± 14*	0.001
Weight (kg)	58.2 ± 7.4	107 ± 26*	0.001
Fat Percentage (%)	22.5 ± 4.4	39.9 ± 6.5*	0.001
Fat Weight (kg)	13 ± 2.6	43.2 ± 14.1*	0.001
DBP (mmHg)	10.7 ± 1.1	13.9 ± 2*	0.001
SBP (mmHg)	7 ± 1.1	8.5 ± 1.3*	0.001

Data are expressed as means ± SD. Data were processed for analysis for One-way Anova followed by Tukey's test. *p < *0.001 compared to the Without MetS group.*

BMI: body mass index; WC: waist circumference; HC: hip circumference; DBP: diastolic blood pressure; SBP: systolic blood pressure; MetS: metabolic syndrome; SD: standard deviation.

Biochemical analyzes

The concentrations of biochemical, inflammatory and oxidative parameters are presented in **Table 2**. The MetS group had a significant increase in insulin, triglycerides, cholesterol, low-density lipoprotein cholesterol (LDL-C), GPT and uric acid

($p < 0.001$), as well as GGT, GOT, HbA1C, HOMA-IR, glucose, IL-6, FRAP, SH groups and TBARS ($p < 0.05$) when compared to the Without MetS group. MetS group presented a significant reduction in IS, ($p < 0.001$) HDL-C and vitamin C ($p < 0.05$) when compared to the Without MetS group. No significant differences were observed in creatinine, urea, GMe and amylase activity between groups. No statistical differences were observed between genders.

TABLE 2 – Biochemical, oxidative and inflammatory characteristics of the experimental groups

	Groups		
	Without MetS	Whit MetS	*p*
Triglycerides (mg/dl)	78 ± 29	235 ± 80**	0.001
Cholesterol (mg/dl)	140 ± 22	206 ± 48**	0.001
HDL-C (mg/dl)	45 ± 10	35 ± 8*	0.05
LDL-C (mg/dl)	71 ± 23	129 ± 51**	0.001
Creatinine (mg/dl)	0.9 ± 0.2	0.9 ± 0.1	0.1
Urea (mg/dl)	30 ± 6.1	34 ± 6.3	0.1
γ-GT (U/l)	23 ± 8.2	56 ± 28*	0.05
GOT (U/l)	19 ± 4.7	26 ± 7.8*	0.05
GPT (U/l)	15 ± 5.2	28 ± 8.1**	0.001
Amylase (U/l)	43 ± 11	45 ± 11	0.39
Insulin (µUI/ml)	7.4 ± 3	30.9 ± 17**	0.001
HbA1C (%)	5.4 ± 0.3	7.4 ± 2.2*	0.05
GMe (mg/dl)	117 ± 8	164 ± 34	0.32
HOMA-IR index	1.4 ± 0.6	10.7 ± 2.3*	0.05
Insulin Sensitivity	0.36 ± 0.02	0.28 ± 0.02**	0.001
Glucose (mg/dl)	78 ± 6	127 ± 23*	0.05
Adiponectin (ng/ml)	65.1 ± 31.3	46.1 ± 28.2	0.05
hs-CRP (mg/dl)	0.3 ± 0.2	1.2 ± 0.7*	0.05
ADA (U/l)	5.9 ± 1.1	7.7 ± 1.5	0.05
IL-6 (pg/ml)	3.4 ± 0.8	6.4 ± 2.1*	0.05
DPP-IV (U/l)	47 ± 5.1	62.9 ± 11.7*	0.05
TBARS (mmol/l)	126 ± 61	252 ± 61.1*	0.05
Vitamin C (mmol/l)	5.5 ± 2.8	1.1 ± 1*	0.05
Uric acid (md/dl)	3.3 ± 0.9	4.9 ± 1.5**	0.001
FRAP (mmol/l)	0.86 ± 0.17	1.2 ± 0.2*	0.05
SH groups (nmol P-SH/ml)	147 ± 15	107 ± 32*	0.05

Data are expressed as means ± SD. Data were processed for analysis, where One-way Anova followed by Tukey's test. *p < *0.05;*** p < *0.001 compared to the Without MetS group.*

HDL-C; high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; γ-GT: gamma-glutamyl transferase; GOT: glutamic-oxaloacetic transaminase; GPT: glutamic-pyruvic transaminase; HbA1C: glycated hemoglobin; GMe: estimated average blood glucose; HOMA-IR: homeostasis model assessment of insulin resistance; hs-CRP: high-sensitive C-reactive protein; ADA: adenosine deaminase; IL-6: interleukin 6; DPP-IV: dipeptidyl peptidase-IV; TBARS: thiobarbituric acid reactive substances; FRAP: ferric reducing/antioxidant power; SH: sulfhydryl; MetS: metabolic syndrome; SD: standard deviation.

DISCUSSION

Multiple mechanisms may contribute to the development of MetS and its comorbidities, including abnormal cytokine production, aberrant oxidative stress, and dysregulated pro-inflammatory response in tissues, such as muscle and liver. As expected, the results of the research showed that the anthropometric characteristics of the With MetS group were significantly higher than the Without MetS group (Table 1).

It was observed a significant increase in HbA1C, HOMA-IR, glucose, IL-6, hs-CRP in the MetS group, and a decrease in IS. It is noteworthy that the reduction of insulin receptor 1 (IRS-1) and glucose transporter type 4 (GLUT-4) in liver and muscle tissues is associated with increased IL-6 levels under high BMI[24]. These changes lead to IR and stimulate the production of hs-CRP[25]. In fact, the action of IL-6 during insulin signaling in adipocytes and hepatocytes causes an increase in free fatty acids by increased lipolysis[26, 27], directly interferes with glucose metabolism[28], besides inducing reduction secretion of adiponectin[29].

The laboratory analyzes revealed a significant increase in insulin, triglycerides, total cholesterol, LDL-C in the MetS group when compared to the Without MetS group, associated with a significant reduction in adiponectin (Table 2). These results are associated with a simultaneous increase in BMI. Adiponectin levels are closely associated with the amount of body fat and its resistance and or decreased stimulation during adipogenesis may modulate several steps during the insulin-signaling pathway leading to IR[30]. Studies suggest that the reduction of adiponectin may induce release of glycerol and fatty acids, which, in excess, are associated with RI[31, 32] and may affect lipid metabolism in adults by stimulating the production of LDL-C in liver cells, as well as, increasing the degradation of LDL-C receptors in the liver[33]. The effect on lipid concentrations in triglycerides blood, on the other hand, can be mediated through its effect on the metabolism of liver fatty acids, which regulate the expression of genes involved in lipid metabolism[34].

A second objective of this study was to explore oxidative biomarkers and, therefore, a reduction of SH groups and vitamin C was observed in the volunteers with MetS, with a significant increase of TBARS, evidencing the decrease in the capacity of the organism with MetS to neutralize free radicals, leading to an increase in reactive oxygen species (ROS) and favoring lipid peroxidation. As vitamin C is a water-soluble vitamin, with the increase of body weight and the concomitant reduction of the ratio between lean mass and fat mass, there is a reduction of the aqueous phase to the lipid phase in the body and, therefore, a decrease in vitamin C concentration, exposing the cells to deleterious effects of oxidative stress[32]. These data are highlighted because the increase of oxidative stress in vascular walls is involved in atherosclerosis, hypertension and induces damage to cellular structures including membranes, proteins and deoxyrribonucleic acid (DNA)[35].

Although it was observed an increase in the serum concentration of FRAP, we believe that this data does not represent an important and reliable antioxidant defense in MetS patients, since the increase of uric acid can interfere significantly in this result, since uric acid can chelate ions metals, such as iron, contributing to total antioxidant capacity[36].

The results of this study reinforce the growing evidence of ADA and DPP-IV increase in patients with MetS when compared with patients without MetS. The increased ADA activity found in this study may be a contributing factor to insulin sensitivity in individuals with MetS, since a reduction in ADA levels reduces glucose transport in adipocytes and interferes with lipid hydrolysis[37]. At the same time, increased DPP-IV activity in individuals with MetS may substantially increase lipolytic activity in adipocytes. For this reason, the increase in serum DPP-IV and ADA activity in these patients could be related to the hyperinsulinemia present in MetS. As previously shown, insulin, glucose, HbA1C and HOMA-IR levels were significantly higher in individuals with MetS, suggesting an important role of ADA and DPP-IV activity in the development of IR in these individuals. In fact, studies have found an association between HbA1C and DPP-IV activity in diabetes *mellitus* type 2 (DM2)[38] and in obese individuals[39].

These results may integrate new knowledge about possible interactions of inflammatory mediators and MetS helping on prevention of future chronic diseases and aggravation of MetS. The results presented here are of relevant clinical importance because, as demonstrated, the evaluation of the several components of MetS (adiposity, dyslipidemia and hypertension) and the biomarkers studied may have beneficial effects in the prevention of DM2, cardiovascular diseases and in the improvement of insulin sensitivity, since they reinforce the need to reduce weight and practice physical activity, confirming the need to develop and strengthen public health policies to prevent early-onset MetS and reduce its effects. However, further longitudinal studies that include assessment of lifestyle, ethnicity, and genetic characteristics of volunteers are needed to promote understanding of this disease and its associations in other populations.

This study emphasized that MetS may predispose significant changes in adipokines, inflammatory and oxidative markers. The combination of IL-6, us-CRP, ADA, DPP-IV, increased TBARS with reduction of vitamin C, SH groups and adiponenctin, favors the infiltration and activation of macrophages in adipose tissue, promote inflammation and compromise IS, therefore, it presents a critical role in the pathogenesis of MetS, development of RI, dyslipidemia and atherosclerosis.

These findings are particularly significant because they may assist in monitoring clinical changes and preventing future cardiometabolic events in individuals with MetS. The results may help determine the pathways involved in inflammation related to this condition and prevent the future development of DM2. These results may also be useful in the identification of inflammatory and oxidative markers that assist in monitoring and especially for obese and overweight individuals, thus preventing the development of MetS or helping the follow-up of patients who already have this disease.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

RESUMO

Introdução: *A obesidade caracteriza-se pela deposição excessiva de gordura no tecido adiposo e está associada ao desenvolvimento de danos patológicos em vários processos metabólicos que estão relacionados com o estresse oxidativo e a inflamação.* ***Objetivo:*** *Avaliar os níveis de adiponectina, marcadores inflamatórios e marcadores oxidativos, com o objetivo de determinar um perfil de biomarcadores em adultos que influencie o risco metabólico de desenvolver síndrome metabólica (SMet).* ***Métodos:*** *Os grupos estudados incluíram 84 adultos (48 sem SMet e 36 com SMet). Parâmetros gerais e bioquímicos foram determinados. Níveis de adiponectina, marcadores inflamatórios [proteína C reativa ultrassensível (PCR-us), interleucina 6 (IL-6), adenosina deaminase (ADA), dipeptidil peptidase-IV (DPP-IV)] e marcadores oxidativos [*thiobarbituric acid reactive species *(TBARS),* sulfhydryl *(SH) grupos,* total antioxidante capacity ferric *(FRAP) e vitamina C] também foram medidos.* ***Resultados:*** *O grupo com SMet apresentou aumento*

significativo de insulina, triglicerídeos, colesterol, colesterol da llipoproteína de baixa densidade (LDL-C), transaminase glutâmica pirúvica (TGP) e ácido úrico, bem como gamaglutamiltransferase (GGT), transaminase glutâmica oxalacética (TGO), hemoglobina glicada (HbA1C), homeostasis model assessment of insulin resistance *(HOMA-IR), glicose, SH e TBARS, e redução significativa de sensibilidade insulínica (SI), lipoproteína de alta densidade (HDL-C) e vitamina C.* ***Conclusão:*** *A combinação de IL-6, PCR-us, ADA, DPP-IV e o aumento de TBARS, com a redução de vitamina C, grupos SH e adiponectina promovem inflamação e comprometem a sensibilidade à insulina, apresentando assim um papel ativo na patogênese da SMet. Esses achados são significativos porque podem auxiliar no monitoramento de alterações clínicas, na prevenção de futuros eventos cardiometabólicos em indivíduos com SMet e na identificação de marcadores inflamatórios e oxidativos que auxiliam no monitoramento e na prevenção da SMet.*

Unitermos: *resistência à insulina; obesidade; diabetes* mellitus.

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