Characteristics of metabolic syndrome associated with hepatic steatosis

Características da síndrome metabólica associados à esteatose hepática

José knopfholz[1], Gustavo R Marques[2], Tadeu A Fernandes[2], Vinícius B Seleme[2], Caio C D Disserol[2]

ABSTRACT

Design of the study: a cross-sectional population-based study performed at clinics and healthy units at Curitiba - Paraná - Brazil.

Objectives: to evidence which are the major cardiovascular risk factors of MS related with the hepatic steatosis (HS).

Methodology: 75 patients with metabolic syndrome (MS), in treatment or not for this condition, after meeting the criteria of inclusion or exclusion for the research, were submitted to ultrasound exam of the liver to quantify the degree of HS. Then, the relationship between HS and cardiovascular risk factors of MS was evaluated.

Results: regardless of age, gender and hypertension, patients with glucose intolerance or diabetes mellitus have a higher risk for HS than patients with normal serum glucose ($p<0,05$). Less relevant, hypertension also demonstrated association with HS.

Conclusions: Cardiovascular risk factors of MS may be associated with HS. The alteration of glucose metabolism appears to be the main risk factor of MS associated with HS.

Keywords: Metabolic Syndrome X. Fatty Liver . Risk Factors / Cardiovascular Diseases. Ultrasonic Imaging. Diabetes Mellitus, Type 2. Insulin Resistance.

1. Introduction

The relationship between metabolic syndrome (MS) and non-alcoholic fatty liver disease (NAFLD) has become increasingly recognized. In this context, hepatic steatosis (HS), also called fatty liver, is the main example of this relationship. It has been suggested that liver disease is not a modest marker of cardiovascular disease, but also a contributing factor for its development.[1,2]

MS is defined as a complex disorder represented by a set of cardiovascular risk factors usually associated to the central deposition of fat and insulin resistance.[3] The features of MS are: abdominal obesity, atherogenic dyslipidemia (elevated triglycerides and small particles of low-density lipoprotein cholesterol

1. PhD in Internal Medicine and Adjunct Professor of Pontifícia Universidade Católica do Paraná
2. Medical Students of Pontifícia Universidade Católica do Paraná

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Address for correspondence:
Caio César Diniz Disserol
Rua Santa Catarina 101, apto 1302
Água Verde - Curitiba (PR) - Brasil
CEP: 80620-100

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[LDL-c], low high-density lipoprotein cholesterol [HDL-c]), insulin resistance and increased blood pressure.[1-5]

However, no one know for sure (although there are several hypothesis) why patients with MS are at higher risk for NAFLD. Probably, the mechanism of liver injury consists in the abnormality of glucose and lipid metabolisms. The alterations of lipid metabolism are the increased liver input and biosynthesis of free fatty acids.[1,2,6]

NAFLD, the most common chronic liver disease, is characterized by increased deposition of triglycerides in hepatocytes.[1,2,7,8] HS is the main representative of this entity.

The connection between liver injury and increased cardiovascular risk can be demonstrated through several examples in the literature. Patients in this condition commonly have intra-abdominal visceral fat in excess and inflammatory changes in adipose tissue, closely related features to atherosclerosis.[1,2]

This study was based in the analysis of the importance of each MS risk factor supposed to participate in liver injury, represented mainly by HS. Then, the knowledge of which MS component impairs liver function in a higher degree is fundamental for optimizing treatment.

2. Objective

To define the main cardiovascular risk factors of MS related to HS.

3. Material and methods

Study Design

A cross-sectional population-based study, whose target population are adults having 30 to 80 years of age, of both genders, living at Curitiba, Paraná, Brazil. The data analysis was performed qualitatively.

Sampling Plan

Sample composed by 75 patients, all with MS according to the diagnostic criteria of NCEP-ATPIII (National Cholesterol Education Program - Adult Treatment Panel III)3, even if they are treating some or all of its factors. The patients were included in the research regardless having or not HS, because this condition was further evaluated by liver ultrasound.

Procedure for data collection

The research was approved by Comitê de Ética em Pesquisa em Seres Humanos do Setor de Ciências da Saúde da Universidade Federal do Paraná.

The patients were selected, after observing the inclusion and exclusion criteria (Table 1), at clinics and healthy units at Curitiba, Paraná by physicians and medical students of Pontifícia Universidade Católica do Paraná. After being selected, they were included in the research signing the Statement of Consent, and then answered the questionnaire. The questions were about personal information of the patients (full name, phone number, age, gender, weight, height, Body Mass Index) and clinic factors that are components of MS (blood pressure, fasting glucose, triglycerides, HDL-c and waist circumference). Therefore, the questionnaire was completed after the quantification, clinical or laboratorial, of the cardiovascular risk factors of this syndrome.

Table 1
Inclusion and exclusion criteria of patients

Inclusion criteria

1) To present or to be in treatment for at least three of the defining criteria for metabolic syndrome:
   - Diabetes mellitus or fasting glucose above 100 mg/dL;
   - Hypertension or blood pressure greater than or equal to 130x85 mmHg;
   - Triglyceride value greater than or equal to 150 mg/dL;
   - HDL-c less than 40 mg/dL for men and less than 50 mg/dL for women;
   - Waist circumference greater than 102 centimeters for men and greater than 88 centimeters for women.
2) Age between 30 and 80 years.

Exclusion criteria

- Present or be in treatment for any autoimmune, metal deposition or chronic viral liver disease;
- Be in treatment with potentially hepatotoxic medication for na illness unrelated to the cardiovascular system;
- Positive serology for hepatitis B or C;
- Established coronary disease;
- Symptoms suggestive of angina pectoris or medical history of coronary atherosclerosis;
- Alcohol intake greater than 20 grams per day.

The blood pressure measurement was done according to the brazilian guideline of arterial hypertension (V Diretrizes Brasileiras de Hipertensão Arterial - *V Brazilian Guideline of Hypertension*).[9] The mensuration of the waist circumference was performed with tape line (standardized among researches) with the patient in orthostatic position, and was considered increased in men when exceeded 102 centimeters and in women when exceeded 88 centimeters.[4]

The serum glucose and the lipidogram were evaluated after blood collection at healthy units (nearest to their houses) and measured at Laboratório Municipal de Curitiba. The serum glucose was considered abnormal when exceeded 100mg/dL (regardless if exceeded or not 126mg/dL, characterizing diabetes mellitus or intolerance to glucose).[3,4] The HDL-c was considered low when below 40mg/dL in men and below 50mg/dL in women. Elevated triglycerides was considered when over 150mg/dL.[3,4]

Serology screening for hepatitis B and C, through Anti-HCV, Anti-HBs and HBsAg was also performed.

Ultrasonic imaging

Once selected, each patient was subjected to real-time ultrasonic imaging after at least six hours of fasting (to reduce intestinal gas and to prevent contraction of the gallbladder) with a curved transducer and appropriate intercostal approach (frequency of 2.5 to 5 MHz). The images were obtained in supine position and right anterior oblique and/or left lateral decubitus. Furthermore, images were acquired during apnea to improve the visualization of the hepatic dome.

The defining ultrasound finding of HS was the liver increased echogenicity.[10]

Statistical analysis

Results were expressed as means and standard deviations (quantitative variables) or as frequencies and percentages (categorical variables). To evaluate the association of dichotomist categorical variables with the presence or not of HS, the Fisher's exact test was considered. For the assessment of age was used Student's t test for independent samples.

In a multivariate analysis a Logistic Regression model was adjusted, considering Wald's test for decision-making about the included factors in the model. After adjusting, the values for odds ratio (OR) and corresponding confidence interval of 95% were estimated. P values <0.05 were considered statistically significant. The data were organized into an Excel spreadsheet and analyzed with the software Statistica v.8.0.

4. Results

The sample comprises 75 30 to 80 years old patients (55 women and 20 men), all with MS.

Forty and two patients (56%) had altered glucose levels (glucose intolerance or diabetes mellitus). Of these, 35 (nearly 83%) showed HS, in the hepatic ultrasound exam (p value= 0.025, regardless the severity of this condition). However, considering the patients that did not have glucose alteration (33 subjects, 44% of total sample), about 60% had fatty liver damage in the exam.

Arterial hypertension also proved being one of the main risk factors of MS associated to HS development. This is explained by the fact of, under statistic analysis, this information revealed marginal tendency close to positivity, both in univariate (Table 2) and in multivariate (Table 2) statistics. Accordingly, about 78% of the patients with increased blood pressure (above 130x85 mmHg or under pharmacological treatment for hypertension), had liver injury confirmed by ultrasonic imaging.

Data for waist circumference were not analyzed because almost all patients exhibited increased values (above 102 centimeters for men and above 88 centimeters for women).

5. Discussion

It is evident in the literature that there is a correlation between the development of NAFLD and MS. Through pathophysiological mechanisms not yet well understood, but which are interconnected, is discussed that both conditions are spectrum of the same disease.[1]

Metabolic Syndrome and fatty liver injury

MS is a range of risk factors that, interrelated, would be directly involved in the development of atherosclerotic cardiovascular disease. This group of metabolic disorders includes the following pathophysiologic process: obesity state (especially increased abdominal circumference), insulin resistance with or without glucose intolerance, hyperinsulinemia, dyslipidemia and prothrombotic and pro-inflammatory states.[1-5,8,11,12,13]

As mentioned, it is not fully elucidated how these factors trigger disease onset. It is believed that MS has three relevant etiologic factors: obesity and the fat tissue functional alteration, peripheral insulin resistance and independent factors produced in the liver or in the immune or vascular systems.[1,2,8,13]

Table 2
Univariate analysis of risk factors for metabolic syndrome and its relevance for the development of hepatic steatosis

Variable	Classification	n	n (%) Without steatosis 20	n (%) With steatosis 55	*p* value
Gender	Female	55	14 (25.4)	41 (74.6)	
	Male	20	6 (30.0)	14 (70.0)	0.770
Age	Years	X	52.4 ± 11.9	55.5 ± 9.6	0.256
Glycemia	Normal	33	13	20	
	Increased	42	7	35	0.025
Triglycerides	Normal	23	7	16	
	Increased	52	13	39	0.778
Hypertension	Absent	11	6	5	
	Present	64	14	50	0.058
	Normal	16	4	12	
HDL-c	Low	58	16	42	1.000

HDL-c = High-density lipoprotein cholesterol;
Fisher's exact test or chi-square test, p<0.05; P=0.256 (Student's t test for independent samples)

Table 3
Multivariate analysis: including factors with p<0.25 in the univariate analysis

Variable	*p* value	Risk classification	OR	Lower limit	Upper limit
Age	0.982	x	1	0.94	1.06
Gender	0.782	x	0.84	0.24	2.97
Glycemia	0.047	GI or DM	3.19	1	10.27
Hypertension	0.061	Yes	4.06	0.91	18.07

OR = Odds Ratio; GI = Glucose intolerance; DM = Diabetes mellitus

Visceral obesity leads to an ectopic deposit of fat tissue. This visceral adipose tissue allows an increased flux of free fatty acids, and can be considered an endocrine organ. This is evidenced by the capacity of hormone and pro-inflammatory cytokines production, including tumor necrosis factor-alpha, which is an important mediator of insulin resistance associated to obesity. Consequently, leads to a significant decrease of glucose transporters functionally available.[1,2,5,7,8,12-16]

Insulin resistance, considered the main factor, seems to act as a regulatory element of our body, trying to block the gain fat, producing a higher quantity of insulin. Nevertheless, maintaining this mechanism is pathogenic to own metabolism. This hyperinsulinemic state is pro-inflammatory and atherogenic. The

pro-inflammatory condition associated to other factors, such as hypertension and dyslipidemia, can cause endothelial damage. In turn, this endothelial dysfunction caused by increased oxidative stress, results in a reduced amount of endothelial nitric oxide, which increases the vascular injury and, consequently, the progression of atherosclerosis.[8,11,13,14,15,17-20]

Therefore, there is a dysfunction state of the metabolism and, the liver, as responsible for the processing of fatty acids through their uptake, oxidation or metabolism, also suffers the consequences of this imbalance.21 This change would be translated by NAFLD onset.[5,7,8,12,14,15,22]

The relationship of these two entities has been showed in this study. Based on a total sample of 75 patients with MS, approximately 73% of the subjects had HS. These values corroborate the literature and make us believe that, possibly, HS and MS are components of the same disease and insulin resistance is his great precursor.

Insulin resistance and NAFLD

Insulin resistance is considered by Hermes et al[13] as the MS epicenter. According to him, "the insulin resistance reflects the insulin inability to promote glucose metabolism homeostasis." The clinical translation of this change is the appearing of impaired fasting glucose, a component of the diagnostic criteria for MS. Insulin also plays a role in lipids and proteins metabolism.[7,8,13] Lipids have in their composition free fatty acids, and, in obese individuals, there is an increased mobilization and oxidation of these acids in the tissues sensitive to insulin.[7,16] In the liver, the free fatty acids metabolism increases gluconeogenesis. In the muscles, the increased oxidation results in insulin resistance. As outcomes of these changes hyperglycemia and hyperinsulinemia are established.[7,8,11,16]

Hyperinsulinemia associated to insulin resistance can contribute to HS induction, because eventually it will increase mitochondrial oxidation of fatty acids that will be accumulated in the liver. As free fatty acids excess is toxic to hepatocytes, they lead to inflammation (pro-inflammatory cytokines release, particularly tumor necrosis factor-alpha) and fibrosis.[23]

In this study, 56% of patients had impaired fasting glucose (translated as diabetes or glucose intolerance). Of these, about 83% had HS (p= 0,025). Thus, insulin resistance, fundame ntal and well described mechanism of liver injury in patients with MS[1,2,5,13,16,23], proved being essential, as well, in this project.

Arterial hypertension and NAFLD

As a consequence of the pathophysiological mechanism already mentioned, besides obesity and insulin resistance, hypertension also appears to influence the onset of NAFLD in patients with MS.[24] It is known that the accumulation of abdominal fat contribute to the emergence of insulin resistance, although the mechanisms are not fully understood.[1,16] Donati et al[25] considered that hypertension may result from insulin resistance state, and that about 50% of patients with hypertension have insulin resistance and hyperinsulinemia. The hyperinsulinemia culminates in two changes: increased sympathetic activity mediated by changes in the renin-angiotensin system[16] and increased sodium tubular reabsorption (which induces peripheral vascular resistance increment, that proceeds with increased blood pressure).[26]

Endothelial damage appears to lead to a range of disorders, including the generation of reactive oxygen species, which are fundamental in the pathophysiology of NAFLD.[17-20]

Donati et al[25] proved a higher prevalence of fatty liver damage in patients with hypertension (30,9%) than in control patients (12,7%). However, the liver fat injury is associated to obesity and the insulin resistance occurs most frequently in patients with hypertension.[24]

Thereby, the research in discussion presented about 85% prevalence of hypertension in the studied population. This cardiovascular risk factor also presented highlighted regarding HS, because 78% of hypertensive patients had fatty liver injury (with a tendency to statistical significance).

Study limitations

The project was developed with a sample of 75 patients, considered small to be used as basis for an entire population. Thus, studies with larger numbers of patients should be done to be used as references in daily clinical practice.

The liver ultrasonic imaging, by the cost-effectiveness and accessibility, was the method of choice for the analysis in this study. When compared with others imaging studies, such as computed tomography and magnetic resonance imaging, it better assesses diffuse fatty changes. Nevertheless, the accuracy is markedly low when less than 33% of hepatocytes are committed by NAFLD, what may underestimate the results. Sensitivity and specificity of ultrasound exam

is much lower than liver biopsy.[21,27] Although the gold standard test is the liver biopsy, it was not used in this project because it is rarely used in clinical practice for the analysis of NAFLD.

Data analysis was performed as qualitative statistic. If the project was analyzed in a quantitative statistic manner, likely p values would be smaller and further statistically relevant ($p<0,05$).

6. Conclusion

Cardiovascular risk factors of MS may be involved in the development of HS. Regardless of age, gender and presence of arterial hypertension, patients with glucose intolerance or diabetes mellitus are more likely for having HS than patients with normal glucose levels.

RESUMO

Modelo do estudo: estudo transversal de base populacional realizado em ambulatórios e unidades de saúde em Curitiba - Paraná - Brasil.
Objetivo: evidenciar quais são os principais fatores de risco cardiovascular da síndrome metabólica (SM) associados à esteatose hepática (EH).
Metodologia: 75 pacientes com SM, sob tratamento ou não, após preencher os critérios de inclusão ou exclusão da pesquisa, foram submetidos à ultrassonografia hepática para investigar a presença de EH. Em seguida, foi avaliada a relação entre EH e fatores de risco cardiovasculares da SM.
Resultados: independente da idade, gênero e hipertensão arterial, pacientes com intolerância à glicose ou diabetes mellitus têm maior risco de EH que os pacientes com glicose sérica normal ($p<0,05$). Menos relevante, hipertensão também demonstrou associação com EH.
Conclusão: fatores de risco cardiovasculares da SM podem estar associados à EH. A alteração no metabolismo da glicose parece ser o fator fisiopatológico mais importante associado ao dano hepático.

Palavras-chave: Síndrome X Metabólica. Esteatose Hepática. Fatores de Risco / Doenças Cardiovasculares. Ultrassonografia. Diabetes Mellitus Tipo 2. Resistência à Insulina.

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