68 - EVALUATION OF SERUM INTERLEUKIN-6 IN PATIENTS WITH METABOLIC SYNDROME AND ITS ASSOCIATION WITH CARDIOVASCULAR DISEASE

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INTRODUCTION

Metabolic Syndrome (MetS) is related to central adiposity and insulin resistance, a complex metabolic disorder being represented by a set of factors that increase cardiovascular risk (REAVEN, 1988). Adipose tissue and visceral accumulation are the greatest predictors of cardiovascular events and that the measure of waist circumference is the best measurement to identify the disease in individuals (ESPINOLA-KLEIN et.al., 2011).

As endocrine organ, fatty tissue secretes a variety of hormones, and adipokines, as the release of the inflammatory cytokine IL-6 (GOYENECHEA et.al., 2005). Such substances with high biological activity and with great potential to adversely affect glucose and lipid metabolism (CURAT et.al., 2004) play an important role in the pathogenesis of insulin resistance, Diabetes Mellitus (DM), type 2 endothelial dysfunction and atherosclerosis (WOZNIAK et.al., 2009; WURM et.al., 2007; LIU et.al., 2011) favoring the development of cardiovascular disease (CVD) (GOYENECHEA et.al., 2005).

Thus, it is considered that the dysfunction of the endothelium is the first step in atherosclerosis. This occurs in response to elevated levels of circulating oxidized LDL- cholesterol in the blood stream, or by injury or infection of endothelial cells and smooth muscle cells, which synthesize pro-inflammatory proteins, including chemokines, cell adhesion molecules, cytokines, factors growth, as well as pro-thrombotic substances (MÜNZEL et.al., 2008; ROSS, 1999; HANSSON, 2005). That is, these inflammatory cells contribute to the formation of atherosclerotic plaque consisting of a set of lipids protected by a fibrous cap (ROSS, 1999).

Interleukin-6 (IL-6) is described as a multifunctional inflammatory cytokine that regulates the immune response of the organism and acute, inflammation, and hematopoiesis (ESPINOLA-KLEIN et.al., 2011). It has been proposed as an inflammatory marker associated with MetS and involves many changes related to excess weight gain, which may represent also a factor in inducing hormone in muscle strength, mediated by adiposity (GOYENECHEA et. al., 2005; ELKS & FRANCIS, 2010).

Thus, IL-6 is an important modulator of lipid metabolism, being directly associated with MetS due to increased fat oxidation and re-esterification of fatty acids, suggesting thus, its association with the development of CVD (BAYTURAN et.al., 2010; PRESTES et.al., 2006).

However, the aim of this study was to evaluate serum levels of IL-6 in patients with MetS and its association with the development of CVD.

METHODOLOGY

IL-6 serum levels were measured in 80 patients with MetS, 40 patients without CVD and 40 patients with CVD established, which were followed on Cardiometabolic Risk Clinic of the Hospital São Lucas (HSL/PUCRS) from March 2008 to December 2011. This study was approved by the Research Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul under the Protocol 11/05731. Patients with acute chronic disease, cancer, chronic liver disease, chronic renal failure and heart failure class IV (according to the New York Heart Association) were excluded. Based on the NCEP-ATPIII (The National Cholesterol Education Program Adult Treatment Panel III) (NCEP, 2002; GRUNDY et. al., 2005) MetS was defined by the presence of three or more of these criteria: abdominal obesity ≥ 102 cm in men and ≥ 88 cm in women; hipertigliceridemia ≥ 150 mg/dL; HDL cholesterol <40 mg / dl in men and <50 mg/dL in women; systolic blood pressure (SBP) ≥ 130 mmHg and diastolic blood pressure (DBP) ≥ 85 mmHg; The established CVD was defined as a history of acute myocardial infarction (AMI) confirmed by electrocardiogram, myocardial scintigraphy or coronary angiography, previous myocardial revascularization; revascularization catheter (angioplasty with or without stenting). Clinical parameters were selected as Systolic and Diastolic Blood Pressure, Hypertension (SAH), type 2 diabetes, fasting blood glucose, total cholesterol, LDL-Cholesterol, HDL-Cholesterol and Triglycerides and as anthropometric parameters, Body Mass Index (BMI), Abdominal Circumference (AC), waist/hip ratio and also other variables that describe characteristics of the population: Age, Sex, Glucose subsidiary and the use of simvastatin to patients. To collect blood of laboratory tests, 12-hour fast of patients were needed. Thus, to obtain the values corresponding to each variable selected in the study, the following steps were taken: for the measurement of variables such as fasting glucose and lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides) was used Chemical method Drought 5.1 VITROS Chemistry®, Johnson & Johnson, from sample serum of these patients, that is if stored at -70°C Research Institute, PUCRS. Diagnosis of type 2 diabetes was defined as fasting blood glucose ≥ 126 mg/dL or; 2h blood glucose after ingestion of 75 g glucose ≥ 200 mg/dL or; glycosylated hemoglobin (HbA1c) or ≥ 6.5%; Random glucose ≥ 200 mg/dL with classic symptoms of hyperglycemia. Systolic and diastolic blood pressures were measured in a sitting position and performed two sequential steps with 3 minute intervals between measurements. An automatic blood pressure monitor brand BIC, Aneroid type was used and the values obtained, it was decided to use the second measurement, they suggest a more stable measure. IL-6 serum levels were assessed by ELISA at the Biophysics Laboratory of PUCRS.

BMI was calculated according to the formula: weight (kg)/height2 (m). Weight (kg) and height (cm) were measured using a precision scale of Filizola without wearing shoes and wearing light clothing. Waist circumference was performed in the supine position after expiration, determined at the midpoint between the last rib and the iliac crest. Still, the relationship waist/hip was obtained from the abdominal circumference values divided by the circumference of the hip. The reference value for men and women were found to fulfill the criteria of the NCEP-ATP III.

As for statistical analysis, quantitative data were described as mean and standard deviation. Categorical variables were expressed as counts and percentages. The study of normality of the data was by the Kolmogorov-Smirnov test. The interleukin-6 values, due to their strong asymmetries, were log transformed prior to analysis. These data were expressed as geometric mean and minimum and maximum value.

Comparisons of quantitative data between two independent groups were performed using Student's t test. Categorical data were compared using the Fisher test of the chi-square or exact when needed. For adjustment covariates on IL-6

values we used the analysis of covariance with robust standard error procedure.

To obtain the results, the significance level was 5%. Data were processed and analyzed using SPSS version 21.0.

RESULTS

We evaluated 80 patients with MetS, of these, 40 with established CVD and 40 without episode of CVD. The baseline characteristics of these patients are shown in Table 1.

Table 1 - Baseline characteristics of the groups of patients with Metabolic Syndrome second absence or presence of Cardiovascular Disease

Caracteristicas	Without DCV (n=40)	With DCV (n=40)	p
Age, years	60±10	58±9	0,48§
Male Sex, nº (%)	14 (35)	18 (45)	0,49¥
BMI Kg/m^2	32,6±5,0	31,4±4,7	0,29§
abdominal circumference, cm	107±11	107±11	0,95§
relationship C/Q	0,98±0,09	1,00±0,07	0,22§
SBP, mmHg	155±27	147±24	0,22§
DBP, mmHg	92±11	84±14	0,008§
SAH, nº(%)	19 (47,5)	22(55,0)	0,66¥
diabetes mellitus 2, nº (%)	16 (40,0)	23 (57,5)	0,18¥
Fasting glucose, mg/dL	140±71	146±76	0,74§
Glicose Cont.≤ 99mg/dL, nº (%)	28 (70,0)	28 (70,0)	>0,99¥
Total Colesterol, mg/dL	208±47	187±54	0,081§
LDL, mg/dL	129±39	107±46	0,026§
HDL, mg/dL	46±12	42±8	0,095§
triglycerides, mg/dL	212±99	202±138	0,72§
Use of simvastatin, No. (%)	11(27,5)	33 (82,5)	<0,001¥

Data are presented as mean ± standard deviation or counts (percentage)
§ Student t test for independent groups;
¥ Pearson's chi-square test

In an overview, it is noted that patients with Mets and without CVD present DBP and LDL- cholesterol higher compared to the group with CVD. This makes it evident that the group with established CVD, 82.5% of patients make use of simvastatin and are controlled by the use of this medication. Since, in the group without CVD only 27.5% of patients make use of this anti-inflammatory medication, which corresponds to only a quarter of these individuals and which explains possibly high values for DBP and LDL-cholesterol in this group of patients . Finally, the two groups have similar characteristics and do not differ significantly in the other variables analyzed. When IL-6 was associated with the development of CVD, the results showed a statistically significant difference (p=0.039), showing that the group with CVD (23.52 ± 59.78; median: 9.80) is concentrating higher levels this inflammatory cytokine, compared to without CVD group (10.39 ± 3.50; median: 9.40) as shown in Table 2.

Table 2: Mean, standard deviation, median and range for the values of IL-6 in patients with MetS with and without CVD

IL-6 Levels / values	Metabolic syndrome		
	With CVD (n=40)	Without CVD (n=40)	p£
Mean ± standard deviation	23,52±59,78	10,39±3,50	0,039£
Median (1st-3rd quartile)	9,80 (9,20-15,97)	9,40 (8,6-10,7)	
Amplitude	7,80 – 382,31	7,90 – 27,90	

DISCUSSION

MetS is defined as a metabolic disorder characterized by factors that increase the predisposition and risk for the development of CVD (ESPINOLA-KLEIN et.al., 2011). Overweight and obesity (YORK et.al., 2004; CELORIA, 2013), along with three or more criteria as type 2 Diabetes Mellitus (WOZNIAK et.al., 2004), hypertension, high triglycerides and low levels of HDL - cholesterol, classify the individual with MS, and these criteria, when combined, are considered high risk because they increase inflammation and the chance of occurrence of new cardiovascular events in these individuals (ESPINOLA-KLEIN et.al., 2011).

This relationship between CVD and abdominal obesity risk factor has been established in some epidemiological studies. In a cohort study of 44.702 women in the USA, Coronary Artery Disease (CAD) free, aged 40-65 years, we observed the evolution of this group of women during the period 1986-1994 and were reported 251 myocardial infarctions and 69 deaths related to CAD and increased waist circumference was independently associated with the risk of CAD in the study group (REXRODE et.al., 1998).

Another French study, a sample of 7.079 men, found that abdominal obesity was measured by abdominal sagittal diameter was significantly associated with increased risk of sudden death, even after they have been made adjusting for age, body mass index, fat trunk subcutaneous, sagittal diameter thoracic, smoking, systolic blood pressure, diabetes and cholesterol levels (EMPATHY et.al., 2004).

Similarly, a systematic review with 3.459 individuals with and without MetS, comparing the relationship between the clinical features and progression of atherosclerotic plaque between groups. These were monitored by intravascular ultrasound for 7 clinical trials. The result found was that more than half of the subjects (57.8%) were patients with MetS, mean age 57.3 + 9.2 years, and 32.3% were female. The group of patients with MetS, was highly prevalent and associated with greater progression of coronary atherosclerosis than the group without MetS (BAYTURAN et.al., 2010).

However, these risk factors can also lead to endothelial imbalance (BARZILAY & FREEDLAND, 2003) releasing cytokines in the bloodstream, such as IL-6 biomarkers (MÜNZEL et.al, 2008). The inflammatory markers are responsible for endothelial damage and platelet aggregation, and as a consequence can lead to atherosclerotic plaque formation (ESPINOLA-KLEIN et.al., 2011).

However, this study looked at the CVD associated with increased serum levels of IL-6 in patients with MS and for this we were then evaluated and compared serum levels of IL-6 in these patient groups.

In this study IL-6 levels were more elevated in patients with established MetS and CVD, reinforcing the association between MetS and the other risk factors traditional to CVD, indicating possibly that these individuals may be more susceptible to new cardiovascular events such as the AMI and stroke, as previously described in the literature (REXRODE et.al., 1998).

However, this increase in serum levels of IL-6 in patients with MetS and with established CVD was four times higher than in the group without CVD which can be explained by the association of MetS with increased release of inflammatory cytokines such as IL 6 by adipose tissue, increasing prevalence of cardiovascular disease in these patients, with this combination of high risk 2.5 fold increased mortality in the world population (LAKKA et.al., 2002).

Elevated levels of IL-6 are associated with an increased risk of cardiovascular events in the evolution of patients after

Acute Coronary Syndrome, however, the prognosis value of the IL-6 serum levels are not defined in patients with coronary artery disease chronic stable coronary (FAIN , 2010).

IL-6 plays an important role in the pathogenesis of coronary artery disease and elevated levels inflammatory biomarker that found in humans with atherosclerotic plaques. Still, high IL-6 levels are correlated with increased mortality, such as unstable angina, left ventricular dysfunction, prone to diabetes and its complications, hypertension, obesity and various cancers (FISMAN & TENENBAUM, 2010).

Seeking a new association to be addressed, one can still comment on the use of simvastatin in the groups with and without CVD. One can see that even with the use of this medication in almost any sample with established CVD (82.5%), LDL-cholesterol and DBP were shown to be lower in the group without CVD. This leads us to understand that in these patients with CVD, the inflammatory process is still very expressive, suggesting the high risk of the occurrence of new cardiovascular events. That is, even with anti-inflammatory medication, we can see that the group of patients with CVD have high cardiometabolic risk and inflammation seems to be constant in these individuals.

In this context, the study of inflammatory biomarkers such as IL-6, are of great value for the population, since they aim to identify early cardiovascular risk in individuals. It is also appropriate, the use of knowledge about biomarkers, aiming at a more accurate diagnosis and effective, exerting a greater therapeutic control with regard to preventing the occurrence of new cardiovascular events, aiming to reduce these episodes and improvement the overall health of these individuals.

Additional work, to report the same characteristics of this study, were not found. This, has limitations as any cross-sectional study. Thus, further research is necessary they can better identify the association of biomarker IL-6 and CVD in patients with MetS because, exposures and outcomes assessed were performed at a single time, which may make it impossible to establish more specific relations of the studied context .

CONCLUSION

The study showed that patients with established CVD and MetS showed serum levels of the inflammatory cytokine IL-6 higher, confirming its association with the development of CVD. These patients, even making use of statins, with potential anti-inflammatory action, persist with elevated IL-6 levels, giving greater risk and probability of occurrence of new cardiovascular events.

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EVALUATION OF SERUM INTERLEUKIN-6 IN PATIENTS WITH METABOLIC SYNDROME AND ITS ASSOCIATION WITH CARDIOVASCULAR DISEASE.

ABSTRACT

Introduction: Metabolic syndrome (MetS) is a metabolic disorder associated with the production and accumulation of adipose tissue related to insulin resistance and release of inflammatory cytokines. IL-6 is an inflammatory cytokine produced in visceral adipose tissue and with high biological activity, adversely affecting lipid and glucose metabolism, creating a favorable environment for the development of cardiovascular disease (CVD). Methodology: Interleukin-6 was evaluated in 80 patients with MetS, 40 patients without CVD and 40 patients with established CVD, in a controlled cross-sectional study consisting of a historical sample of the database Outpatient Cardiometabolic Risk PUCRS. Results: Serum IL-6 levels were higher in patients with established CVD (P=0.036). The average age is higher in the group of patients without CVD (60±10 years). Were also statistically significant values of DBP (P=0.008), LDL cholesterol (P=0.026) in the group without CVD. For patients with CVD use of simvastatin is performed by most patients, showing statistical significance, respectively (P=0.001). Conclusion: Patients with MetS and with established CVD, showed serum levels of IL-6 higher, confirming its association with the development of CVD. These patients, even making use of statins also show levels of IL-6 increased, improving risk and probability of occurrence of new cardiovascular events.

KEYWORDS: Interleukin-6, cardiovascular disease, metabolic syndrome.

ÉVALUATION DES NIVEAUX DE SERUM INTERLEUKINE-6 CHEZ LES PATIENTS PRÉSENTANT UN SYNDROME MÉTABOLIQUE ET LEUR ASSOCIATION AVEC MALADIES CARDIOVASCULAIRES.

RÉSUMÉ

Introduction: Le syndrome métabolique est un trouble métabolique associé à la production et l'accumulation de tissu adipeux, associées à l'insulino-résistance et la libération de cytokines inflammatoires. IL-6 est une cytokine inflammatoire produite dans la graisse viscérale et l'activité biologique élevée, ce qui affecte le métabolisme des lipides et du glucose, la création d'un environnement propice au développement de maladies cardiovasculaires. Méthodologie: L'interleukine-6 a été évaluée chez 80 patients atteints de syndrome métabolique, 40 patients sans maladie cardiovasculaire et 40 patients atteints de maladies cardiovasculaires dans une étude transversale contrôlée compose d'un échantillon historique de base de données clinique de risque cardiométabolique des PUCRS. Résultats: Les taux sériques d'IL-6 étaient plus élevés chez les patients atteints de maladies cardiovasculaires (P=0,036). L'âge moyen est plus élevé chez les patients sans maladie cardiovasculaire (60±10). Il y avait aussi statistiquement significative les valeurs de la la pression artérielle diastolique (P=0,008), taux de cholestérol LDL (P=0,026) dans le groupe sans maladies cardiovasculaires. Pour le groupe maladies cardiovasculaires avec l'utilisation de la simvastatine est effectuée par la plupart des patients, montrant la signification statistique, respectivement (p=0,001). Conclusion: Les patients atteints syndrome métabolique et ayant subi un maladie cardiovasculaire, ont montré des niveaux sériques de l'IL-6 supérieur, confirmant son association avec le développement des maladies cardiovasculaires. Ces patients, même en faisant usage des statines, démontrent en outre l'IL-6 niveaux ont augmenté, donnant un plus grand risque et la probabilité de survenue de nouveaux événements cardiovasculaires.

MOTS-CLÉS: l'interleukine-6, les maladies cardiovasculaires, le syndrome métabolique.

EVALUACIÓN DE CONCENTRACIONES SÉRICAS DE INTERLEUCINA-6 EN PACIENTES CON SÍNDROME METABÓLICO Y SU ASOCIACIÓN CON ENFERMEDAD CARDIOVASCULAR.

RESUMEN

Introducción: El síndrome metabólico es un trastorno metabólico asociado con la producción y la acumulación de tejido adiposo, relacionado con la resistencia a la insulina y la liberación de citoquinas inflamatorias. IL-6 es una citoquina inflamatoria producida en la grasa visceral y la alta actividad biológica, afectando negativamente el metabolismo de los lípidos y la glucosa, la creación de un entorno propicio para el desarrollo de la enfermedad cardiovascular. Metodología: La interleucina-6 se evaluó en 80 pacientes con síndrome metabólico, 40 pacientes sin enfermedad cardiovascular y 40 pacientes con enfermedad cardiovascular establecida en un estudio transversal controlada consiste en una muestra histórica de la base de datos de la Clínica de riesgo cardiometabólico de la PUCRS. Resultados: Los séricas de IL-6 niveles fueron más altos en los pacientes con enfermedad cardiovascular establecida (P= 0,036). La edad media es mayor en pacientes sin enfermedad cardiovascular (60±10). También fueron estadísticamente significativos los valores de la presión arterial diastólica (p=0,008), el colesterol LDL (P=0,026) en el grupo sin enfermedad cardiovascular. Para el grupo com enfermedad cardiovascular el uso de simvastatina se realiza por la mayoría de los pacientes, mostrando la significación estadística, respectivamente (P=0,001). Conclusión: Los pacientes con síndrome metabólico y con enfermedad cardiovascular establecida, mostraron niveles séricos de IL-6 más alta, lo que confirma su asociación con el desarrollo de las enfermedades cardiovasculares. Estos pacientes, incluso

haciendo uso de las estatinas, demuestran una vez más la IL-6 niveles aumentaron, dando mayor riesgo y la probabilidad de ocurrencia de nuevos eventos cardiovasculares.

PALABRAS CLAVE: interleucina-6, la enfermedad cardiovascular, síndrome metabólico.

AVALIAÇÃO DOS NÍVEIS SÉRICOS DE INTERLEUCINA-6 EM PACIENTES COM SÍNDROME METABÓLICA E SUA ASSOCIAÇÃO COM DOENÇA CARDIOVASCULAR.

RESUMO

Introdução: A Síndrome Metabólica (SM) é um distúrbio metabólico, associado a produção e acúmulo de tecido adiposo, relacionado a resistência à insulina e liberação de citocinas inflamatórias. A IL-6 é uma citocina inflamatória produzida no tecido adiposo visceral e com alta atividade biológica, afetando adversamente o metabolismo lipídico e glicêmico, criando um ambiente propício para o desenvolvimento de Doença Cardiovascular (DCV). Metodologia: A Interleucina-6 foi avaliada em 80 pacientes com SM, 40 pacientes sem DCV e 40 pacientes com DCV estabelecida, em um estudo transversal controlado composto por uma amostra histórica do banco de dados do Ambulatório de Risco Cardiometabólico da PUCRS. Resultados: Os níveis séricos de IL-6 foram maiores em pacientes com DCV estabelecida (P=0.036). A idade média é maior no grupo de pacientes sem DCV (60+10 anos). Foram, ainda, estatisticamente significativos os valores de PAD (P=0,008), LDL- Colesterol (P=0,026) no grupo sem DCV. Para o grupo com DCV, o uso de Sinvastatina é realizado pela maior parte dos pacientes, demonstrando significância estatística, respectivamente, (P=0,001). Conclusão: Pacientes com SM e com DCV estabelecida, apresentaram níveis séricos de IL-6 mais elevados, confirmando sua associação com o desenvolvimento de DCV. Esses pacientes, mesmo fazendo uso de estatinas, ainda demonstram níveis de IL-6 aumentados, conferindo maior risco e probabilidade de ocorrência de novos eventos cardiovasculares.

PALAVRAS-CHAVE: Interleucina-6, Doença Cardiovascular, Síndrome Metabólica.