Risk factors associated with hepatic steatosis; a study in patients in the Northeast Brazil

M.ª do S. Alves de Carvalho1, P. Coelho Cabral1, I. Kruze Grande de Arruda1, M.ª Goretti Pessoa de Araújo Burgos1, A. da Silva Diniz1, J. R. Barros Pernambuco2 and P. I. Cabral de Lira1

2Department of Clinical Medicine. UFPE. Recife. PE. Brazil.

Abstract

Introduction: Although there are several studies in the international literature regarding hepatic steatosis, few large-scale studies of risk factors are available.

Objective: To verify potential risk factors associated with hepatic steatosis, such as: alcohol consumption, overweight, dyslipidemia, hypertension, and type 2 diabetes mellitus.

Methods: This is a case series study including a control group (without hepatic steatosis), carried out at the gastroenterology outpatient clinic in Northeast Brazil. The sample was composed of 219 patients with hepatic steatosis and 82 without the disease.

Results: There was an association between hepatic steatosis and socioeconomic status. Prevalence Ratio (PR) for family income ≤ 2 minimum wage was (PR = 1.35 CI 95%, 1.18-1.54) and education level < primary education (PR = 1.44, CI 95%, 1.27-1.64). Regarding anthropometric and clinical characteristics and lipid profile, there was an association with overweight (PR = 1.59, CI 95%, 1.38-1.83), abdominal circumference in the range of very high risk (PR = 2.28, CI 95%, 1.68-3.09), hypertension (PR = 1.30, CI 95%, 1.15-1.48) and type 2 diabetes mellitus (PR = 1.23, CI 95%, 1.07-1.64), low HDL-cholesterol (PR = 1.96, CI 95%, 1.55-2.48), hypertriglyceridemia (PR = 2.10, CI 95%, 1.64-2.68). In the regression model three variables remained independently associated to hepatic steatosis, abdominal circumference in the range of very high risk (PRAdjusted = 1.74), low HDL-cholesterol (PRAdjusted = 1.39) and overweight (PRAdjusted = 1.28).

Conclusion: The results showed an association of hepatic steatosis with some risk factors, being abdominal circumference (very high risk) the most strongly associated, followed by low HDL-cholesterol and overweight.

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Key words: Hepatic steatosis. Risk factors. Abdominal obesity. Body mass index.

Correspondence: Poliana Coelho Cabral.
Rua Conselheiro Eribas 131 Casa Amarela.
CEP: 52070-400. Recife. PE. Brazil.
E-mail: cabralpc@yahoo.com.br

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Resumen

Introducción: Aunque hay varios estudios en la literatura internacional sobre la esteatosis hepática, pocos estudios a gran escala de factores de riesgo están disponibles.

Objetivo: Verificar potenciales factores de riesgo asociados a la esteatosis hepática como: consumo de alcohol, exceso de peso, dislipidemia, hipertensión arterial y diabetes mellitus tipo 2.

Método: Estudio del tipo serie de casos, incluyendo un grupo control (sin esteatosis hepática), realizado en el ambulatorio de gastroenterología de un hospital universitario en el nordeste brasileño, compuesto por 219 pacientes con esteatosis hepática y 82 sin la enfermedad.

Resultados: Hubo asociación entre la esteatosis hepática y condiciones socioeconómicas, el renta familiar ≤ 2 salarios mínimos presentó Razón de Prevalencia (RP) = 1,35, IC 95%, 1,18-1,54) y escolaridad < 1º grado (RP = 1,44, IC 95%, 1,27-1,64). Respecto a las características antropométricas, clínicas y perfil lipídico, hubo asociación con exceso de peso (RP = 1,59, IC 95%, 1,38-1,83), circunferencia abdominal en el intervalo de muy alto riesgo (RP = 2,28, IC 95%, 1,68-3,09), hipertensión arterial (RP = 1,30, IC 95%, 1,15-1,48) y diabetes mellitus (RP = 1,23, IC 95%, 1,07-1,64), bajo HDL-colesterol (RP = 1,96, IC 95%, 1,55-2,48), hipertrigliceridemia (RP = 2,10, IC 95%, 1,64-2,68). En el modelo de regresión con factores de riesgo para la esteatosis hepática, se constató que tres variables permanecieron independientemente asociadas, circunferencia abdominal en el intervalo de muy alto riesgo (RP ajustada = 1,74), bajo HDL-colesterol (RP ajustada = 1,39) y sobrepeso (RP ajustada = 1,28).

Conclusión: los resultados muestran una asociación de la EH con algunos factores de riesgo, destacándose la circunferencia abdominal en el intervalo de muy alto riesgo seguidos por el bajo HDL-colesterol y el exceso de peso.

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Palabras clave: Estesatosis hepática. Factores de riesgo. Obesidad abdominal. Índice de masa corporal.
Risk factors and hepatic steatosis


Abbreviations

HS: Hepatic steatosis.
NAFLD: Non-alcoholic fatty liver disease.
T2DM: Type 2 diabetes mellitus.
USG: Ultrasonography.
MHz: Mega hertz-frequency measurement.
AST: Aspartate aminotransferase.
ALT: Alanine aminotransferase.
GGT: Gamma-glutamyl transferase.
ALP: Alkaline phosphatase.
AgHbs and Anti-HCV: Viral hepatitis markers.
TC: Total cholesterol.
LDLc: LDL cholesterol.
HDLc: HDL cholesterol.
TG: triglycerides.
FG: fasting glucose.
BMI: Body Mass Index.
WHO: World Health Organization.
AC: Abdominal circumference.
NCCD: Non-communicable chronic diseases.
PR: Prevalence ratios.
NCEP: National Cholesterol Education Program.
MS: Metabolic syndrome.
IR: Insulin resistance.

Introduction

Hepatic steatosis (HS) is characterized by lipid deposition, particularly triglycerides, in the hepatocytes of the liver parenchyma, exceeding 5% of the liver weight. HS can be accompanied by cellular inflammatory infiltration, hepatocellular ballooning as well as pericellular and perisinusoidal fibrosis, including cirrhosis, similar to that observed in alcoholic hepatitis. Although HS has been identified as a component of non-alcoholic fatty liver disease (NAFLD), it can affect both alcoholic and non-alcoholic patients and other associated factors, such as obesity, type 2 diabetes mellitus (T2DM), and hyperlipidemia.

HS occurs in parallel to obesity and insulin resistance, the two most common risk factors of this type of hepatic disease, and ranges from 16.4% in normal-weight population to 75.8% in obese patients who do not consume alcohol. In Europe, Australia, North America, South America and Japan, it affects 10 to 40% of the population. In Brazil, data regarding HS prevalence are lacking; however, it is thought to be high since there is an increasing prevalence of obesity, type 2 diabetes, and metabolic syndrome in the population.

Also, the central obesity or more precisely the visceral fat appears to be involved in the development of HS independent of overall obesity, which is defined by the BMI. In addition, visceral adipocytes have been shown to be more resistant to insulin and associated with elevated levels of inflammatory mediators compared with subcutaneous adipocytes.

Recent evidence supports an association between HS and atherosclerosis. In a large study by Kim et al., HS was an independent predictor of elevated coronary artery calcification and there was a positive correlation between level of this calcification and severity of HS.

Although there are several studies in the international literature regarding HS, few large-scale studies of risk factors are available, especially in the Brazilian context. Therefore, the objective of this study was to identify the potential risk factors associated with HS in patients assisted at outpatient clinics in order to contribute to the development of strategies based on preventive and therapeutic measures, thus, leading to improved quality of life and reduced public health costs.

Methods

Study design and sample

This case series study was carried out at the gastroenterology outpatient clinic of a university hospital in Northeast Brazil between November 2009 and April 2010. 219 patients were select for the study under the following eligibility criteria: (i) patients diagnosed with HS assessed by ultrasonography (USG); (ii) to be 20-80 years old; (iii) to have no viral or non-viral chronic hepatitis, metabolic, self-immune hepatic diseases, chronic and consumptive pathologies; (iv) to have no toxicodependency, exposure to toxic substances, hemochromatosis or other diseases that may affect the hepatic histology; (v) to have no use of medications such as corticoids, diltiazem nifedipine, amiodarone.

The control group comprised 82 apparently healthy volunteers of both sexes, selected from among employees of the same hospital or among relatives of patients, with socioeconomic status and age similar to those of the patients with HS. To compose the control group all participants also underwent a USG, with negative diagnosis for HS. The choice of age range between 20 and 80 years was due to the fact that the gastroenterology clinic where the study was conducted only attends patients over 20 years and we found no case more than 75 years old.

The diagnosis criteria for HS included liver echogenicity exceeding that of the renal cortex and spleen, attenuation of the ultrasound wave, loss of definition of the diaphragm and poor delineation of the intrahepatic architecture. USG was performed using Aloka SSD 500 with 3.5 MHz convex transducer (mega hertz-frequency measurement unit equivalent to 1 million cycles per second), which is related to the speed of the device processor.

All patients diagnosed with HS, who represented the case group, were included and the information from medical records was transcribed for the filling out of a questionnaire regarding socioeconomic, demographic, clinical, and biochemical conditions, as well as hepatic...
and anthropometric functions of each patient. Data from subjects without HS (control group) were obtained from interviews with volunteers according to the eligibility criteria.

Laboratory examinations included aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), viral hepatitis markers (AgHbs and Anti-HCV), total cholesterol (TC), LDL cholesterol (LDLc), HDL cholesterol (HDLc), triglycerides (TG) and fasting glucose (FG).

Weight was measured using a FILIZOLA® scale with capacity of 150 kg and precision of 100 g. Height was measured using an anthropometer attached to the scale, with capacity of 1.90 m with precision in millimeters. All patients were asked to stand straight with their heels together wearing no shoes. Both weight and height were measured according to techniques described by Lohman et al.10 BMI was obtained by weight in kg divided by height squared in meters. Participants were considered overweight if BMI ≥ 25 kg/m², according to the classification for adults proposed by the World Health Organization (1997).11 For older individuals, Lipschitz (1994) classification was used, considering the cutoff points of BMI ≥ 27 kg/m².12 Abdominal circumference (AC) was obtained using a non-extensible tape measure positioned at the midpoint between the iliac crest and the last rib. Abdominal obesity was diagnosed by the cutoff points established by the WHO (1997)13 as follows: very high risk ≥ 102 cm (males), ≥ 88 cm (females) for both groups, since they were patients with high prevalence. All anthropometric measurements were collected by the researcher responsible for the study.

Both case and control patients were considered hypertensive if they had been previously reported on medical records and/or interviews and were taking antihypertensive medications, considering systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg.14 The American Diabetes Association ADA (2004)15 criteria were used for diagnosing diabetes mellitus, as well as data from records with defined diagnosis (case) and interview answers based on medical history. Diagnosis for dyslipidemia was interpreted using the reference values for National Cholesterol Education Program-NCEP.16 Individuals who smoked at least one cigarette a day for at least one year were considered smokers. Those who had never smoked were considered non-smokers. Alcohol consumption was collected according to data from medical records (case) and interview answers (control), by means of affirmative and negative answers.

Data were double entered and verified using VALIDATE module (Epi-info, version 6.04) to check data consistency and validation. Statistical analysis was performed using EPI-INFO, version 6.04, SPSS version 13.0 and STATA 7.0. Poisson regression was used to assess the association between risk factors and the prevalence of HS. Prevalence ratios (PR) were calculated using a robust method and the confidence interval of 95% was presented. The significance level was set at 5%. All variables with p < 0.20 at the non-adjusted univariate analysis were selected for multivariate analysis. Stepwise backward was used in adjusted analysis. The final model was composed of variables associated with a value of p < 0.05.

This study was approved by the ethics committee of the Health Sciences Center of the Federal University of Pernambuco (Process Nº 333/2009), being the procedures employed in the study in accordance with the ethical standards for human studies. All participants signed a written informed consent.

Results

A sample of 219 patients and 82 controls were studied. The losses occurred in the control group (n = 20) during the diagnosis phase, because when performing ultrasound showed HS giving rise to the case group. The demographic variables gender and age were not considered in the analysis due to the large heterogeneity found between case and control groups which could distort the results. When assessing hepatic function by enzyme analysis, AST was altered in 24.3% and 2.8% of case and control patients, respectively (PR = 1.73 CI 95% 1.45-2.07) and ALT in 45.6% and 12.0% of case and control groups, respectively (PR = 1.66 CI 95% 1.35-2.05).

Table I shows socioeconomic and lifestyle variables associated with the occurrence of HS in the univariate analysis. Among socioeconomic variables, participants with family income ≤ 2 minimum wage (PR = 1.35 CI 95% 1.18-1.54) and those with education level < primary school (PR = 1.44 CI 95% 1.27-1.64) showed increased risk for HS. Regarding lifestyle variables (alcohol consumption and smoking), no significant statistical association with HS was found.

Table II shows anthropometric, clinical characteristics and lipid profile of the patients. Individuals assessed by the BMI ≥ 25 kg/m² for adults and BMI ≥ 27 kg/m² for older individuals (PR = 1.59, CI 95% 1.38-1.83), as well as individuals assessed by the AC (very high risk ≥ 102 cm (males), ≥ 88 cm (females), showed higher risk for HS (PR = 2.28 CI 95% 1.68-3.09), whereas AC showed twice the risk. The variables hypertension (PR = 1.30 CI 95% 1.15-1.48) and diabetes mellitus (PR = 1.23 CI 95% 1.07-1.64) showed a significant association with HS. Regarding lipid profile, HS showed no significant association with total cholesterol. On the other hand, patients with low HDL-c for values under the cutoff points of normality < 40 mg/dl (PR = 1.96 CI 95% 1.55-2.48), had a two-fold risk for the development of the disease.

Table III shows that after adjustment of the final model obtained by Poisson regression, three variables remained independently associated with HS, AC – very high risk; low HDLc and overweight. According to this model, AC in the range of very high risk is strongly
associated with HS (PR\_adjusted = 1.74 CI\_95\% 1.11-2.08), followed by low HDLc (PR\_adjusted = 1.39 CI\_95\% 1.01-1.93) and overweight (PR\_adjusted = 1.28 CI\_95\% 1.01-1.68).

Discussion

HS is most of the times asymptomatic and can be observed in both men and women and in several age groups. It is diagnosed by USG and serum biochemical alterations of hepatic enzymes in routine examinations, although normal exams do not exclude the presence of the disease, increases in ALT and AST are the most frequent markers.2

In our sample, alterations in the AST (24.3%) and ALT (45.6%) were found in patients with HS. Similar results were found by Bellentani,3 that observed high prevalence of ALT alterations, with a 3.76 times higher risk for patients with HS.

It is interesting to note that the patients of the present study have low socioeconomic status which may increase the risk of developing HS. This can be explained by the fact that low family income leads to high-calorie food intake, including saturated fat and simple carbohydrates.7 In our data, the lower education levels raised the risk for the development of HS by over 1.5. Obesity is the most significant single risk factor for the development of HS. Other predictive factors for liver disease progression have been identified, but at present the treatment of obesity remains the most effective preventive strategy.21 Because of the increased prevalence of HS with metabolic syndrome (MS) components such as obesity, type 2 diabetes mellitus (T2DM) and dyslipidemia, one can understand that the etiopathogenic mechanisms between these variables can be common.22

Besides BMI, the AC measurement is used to assess body fat distribution and nutritional risk, which determines a significantly statistical association with the amount of visceral fat, which is responsible for insulin resistance and MS.23 Increases in BMI and AC are associated with metabolic alterations (hyperinsulinemia, hypertriglyceridemia and T2DM) and HS. These indexes are related to the severity of steatosis and steatohepatitis.24

In the regression model, AC was considered an independent risk factor for steatosis. Similar results were

### Table I

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<td>77</td>
<td>29.3</td>
<td>263</td>
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</table>

Note: Total number of participants in each variable is different because of the number of respondents.

†Prevalence Ratio.
‡Confidence Interval.
Family Income = Minimum wage was US$ 425.00 at the time of the study.
*Pearson’s chi-squared test.
found by Bellentani et al., with a risk almost three times higher. However, Gunji et al. found no association between HS and AC, which could explain by the methodological differences among the studies.

The results of the present study showed that hypertension is associated with HS, with prevalence of 30% in these patients, who presented risk higher than 1.0 for HS. Similar findings were shown by Donati et al., who demonstrated that obese and hypertensive patients were at high risk of developing HS, which was observed in 30.9% of the hypertensive non-diabetic patients compared to 12.7% of normotensive controls. Nevertheless, HS is not commonly investigated in hypertensive patients, mainly those who do not have components of MS. As for hypertensive patients, it is important to consider the diagnosis for HS and examine the slightest increases in serum transaminases. This is important for patients at risk of HS progression, mainly those with central obesity and T2DM. It is important to note that hypertension is a known aspect of MS and that 50% of non-obese hypertensive patients have hyperinsulinemia. In addition, abdominal (central) obesity is a characteristic of MS, being a risk factor for hypertension.

Regarding T2DM, the results of the present study showed an association with HS, with a prevalence of 23% and a significant risk factor, which can be explained by the metabolic disorders derived from the disease, as well as insulin resistance. It is interesting to note that HS is an indicative of T2DM, independent on the classic risk factors, suggesting an early marker of atherosclerosis, caused by the increase of hepatic enzymes preceding cardiovascular disease. Mechanisms that explain the association between HS and insulin resistance are the lipid metabolism harmed at hepatic level and the increase of oxidative stress.
can lead to fat deposition and activation of several cytokines. These factors would consequently lead to the progression of the inflammatory process and fibrosis.28

Dyslipidemia was associated with HS, i.e. low HDLc showed almost two-fold the risk and TGR over two-fold the risk for the development of the disease. Although high CT and LDLc showed no statistical significance in this study, the literature has shown strong evidence that metabolic disorders are considered risk factors for HS.29 Similar results were found by Cotrim et al.,30 suggesting that dyslipidemia is one of the most frequent and significant risk factor for the development of HS, correlating with TG levels, MS, and severity of hepatic disease. Since these factors are associated with MS, HS has been suggested to be another component of this syndrome.

Although LDL was not significantly associated with HS, triglycerides showed significant clinical importance, with a two-fold risk for HS. Similar results were observed by Bellentani et al.,3 with values 2.48 higher.

The association between HS and dyslipidemia ranges from 20 to 92%, being the hypertriglyceridemia the most common alteration.30 Altered triglycerides and diabetes have shown to be an important trend as predictors. It is important to emphasize that in this study we used in the estimation of HS risk the Poisson regression, while other studies has estimated by means of odds ratio and logistic regression, which overestimate the risk.

Among the study limitations, we can mention, the first non-use of liver biopsy in case definition, because it is an invasive, expensive and not without risk. Second, the fact we have not evaluated the dietary intake of patients nor the physical exercise and finally the small number of individuals in the control group due to the difficulty in finding people that met the inclusion criteria for the control group in the hospital environment. With respect to food intake data from an unpublished master’s thesis with a similar population and environment. With respect to food intake data from an unpublished master’s thesis with a similar population and environment.

In the adjusted Poisson regression, AC in the range of very high risk was considered the most significant risk factor independently associated to steatosis, showing an \( \text{PR}_{\text{adjusted}} = 1.74 \text{ CI}_95\% 1.11-2.08 \), followed by HDLc \( \text{PR}_{\text{adjusted}} = 1.39 \text{ CI}_95\% 1.01-1.93 \), and overweight \( \text{PR}_{\text{adjusted}} = 1.28 \text{ CI}_95\% 1.01-1.68 \).

Final considerations

Overweight and central obesity and low HDL are risk factors for HS, showing the need for screening these individuals for HS aimed at early diagnosis and no the disease progression for complications such as steatohepatitis and fibrosis. Thus, prevention and control of excess body weight are highly recommended to combat the problem.

References