The spectrum of non alcoholic fatty liver disease in morbidly obese patients. Prevalence and associate risk factors[1]

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ABSTRACT

PURPOSE: To determine the prevalence of non alcoholic fatty liver disease (NAFLD) and non alcoholic steatohepatitis (NASH) in morbidly obese patients undergoing bariatric surgery and to identify risk factors associated with the disease spectrum.

METHODS: Liver biopsy was performed in 60 patients who underwent bariatric surgery, after other causes of liver disease were excluded. Clinical, biochemical and histological features were evaluated.

RESULTS: NAFLD was detected in fifty-seven patients (95%) of the sample and forty patients (66.7%) of the total sample met the criteria for NASH. Perisinusoidal fibrosis was only found in three (7.5%) patients with NASH. The γGT was an independent predictive factor associated with the degree of hepatic steatosis. The variables such as dyslipidemia and ALT were independently associated with the presence of Mallory's corpuscles with the following values, respectively, OR 0, 05, 95% CI 0.002 to 0.75, P = 0.031 and OR 10, 99, 95% CI 1.44 to 83.93, P = 0.021.

CONCLUSIONS: Non alcoholic fatty liver disease seems to be an obese-related condition with approximately half of asymptomatic morbidly obese patients having histological NASH. The γGT was an independent predictor of the degree of steatosis.

Key words: Fatty Liver. Obesity, Morbid. Bariatric Surgery.

Introduction

Non alcoholic fatty liver disease (NAFLD) is considered one of the most common liver diseases, affecting a wide spectrum of patients worldwide. The estimated prevalence in the general population is 10 to 24% and 70% in obese patients[1,2]. Given the obesity epidemic, it is thought to become one of the most important liver pathology in the future. Increased levels of insulin, triglycerides, low HDL, type 2 diabetes glucose intolerance are considered important risk factors.

Approximately 60% of patients with NAFLD have metabolic syndrome[3]. There is a wide spectrum of liver histology in NAFLD, ranging from steatosis to steatohepatitis (NASH), advanced fibrosis and cirrhosis[4]. NASH is characterized by zone 3 macrovesicular steatosis, inflammatory changes, fibrosis and may ultimately lead to cirrhosis and hepatocelular carcinoma in the absence of alcohol use. NASH histological findings resolve or remit in approximately 80% of patients after bariatric surgery[5]. Several studies have been conducted to evaluate the real prevalence of NASH and NAFLD in severely obese patients that underwent bariatric surgery, when liver biopsy was performed. The prevalence varied from 27 to 98%, which may be explained by recent modifications in histological classification[6].

It is of great importance to predict and prevent the development of advanced liver diseases in obese patients. Determining the prevalence of NASH in those patients and further understanding the role of certain predictor factors is of interest, since it would aid in identifying patients at high risk. Furthermore, patients undergoing bariatric surgery would greatly benefit from strategies to allow detection of NASH and closer follow-up given they are at increased risk for the development of cirrhosis, hepatocellular and end-stage liver disease.

Therefore, the aim of this study was to prospectively determine the prevalence of NASH in obese patients undergoing bariatric surgery from Northeastern of Brazil and to identify risk factors involved in the development of NASH.

Methods

The study was approved by the Ethics Committee for Clinical Investigation at Ceara Federal University of Brazil. Informed written consent was obtained from every patient. Seventy eight patients from the Nucleus of Obese of state of Ceara scheduled to perform gastric bypass, after consultation with the surgeon, anesthetist and dietitians, were invited to participate in the study. Eighteen failed to meet the selection criteria. The study was composed of sixty consecutive patients with obesity class II or III, defined as a BMI> 35kg/m2 that were submitted to bariatric gastric bypass surgery type. Intraoperative fine-needle liver biopsy was performed in all patients using 16 gauge biopsy needle-22mm (Bard - Max Core).

Exclusion criteria included: daily alcohol consumption > 30 g in men or > 20 g in women[7] at the time of evaluation or for a period longer than two years at any time, hepatitis B or C, previous history or laboratory evidence for a specific liver disease, had undergone extensive small bowel resection or jejuno-ileal or were on parenteral nutrition. Additionally, patients were excluded if on tamoxifen, amiodarone, corticosteroids, high doses of estrogen, methotrexate and cyclins in the last six months or for a prolonged period of time.

Serology for hepatitis C (anti HCV), hepatitis B (HBsAg, anti HBc IgM, anti HBc IgG, anti HBs), autoantibodies (antinuclear antibodies, anti smooth muscle and anti mitochondrial) was performed. Iron study (ferritin and tranferrin saturation index), serum levels of AST, ALT, γGT, alkaline phosphatase, total bilirubin and fractions, protein fractions, and prothrombin time, ceruloplasmin, uric acid and TSH were evaluated. Additionally, lipid profile (HDL, LDL, VLDL and triglycerides) and glycemic profile (fasting glucose, glycated hemoglobin A1c). An assessment of past and present alcohol consumption was done.

Patients were diagnosed with diabetes mellitus (DM) type 2 or impaired fasting glucose, according to previous classification[8], with hypertension when they had a resting blood pressure greater than or equal to 140/90 mmHg in two measurements. Patients were considered dyslipedemic based on the criteria of the Brazilian Society of Cardiology. The diagnosis of metabolic syndrome followed the criteria established by the International Diabetes Federation 2005 [9]. Alcohol consumption was classified into four categories: 0, no consumption or sporadic, 1, and 10 g / day; 2 between 10-20g/dia; 3 between 20-30g/dia.Waist circumference (WC) was considered at risk when greater than 102cm in men and> 88cm in women and waist-quandril (WHR) was assessed by measuring waist circumference divided by hip circumference[10].

Histological evaluation

The material was fixed in paraffin and stained: H&E; periodic acid-Schiff after diastase (PAS-d), Perl's and Masson's trichrome, and examined by a single pathologist (RP), without prior knowledge of clinical and laboratory data of patients. All samples were deemed sufficient for the study by the pathologist. Patients were defined as having NAFLD when they had at least

steatosis on biopsy, which was graded on a scale of 0 to 3 according to the criteria of Brunt *et al*.[11]. NASH diagnosis was defined as the presence of at least three of the four initial histological findings: 1) predominant macrovesicular steatosis in zone 3, 2) neutrophilic lobular inflammation 3), hepatocellular ballooning, 4) perisinusoidal fibrosis, according to the criteria of Brunt *et al*.[11]. The degree of steatosis was assessed on a scale of 0 to 3. Zero, indicating no: 1, mild (<33% of parenchyma involved) 2, moderate (33% to 66% of parenchyma involved), and 3, severe (> 66% of parenchyma involved). The degree of lobular inflammation was also graded on a scale of 0 to 3. Zero: no inflammation; 1: 1 to 2 inflammatory foci; 2: 2 to 4 inflammatory foci; 3: > 4 inflammatory foci. The stage classification required an assessment of the degree of perisinusoidal fibrosis / portal on a scale of 1 to 4. Stage 1, perisinusoidal fibrosis / pericellular in zone 3, focal or extensive; 2 fibrosis, perisinusoidal / pericellular in zone 3 and portal fibrosis, focal or extensive, 3, perisinusoidal fibrosis / pericellular fibrosis in zone 3 and portal w / fibrosis bridge, focal or extensive; 4, cirrhosis with or without perisinusoidal fibrosis. The presence of portal fibrosis in the absence of perisinusoidal fibrosis was not considered for the stage of NASH but was studied separately. Thus, histological variables described above, constitute the system of grade and stage of NASH: Grades on a scale of 1 to 3 and stage scale of 1 to 4[11].

Statistical analysis

Results are expressed as mean ± SD and percentages. Comparisons of quantitative variables were made using the Student's *t*-test. Categorical variables and proportions were tested by the qui square and Fisher exact test. Multivariate analysis was tested using logistic regression. A *p*-value <0.05 was considered as statistically significant All variables with a P value of 0.25 or less in univariate analyses were included in the full logistic regression model, according to recommendations of the Hosmer-Lameshow goodness-of-fit-test. Odds ratio (OR) and 95% confidence intervals (CI) were used as an estimated of the risk. Analysis was performed with SPSS computer software (version 11.5).

Results

Seventy-eight patients were invited to participate in the study, one was excluded for presenting positive marker for hepatitis C during the evaluation, four decided not to carry out with surgery, two had inadequate biopsy sample and eleven did not undergo biopsy for various technical reasons. A total of 60 patients,

(73% female and 27% male) were considered for analysis.

Laboratory and demographic evaluation

Patients (mean age ± SD, 38.4 ± 11.6,) had a mean ± SD BMI of 44.2 kg/m2 ± 5.5, mean ± SD CC 125.8 ± 15.2 cm and mean ± SD of the sample WHR 0.93 ± 0.11. Thirty-four (56.6%) were hypertensive, twenty (33%) were type 2 diabetic or had changes in fasting glucose, thirty-one (51.7%) had altered lipid profile and thirty-nine (65%) fell within the metabolic syndrome. Thirty-eight (63.3%) were abstainers, and only one (1.67%) consumed between 20 - 30 g alcohol / day. The liver function tests together (AST, ALT, GGT, total bilirubin and fractions and alkaline phosphatase) were normal in twenty-seven patients (45%). γGT was raised in twenty-three (38.3%), ALT in twenty (33.3%), AST in eight (13.33%). The mean ratio of AST / ALT was 0.76 (range, 0.27 to 1.61), with the ratio AST / ALT> 1 detected in six (10%). Twenty-one (35%) had slightly elevated levels of ferritin and transferrin saturation index normal in all.

Histological data

Liver biopsy results are summarized in Table 1. Fifty-seven patients (95%) had hepatic steatosis. Twenty-four (42.1%) had moderate to severe steatosis. Forty (66.6%) met all histologic criteria for NASH as described by Brunt *et al*.[11]

TABLE 1 - Hepatic histologic characteristics in 60 morbidly obese patients.

Characteristics		Patients N	(%)
Steatosis -		57	95
Degree	Mild	33	57.9
	Moderate	18	31.6
	Severe	06	10.5
Inflammation		50	83.3
	Lobular	22	44.0
	Mixed	21	42.0
	Portal	07	14.0
Balooning		52	86.7
Mallory's Corpuscules:		09	15.0
NASH		40	66.7
Degree of NASH	I	32	80.0
	II	06	15.0
	III	02	5.0
Degree of fibrosis	1	02	5.0
	2	00	0.0
	3	01	2.5

Factors associated with the degree of hepatic steatosis

Table 2 shows the association of demographic, anthropometric and laboratory variables with the degree of hepatic steatosis (I versus II and III degree of hepatic steatosis). γGT was the only variable significantly and independently associated with the degree of steatosis (OR 0.04, 95% CI 0.003 to 0.63, P = 0.022)

TABLE 2 - Crude and adjusted odds ratios (OR) with 95% confidence interval (CI) for the degree of steatosis according to clinical and biological variable

Variables	Crude Odds Ratio (OR)			Adjusted Odds Ratio (OR)		
	OR	95% IC	*P*	OR	95% IC	*P*
Age	1.04	0.99 – 1.09	0.075	0.94	0.86 – 1.04	0.273
Gender	0.31	0.09 – 1.03	0.057	8.96	0.35 - 230,	0.186
Diabetes	2.26	0.74 – 6.84	0.151	0.65	0.07 – 5.59	0.693
BMI	2.52	0.97 – 6.56	0.059	0.26	0.04 – 1.81	0.174
WC	1.03	0.99 – 1.07	0.075	1.01	0.92 – 1.11	0.820
HDL-C	0.93	0.87 – 0.99	0.036	1.07	0.97 – 1.19	0.186
Triglycerides	1.00	0.99 – 1.01	0.099	1.00	0.99- 1.01	0.720
Uric acid	4.50	1.36 – 14.83	0.013	0.19	0.02 – 1.74	0.140
AST	5.17	0.94- 28.35	0.059	1.17	0.1 – 12.75	0.985
ALT	14.50	3.77– 55.74	0.000	0.15	0.02 – 1.07	0.059
γGT	5.21	1.65 – 16.41	0.050	**0.04**	**0.00 – 0.63**	**0.022**
Ferritin	6.30	1.89 – 20.92	0.003	0.28	0.04 – 1.84	0.184

Factors associated with histological features present in NAFLD

In univariable analysis Mallory corpuscles were significantly associated with type 2 diabetes (p = 0.036), dyslipidemia (p = 0.017), glucose (p = 0.008), HbA1c (p = 0.027), ALT (p = 0.004) and ferritin (p = 0.040). After subjecting these values to the logistic regression variables dyslipidemia and ALT were confirmed as factors independently associated with presence of Mallory's corpuscles with the following values, respectively, OR 0.05, 95% CI 0.002 to 0.75, P = 0.031 and OR 10.99, 95% CI 1.44 to 83.93, P = 0.021. Age was the only variable that was independently associated with the presence of ballooning, OR 0.89, 95% CI 0.79 to 0.99, P = 0.029.

Factors associated with NASH

In the univariable analysis, the factors that were significantly associated with NAFLD were: triglycerides (P = 0.029); group consisting of type 2 diabetes and those with impaired fasting glucose (P = 0.044), fasting glucose greater than 110mg/dL (p = 0.034), ALT changes (p = 0.044). The logistic regression analysis identified no factor independently associated with NASH.

Factors associated with fibrosis

Zone 3 perisinusoidal fibrosis was detected in three cases (7.5%) while the isolated portal fibrosis was seen in seven (11.6%). Figure 1The univariable analysis for factors associated with perisinusoidal fibrosis was compromised by the low prevalence of perisinusoidal fibrosis in the sample. However, taking into account that in morbid obesity, portal fibrosis alone has received attention[12], all type of fibrosis (perisinusoidal, portal, perisinusoidal and portal) were analyzed as a group, and portal fibrosis also been analyzed separately. Only the elevation of γ GT showed a significant association with the first group (p = 0.035). After logistic regression analysis, γ GT did not remain as a significant independent factor. However, there was a trend towards significancy (OR 5.69, 95% CI 0.99 to 32.73, P = 0.051) Table 3.
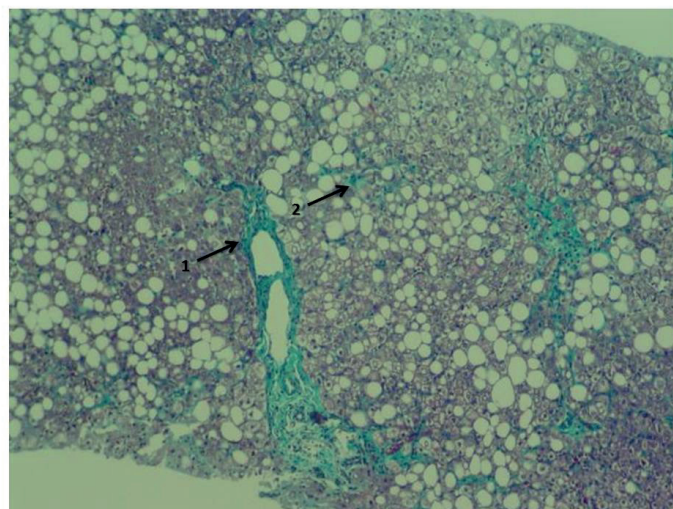

FIGURE 1 - Masson staining of the liver(x100) shows portal fibrosis (**1**) and perisinusinusoidal fibrosis (**2**).

TABLE 3 - Crude and adjusted odds ratios with 95% confidence interval (CI) for *fibrosis* according to clinical and biological variable

Variables	Crude Odds Ratio (OR)			Adjusted Odds Ratio (OR)		
	OR	95% IC	*P*	OR	95% IC	*P*
Age	0.97	0.91 – 1.04	0.382	0.98	0.89 – 1.07	0.597
BMI	1.50	0.47 – 4.78	0.496	1.79	0.46 – 7.02	0.403
Glicose	1.00	0.99 – 1.02	0.340	1.01	0.99 – 1.02	0.402
Uric acidic	0.22	0.03 – 1.85	0.161	0.19	0.02 – 2.13	0.179
ALT > 1	2.33	0.59 – 9.27	0.229	2.16	0.34 – 13.97	0.417
GGT > 1	4.96	1.13 – 21.72	0.034	5.69	0.99 – 32.73	0.051
Alcaline fosfatase	1.01	1.00 – 1.02	0.161	1.01	0.99 – 1.02	0.529
Ferritin	0.41	0.08 – 2.13	0.287	0.32	0.04 – 2.67	0.293

Discussion

The present prospective study evaluated liver biopsies of patients that underwent bariatric surgery. The prevalence of NAFLD in the sample in this study was 95%, and among patients with NAFLD, twenty-four (42.1%) scored higher (Grade II and III).

The γGT was the only laboratory parameter that showed independent association with the degree of liver steatosis after logistic regression analysis. Interestingly. AST did not correlate with the degree of steatosis even in the univariate analysis and an AST / ALT ratio below 1 was found in 90% of cases. These findings suggest that increased levels of γ GT are not associated with alcohol consumption. Recent studies have demonstrated an association of GT levels with NAFLD. However, few studies have evaluated the frequency of such findings[13]. Additionally, increased levels of γ GT have been associated with oxidative stress[14] and insulin resistance syndrome[15]. Our findings highlight the importance of monitoring γ GT levels in patients with NAFLD.

Among the histological findings in NAFLD, the presence of corpuscles Mallory was independently associated with dyslipidemia and ALT changes. Free fatty acids, acting at the intracellular level may result in the formation of corpuscles underlying Mallory[16] The hepatocyte ballooning showed an independent association with age and a trend towards statistical significance with type 2 diabetes. The trend towards an independent association of type 2 diabetes with hepatocyte ballooning as well as and dyslipidemia and ALT in the presence of Mallory's

corpuscles may support the idea of type 2 diabetes, dyslipidemia and ALT as factors associated with NAFLD. The ballooning of hepatocytes and presence of Mallory's corpuscles are now crucial for the framework of NASH in the spectrum of NAFLD[13] It has been suggested that the mechanisms of pathogenesis of steatosis and ballooning are closely linked to insulin resistence, and their long-term evolution may be predicted by early improvement in insulin resistence[17].

The prevalence of NASH in this study was 66.7%, similarly with what was reported from Belo Horizonte Southeastern of Brazil 57%[18], lower than found in Ribeirao Preto also in Southeastern of Brazil 89%[19] and in Greek 98%[20], however it is higher than that reported from Dixon *et al*. 25% [5] and Beymer *et al*. 33%[21] in the USA. In this study we did not find any significant association between NASH and gender unlike others studies that found higher prevalence of NASH in men[22,23].

In the univariable analysis, diabetes mellitus and changes in fasting glucose were significantly associated with NASH, as well as laboratory variables ALT, triglyceride and glucose levels >110mg/dL. However, none of these variables remained significant after the adjusted analysis. The BMI, WHR and waist circumference did not show statistical significance as factors associated with NASH in our study, in accordance with several studies[2,5].

We found a lower rate of fibrosis in this asymptomatic population (12%) than is commonly identified and no evidence of cirrhosis. One explanation may include referral bias in other published reports, but the exclusion criteria that applied to preexisting liver disease may also be related. Another possibility could be due to the fact that the mean age of the patient is lower than the one reported from the studies that found higher fibrosis rates since it has been reported that age could be a predictive factor for fibrosis in several studies[2].

The role of isolated portal fibrosis, despite being reported in patients with NAFLD, has not been fully understood**.** Abrams *et al*.[12] found isolated portal fibrosis in 30.3% of 195 liver biopsies in obese morbid patients and hyperglycemia. Our findings in the isolated portal fibrosis highlight the importance of this type of in the group of morbidly obese patients[13]**.** The two groups of fibrosis studied were not associated with any of the variables evaluated. It has been described a significant association between AST, ALT and γ GT elevation and NASH/fibrosis. However, high proportion of patients with NASH (and even severe NASH and hepatic cirrhosis) may present with normal serum concentrations of hepatic enzymes[20]**.**

Conclusions

Non alcoholic fatty liver disease seems to be an obese-related condition with approximately half of asymptomatic morbidly obese patients having histological NASH. The γGT was an independent predictor of the degree of steatosis.

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