Nonalcoholic fatty liver disease in Brazil. Clinical and histological profile

Helma P. Cotrim,* Edison R. Parise,* Claudia P.M.S. Oliveira,* Natalhie Leite,*
Ana Martinelli,* João Galizzi,* Rita de Cássia Silva,* Ângelo Mattos,* Leila Pereira,* Waldir Amorim,*
Cláudia Ivantes,* Francisco Souza,* Marcelo Costa,* Lizomar Maia,* Mário Pessoa,* Frederico Oliveira*

* Brazilian Group for the Study of the NAFLD-Sociedade Brasileira de Hepatologia-Brazil.

ABSTRACT

Background. The epidemiology and clinical characteristics of nonalcoholic fatty liver disease (NAFLD) in South America are not well known. Brazil is a largest country in this part of the world and the present study aimed to contribute with this information. **Methods.** This descriptive study included patients from medical centers around Brazil, who had diagnosis of NAFLD. They were selected from chart review and also prospectively in Hepatology out-clinics. Patients with history of alcohol intake and others liver diseases were excluded. Histological diagnosis included: steatosis or steatohepatitis (steatosis, ballooning of hepatocytes or fibrosis). The criteria to perform a liver biopsy was ALT or AST > 1.5 x normal levels. **Results.** A total of 1280 patients from 16 Brazilian centers and all five regions were included. The mean age was 49.68 ± 13.59 years; 53.3% were males and 85% were asymptomatic. Hyperlipidemia was observed in 66.8% cases, obesity in 44.7%, overweight in 44.4%, diabetes in 22.7%, and toxins exposure in 10%. Metabolic syndrome was observed in 41.3% cases. Elevated levels of ALT, AST and GGT were observed in 55.8%, 42.2% and 63.1% cases, respectively. Liver biopsy performed in 437 cases showed: isolate steatosis in 42% cases, steatohepatitis in 58% and 27% of them also presented fibrosis. Cirrhosis was observed in 15.4% and hepatocellular carcinoma in 0.7%. **Conclusions.** NAFLD in Brazil is more frequent in asymptomatic males; steatohepatitis with fibrosis and cirrhosis were a significant diagnosis. The genetic predisposition and lifestyle should be influenced in the spectrum; however these findings deserve a future investigation.

Key words. Nonalcoholic steatohepatitis. Metabolic Syndrome. Insulin resistance. NAFLD in South America. NASH. MeSH.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) may affect any age, ethnic group or country. The prevalence estimated of NAFLD in the general population ranges from 3 to 24%.[1,2] This liver disease appears to be most strongly associated with obesity and insulin resistance states including diabetes and with other features of the metabolic syndrome, such as high triglycerides and low HDL. It appears to be more common in men, and it increases with increasing age and after menopause in women. More advanced stages of NAFLD are associated with older age, higher body mass index, diabetes, hypertension, high triglycerides, and/or insulin resistance.[3,4]

The high frequency of NAFLD was described in Mexican Americans when it was compared with African Americans and non-Hispanic Caucasian.[5] The higher incidence of metabolic syndrome also has been described in Amerindian populations,[2] and it may suggest that NAFLD also is common throughout Latin America.

This high prevalence of NAFLD, however, is not exclusive to the Caucasian western population. NAFLD among healthy Japanese adults has reached epidemic proportions in different populations around the world.[6] In the South Asian Indian some studies have shown that this population also is at high risk for development of metabolic syndrome, type 2 diabetes and cardiovascular disease, suggesting the possibility of gene variants that impact shared molecular pathways amongst these clinic entities.[7,8]

In Brazil, over the last two decades, changes in human behavior and lifestyle have resulted in a dra-

Correspondence and reprint request: Helma Pinchemel Cotrim, M.D.
Programa de Pós Graduação em Medicina e Saúde-PPGMS
Rua Padre Feijó. Hospital Prof. Edgar Santos, 5°. andar
CEP: 40110-170-Salvador-Bahia-Brazil
Fone/Fax: 5571-3247-4634
E-mail: hpcotrim@ufba.br

matic increase in the prevalence of obesity. According the last Census by Brazilian Institute of Geography 40% of Brazilian adult population is overweight and 8.9% males and 13% of females were obese.[9,10]

OBJECTIVE

The present study aimed to describe the clinical and histological aspects of NAFLD in Brazil and to contribute with information about characteristics of NAFLD in this part of South America.

PATIENTS AND METHODS

Study design and patients selection

This descriptive study included patients with NAFLD diagnosis from the five regions and sixteen centers around Brazil. They came from states of South (Rio Grande do Sul and Paraná), North (Pará), Northeast (Paraiba, Pernambuco and Bahia), Southeast (São Paulo, Rio de Janeiro e Minas Gerais) and Center (Federal District-Brasilia). The patients were selected from chart review and prospectively from hepatology out-clinics.

The study was approved by the Ethics Committee for Medical Research-Programa de Pós Graduação em Medicina e Saúde–Universidade Federal da Bahia, Brazil and in all centers that the patients were included.

NAFLD criteria

Patients were included when they presented the following criteria: presence of steatosis on abdominal ultrasound and alterations of hepatic enzymes (ALT/ALT); history of alcohol intake ≤ 20 g/day; exclusion of others liver diseases (B and C virus infection, haemochromatosis, Wilson's disease and autoimmune hepatitis).

The pattern of alcohol consumption was obtained from medical consultations with physicians and inter-views with patient relatives.

Patients evaluation

A questionnaire was used to obtain medical history, demographic data, to evaluate alcohol consumption, and the use of medications. Data of physical exam, laboratory tests and abdominal ultrasound were included.

- **Anthropometry.** Body mass index (BMI) was calculated as body weight (kg)/height (m^2) and patients were categorized as normal weight (< 25.0 kg/m^2), overweight (≥ 25.0 and ≤ 29.9 kg/m^2) and obese (≥ 30.0 kg/m^2). The obesity was classified in grade I (30.0-34.9 kg/m^2), grade II (35.0–39.9 kg/m^2) and grade III (≥ 40.0 kg/m^2).[11]
- **Laboratory tests.** ALT, AST, GGT, fasting glucose, insulin, and a lipid profile (triglycerides, total cholesterol, HDL-C (high-density lipoprotein), LDL-C (low-density lipoprotein), serological tests for hepatitis B and C, ferritin and transferin saturation.

Insulin resistance (IR) was evaluated by calculating the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR).[12] To apply HOMA-IR to the insulin resistance index, HOMA-IR ≥ 3 was used.[13]

Metabolic syndrome was evaluated in all cases and defined as ATP III criteria.[14]

Liver biopsy and histological diagnosis

Criteria for liver biopsy included serum ALT/AST determinations greater than 1.5 the normal level in more than two occasions, separate by at least 1 month.

Liver tissue was fixed in 4% formol saline, processed in an auto-technicon apparatus and stained with hematoxylin-eosin (HE) and Masson's trichrome. The following histological variables were analyzed: macro- and microvacuolar fatty change, zonal distribution, foci of necrosis, portal and perivenular fibrosis, and inflammatory and fibrotic infiltrate with zonal distribution. All specimens were scored by a liver pathologist with expertise in NAFLD.

A preview protocol to histological diagnosis was done and sent for all Brazilian pathologists, and the participant's centers have used the same histological score.

The patients were classified as having steatosis; steatohepatitis (steatosis, ballooning of hepatocytes, and inflammatory infiltrated with or without fibrosis). Fibrosis was classified into 5 stages:

- **S 0:** Without fibrosis.
- **S 1:** Fibrosis limited to the perivenular or per sinusoidal area in zone 3.
- **S 2:** Perivenular and per sinusoidal fibrosis with portal fibrosis.

- **S 3:** Bridging fibrosis.
- **S 4:** Cirrhosis.[15]

- **Statistical analysis.** The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA, and Release 16.0.2, 2008) and Prism (Graph Pad Inc., San Diego, CA, USA, Release 5.01) software program, version 12.0, was used to analyze the data in this study.

RESULTS

The study included 1280 patients with diagnosis of NAFLD around Brazil. The mean age of the patients was 49.68 ± 13.59 years, 53.3% were males and 85% were asymptomatic. The evaluation of the risk factors showed: hyperlipidemia in 66.8% cases, obesity in 44.7%, overweight in 44.4% and diabetes in 22.7%. Toxins exposure was related for 10% of the cases. Metabolic Syndrome was observed in 41.3% cases.

Elevated levels of ALT, AST and GGT were observed in 55.8%, 42.2% and 63.1% cases, respectively.

The table 1 shows demographic, clinical and biochemical parameters in patients with isolate steatosis and NASH. Some differences was observed in age and BMI when compared patients with NASH and steatosis. NASH patients also presented increased liver enzymes (AST and GGT), higher HOMA-IR index and lower HDL-cholesterol. However, statistical significance was not achieved.

Liver biopsy was performed in 437 cases of 1280 patients included. Isolate steatosis was observed in 41.7%, steatohepatitis without fibrosis in 31.1% and
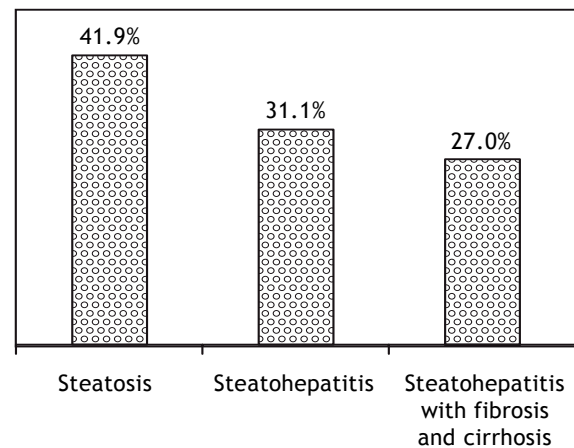
Figure 1. Histological NAFLD diagnosis in Brazilian patients (n = 437).
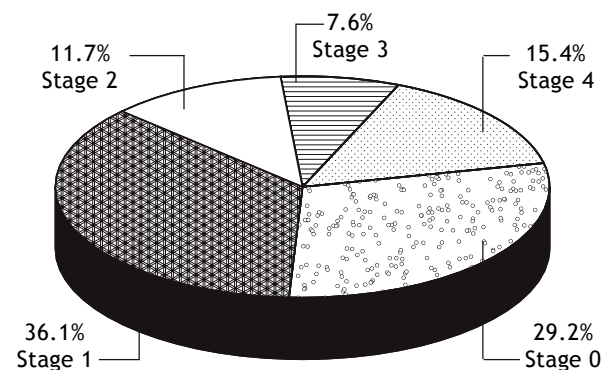
Figure 2. Stages of fibrosis in NAFLD Brazilian patients.

Table 1. Demographic, clinical and biochemical data of patients with histological diagnosis of steatosis and steatohepatitis (NASH) (n=1280).

Variable	Patients with steatohepatitis	Patients with steatosis
Age (years)	56 ± 11.4	49 ± 11.9
Diabetes (glucose>110/dl)	37 (58%)	10 (37%)
Dislypidemia*	48 (75%)	16 (59%)
High-blood pressure*	41 (64%)	9 (33%)
BMI (body mass index)	29.6 ± 4.2	28 ± 3.9
Fasting glucose (mg/dl)	128 ± 52.3	144 ± 99.4
HOMA-IR >2,5	48 (75%)	17 (63 %)
AST (U/L)	46 ± 3	42 ± 3
ALT (U/L)	52 ± 3	64 ± 6
Total cholesterol (mg/dl)	200 ± 43.3	204 ± 38.2
HDL (mg/dl)	43 ± 12.5	52 ± 15.7
LDL (mg/dl)	113 ± 41.7	124 ± 35.1
Triglyceride (mg/dl)	195 ± 138.3	178 ± 108.3
Metabolic Syndrome*	39 (61%)	10(37%)

* ATP III (ref 15).

36

Cotrim HP, et al. Annals of Hepatology, 2011; 10 (1): 33-37

steatohepatitis with fibrosis in 27% (total of NASH-58%) (Figure 1). Stages of fibrosis: S zero: 29.2%, S1: 36.1%, S2 in 11.7%, S3 in 7.6%, cirrhosis in 15.4% (Figure 2) and three of them have hepatocellular carcinoma (HCC).

DISCUSSION

The present study involves a large series of patients with NAFLD in Brazil and represents a contribution to describe a profile of these patients in South America, where there are no much information about this liver disease.

Among the 1280 cases of the NAFLD evaluated, the majority of them were asymptomatic male; with mean age of fifty years, and the most frequent risk factor to NAFLD were hyperlipidemia, obesity or overweight. Metabolic Syndrome (ATP III) was observed in 41.3% cases. All these features in Brazilians NAFLD patients are similar to other countries.[15] A population-based study performed in the USA demonstrates that up to 34% of the adult general population has excessive fat accumulation in the liver, mostly unrelated to alcohol abuse.[17] This high prevalence of NAFLD is most likely a result of the increasing prevalence of obesity, type 2 diabetes and metabolic syndrome in the general US population.[3]

In Japan, the results of a series of cross-sectional data obtained from patients presenting for a health checkup demonstrated that the prevalence of fatty liver on ultrasound increased from 12.6% in 1989, peaked at 30.3% in 1998, and was 28.4% in 2000.[6] Furthermore, unless uniform data are available over the time period, the prevalence estimates of NAFLD could also be affected by a change in ascertainment based on increased awareness. Nevertheless, because of the marked increase in the prevalence of both obesity and type 2 diabetes, it is assumed that the prevalence of NAFLD is increasing as well.[16]

All these studies have suggested that ethnic differences may have a role in susceptibility to NAFLD, especially the progressive disease that cannot be explained simply on the basis of diet or socioeconomic differences. The higher incidence of NASH in US populations of Hispanic origin relative to Caucasian, and a lower incidence in African-Americans, despite a higher rate of obesity have suggested it.[17,18]

This Brazilian study showed that the majority of patients were males, with mean age of fifty years. This result are similar a population-based studies,[19,20] however it is different of others studies also performed in patients selected of out-clinics, that have shown cases of NAFLD most common in middle-aged women.

The ethnic differences were not studied in this simple. In Brazil, the mixed racial heritage is recognized and it is an important subject to be investigated. It would be useful in future to identify them more precisely using accepted racial origin genetic markers, and it will be relevant to be used to set public policy in regards to population screening and/or public health intervention strategies.

Steatohepatitis with fibrosis and cirrhosis was frequent in patients, who underwent liver biopsy and three of hepatocellular carcinoma was observed. These results showed that NAFLD has a high potential of progression in this population. The mechanism of this progression is complex and remains to be understood. It clearly is multifactorial. Many cases are related to a lifestyle; however, it is likely that genetic predisposition plays an important role in determining which individuals have increased risk for development of cirrhosis and HCC in patients with NAFLD.

CONCLUSION

The study contributes with information about characteristics of NAFLD in South America. It describes the profile of NAFLD in Brazil, the largest country in South America. The patients did not present symptoms, however, the most frequent histological diagnosis in these simple was steatohepatitis with fibrosis and a significant number of cases already presented cirrhosis. These findings deserve a future investigation. Although the present study did not aim to evaluate pathogenic mechanisms, the genetic predisposition and lifestyle should be influenced in the spectrum of NAFLD in Brazil and the high frequency of severe disease in this population.

ABBREVIATIONS

- **NAFLD:** Nonalcoholic Fatty Liver Disease.
- **NASH:** Nonalcoholic Steatohepatitis.
- **MS:** Metabolic Syndrome.
- **BMI:** Body Mass Index.
- **IR:** Insulin Resistance.
- **ALT:** Alanine aminotransferase.
- **AST:** Aspartate aminotransferase.
- **GGT:** Gamma Glutamyl Transpeptidase.
- **HDL-C:** High-density lipoprotein cholesterol.
- **LDL-C:** Low-density lipoprotein cholesterol.

- **HOMA-IR-IR:** Homeostasis Model Assessment of Insulin Resistance.
- **SPSS:** Statistical Package for Social Sciences.

ACKNOWLEDGMENTS

REFERENCES

1. Ong JP, ZM Younossi. Epidemiology and natural history of NAFLD and NASH. *Clin Liver Dis* 2007; 11: 1-16.
2. Lazo M, JM Clark. The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis* 2008; 28: 339-50.
3. Adams LA, Angulo P. Recent concepts in non-alcoholic fatty liver disease. *Diabetes Med* 2005; 22: 1129-33.
4. Bugianesi E, Marchesini G, Arthur J. McCullough Insulin resistance: A metabolic pathway to chronic liver disease. *Hepatology* 2005; 42(5): 987-1000.
5. Weston SR, Leyden W, Murphy R, Bass NM, Bell BP, Manos MM, Terrault NA. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology* 2005; 41: 372-9.
6. Kojima S, Watanabe N, Numata M, et al. Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. *J Gastroenterol* 2003; 38: 954-964.
7. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Man CD, Cobelli C, Shulman GI. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci USA* 2006; 103: 18273-77.
8. Amarapurkar DN, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ, Goh KL. How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? *J Gastroenterol Hepatol* 2007; 22: 788-93.
9. Monteiro CA, Conde WL. A tendência secular da obesidade segundo estratos sociais: Nordeste e Sudeste do Brasil, 1975-1989-1997. *Arq Bras Endocrinol Metab* 1999; 186-94.
10. Sichieri R, Coitinho DC, Leão MM, Recine E, Everhart JE. High temporal, geographic, and income variation in body mass index among adults in Brazil. *Am J Public Health* 1994; 84: 793-8.
11. Seidell JC, Flegal KM. Assessing obesity: classification and epidemiology. *Br Med Bull* 1997; 53(2): 238-52.
12. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and b-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412-9.
13. Guidorizzi de Siqueira AC, Cotrim HP, Rocha R, et al. Nonalcoholic fatty liver disease and insulin resistance: importance of risk factors and histological spectrum. *Eur J Gastroenterol Hepatol* 2005; 17(8): 837-41.
14. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *J Am Med Assoc* 2001; 285: 2486-97.
15. Freitas LA & Cotrim HP. Atlas NASH: Esteato-Hepatite Não Alcoólica. São Paulo: Zambon; 2006, p. 1-80.
16. Falck-Ytter Y, Younossi ZM, Marchesini G, et al. Clinical features and natural history of nonalcoholic steatosis syndromes. *Semin Liver Dis* 2001; 21: 17-26.
17. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; 40: 1387-95.
18. Caldwell SH, Harris DM, Patrie JT, Hespenheide EE. Is NASH underdiagnosed among African Americans? *Am J Gastroenterol* 2002; 97: 1496-500.
19. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003; 98: 960-7.
20. Sundborn G, Metcalf PA, Gentles D, Scragg RK, Schaaf D, Dyall L, et al. Ethnic differences in cardiovascular disease risk factors and diabetes status for Pacific ethnic groups and Europeans in the Diabetes Heart and Health Survey (DHAH) 2002–2003 Auckland New Zealand. *NZ Med J* 2008; 121: 28-39.