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RESEARCH ARTICLE

CLINICAL, EPIDEMIOLOGICAL, AND LABORATORY ANALYSIS OF PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS

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ABSTRACT

Background: Non-alcoholic fatty liver disease is characterized by excessive accumulation of fat in the form of triglycerides in the liver. A subgroup of these patients presents, in addition to excess fat, cell damage and inflammation, a condition called non-alcoholic steatohepatitis. **Objective:** Evaluating the clinical and epidemiological aspects of patients with non non-alcoholic fatty liver disease, allowing a better screening of patients who will require liver biopsy. **Methods:** We analyzed the medical records of 73 patients with non-alcoholic steatohepatitis, all diagnosed histopathologically after performing liver biopsy. Patients were grouped according to the degree of hepatic steatosis, being classified as mild, moderate, or severe. The normality test was performed for all cases. The mean values of the clinical epidemiological parameters were used for statistical analysis with ANOVA. The median value of the laboratory parameters was used for statistical analysis with the Kruskal-Wallis test. **Results:** In our study on non-alcoholic steatohepatitis patients, 53.44% (39) showed mild disease, 34.24% (25) showed moderate disease and 12.32% (9) showed severe disease. Among the epidemiological, clinical and laboratory parameters evaluated, a statistically significant difference was observed among the three groups for low density lipoprotein ($p = 0.02$), systemic arterial hypertension ($p = 0.03$), metabolic syndrome ($p = 0.02$), and female sex ($p = 0.03$). **Conclusions:** Most patients of non-alcoholic steatohepatitis are females and present with mild hepatic steatosis, which is associated with raised low density lipoprotein values, in addition to the presence of other risk factors, such as hypertension and metabolic syndrome.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by the presence of hepatic steatosis, in the absence of sufficient alcohol consumption, to explain the accumulation of triglycerides in liver. NAFLD is considered the most common aetiology of chronic hepatic failure worldwide. It is currently estimated to affect up to 30% of adults with the an increasing trend in prevalence (Dietrich and Hellerbrand, 2014). Being the hepatic component of metabolic syndrome (MS), NAFLD has been associated with obesity, insulin resistance / type 2 diabetes mellitus (DM), sedentary lifestyle, diet rich in saturated fats, age > 65 years old, female gender, and genetic predisposition (Sayiner *et al*., 2016; Reccia *et al*., 2017; Perlemuter *et al*., 2007; Schuppan and Schattenberg, 2013). Despite the fact that the majority of the patients with NAFLD

present with hepatic steatosis alone, non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis may also occur (Brunt, 2004). Even if only a few patients develop these complications, those with NAFLD present a markedly greater risk of the development of fibrosis, cirrhosis, and hepatocellular carcinoma (Fleischman *et al*., 2014). Currently, NASH is considered the second most common indication for liver transplantation, but due to its increasing prevalence it is estimated that this disease will be the main indication in the next twenty years (Charlton *et al*., 2011). Although imaging studies such as ultrasonography, magnetic resonance imaging and fibroscan help in clinical evaluation, in addition to elevation of aminotransferases in an asymptomatic patient, liver biopsy is the only procedure which enables differentiation of isolated hepatic steatosis from steatohepatitis and accurate assess the degree of cirrhosis. However, the greatest challenge is to correctly indicate the liver biopsy, since about 30% of the adult population has NAFLD. Another complicating factor is that the presence of hepatocyte inflammation is not directly related to the degree of steatosis, and NASH may occur in patients with mild hepatic steatosis (Perlemuter *et al*., 2007; Kleiner *et al*., 2005). Thus, the importance of the present study is highlighted as it retrospectively analysed epidemiological, clinical, and laboratory characteristics of NASH patients observed at a referral centre in North-eastern Brazil.

MATERIALS AND METHODS

A total of 73 patients were enrolled in the Liver Steatosis outpatient clinic of the Institute of Liver of Pernambuco (IFP / University of Pernambuco) between January and December of 2017. Ours is a retrospective study carried out by reviewing medical records with demographic, anthropometric, clinical and laboratory data. All patients were diagnosed histopathologically with NASH on liver biopsy, showing ballooning degeneration of hepatocytes, according to the criteria adopted American Association for the Study of Liver Diseases, American College of Gastroenterology and American Gastroenterological Association (Chalasani *et al*., 2012). The patients were grouped according to the degree of hepatic steatosis, being classified as mild, moderate or severe, using the criteria proposed by the Nonalcoholic Clinical Research Network (Rinella, 2015).

For the diagnosis of DM, the 2014 guidelines of the American Diabetes Association were adopted (Siminerio *et al*., 2014). The study patients were diagnosed as carriers of metabolic syndrome (MS) according to the criteria adopted by the Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention (Alberti *et al*., 2009). Exclusion criteria were hepatitis C or B, as well as alcohol consumption (>40 g ethanol/day), that could justify alcoholic steatohepatitis. Laboratory tests were performed according to the NASH protocol. The study was approved by the Human Ethics and Research Committee of the University of Pernambuco, in accordance with the Declaration of Helsinki.

Statistical analysis

All data collected were recorded in an Excel database, and statistical analysis was performed using Stata 9.2 SE software. The normality test was performed for all quantitative variables. The clinical parameters (DM, systemic arterial hypertension [SAH], body mass index [BMI], MS) and epidemiological parameters (age, gender) were evaluated using ANOVA. The analysis for laboratory parameters (liver aminotransferases, low and high density lipoproteins [LDL and HDL], total bilirubin and alpha fetoprotein) was performed using the Kruskal Wallis test.

RESULTS

The study included 73 patients registered at the IFP/UPE reference centre (Brazil) with diagnosis of NASH; the patients were aged 16–77 years, with a mean age of 55.6 years. The majority of the patients were women (48,65.7%). We noted a statistically significant variation regarding sex ($p = 0.03$) (Table 1). We observed a higher prevalence of mild hepatic steatosis with 39 patients (53.42%), followed by moderate form (34.24%) and severe form (12.16%). Among the associated clinical parameters, considering both sexes, SAH was the most frequent, affecting 48 patients (65.7%). It was also more prevalent when considering the sexes separately, being present in 15 men (60.0%) and 33 women (68.8%). When considering the degree of hepatic steatosis, SAH was seen to be more frequent in the severe form (8 patients, 88.8%) compared to the mild form (23 patients, 58.9%), with a statistical significance of $p = 0.03$.

Table 1. Epidemiological, clinical and laboratory profile of patients with non-alcoholic steatohepatitis

Variable	Mild (39)	Moderate (25)	Severe (09)	Total (73)	*p* value
Epidemiological					
Age	54±2	57±3	54±4	-----	0,3
Male Gender	13 (68%)	-----	06 (32%)	19	*0,03*
Female Gender	03 (10,30%)	-----	26 (89,70%)	29	*0,03*
Clinical					
DM	18 (75%)	-----	06 (25%)	24	0,12
SAH	23 (74%)	-----	08 (26%)	31	*0,03*
BMI ≥ 25	31 (81,60%)	-----	07 (18,40%)	38	0,49
BMI < 25	09 (81,80%)	-----	02 (18,20%)	11	0,49
MS	17 (70,80%)	-----	07 (29,20%)	24	*0,02*
Laboratorial					
AST	43,76±22,68	44,54±22,65	56,46±26,29	72	*0,02*
ALT	105,5 (30-202)	120,0 (38-195)	76,2 (40-125)	70	0,9
HDL	42,0 (24-93)	44,0 (24-53)	39,0 (27-92)	66	0,44
LDL	108,9±43,67	116,5±39,69	73,69±26,85	73	*0,02*
AFP	3,25(1,17-7,6)	2,75(1,0-13,1)	8,86 (2,3-10,0)	39	0,14
BT	0,6 (0,14-2,0)	0,6 (0,26-1,4)	0,78 (0,27-1,5)	73	0,53

DM: type 2 diabetes mellitus; SAH: systemic arterial hypertension; BMI: body mass index; MS: metabolic syndrome; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HDL: high density lipoprotein; LDL: low density lipoprotein; AFP: alpha-feto-protein; TB: total bilirubin

The second most frequently associated clinical parameter was MS, affecting 39 patients (53.4%), 14 being men (56.0%) and 25 were women (52.0%). Comparing the degree of hepatic steatosis, MS occurred significantly higher among the severe group (7 patients, 77.7%) compared to the mild group (17 patients, 43.6%) (p = 0.02).

Among the laboratory parameters, statistically significant difference of low-density lipoprotein (LDL) (p = 0.02) and AST (p = 0.03) was observed, comparing the three groups (Figure 1). Among patients with high LDL values (reference value: up to 130 mg / dL), 47.05% had mild hepatic steatosis and 52.95% had moderate or severe hepatic steatosis.

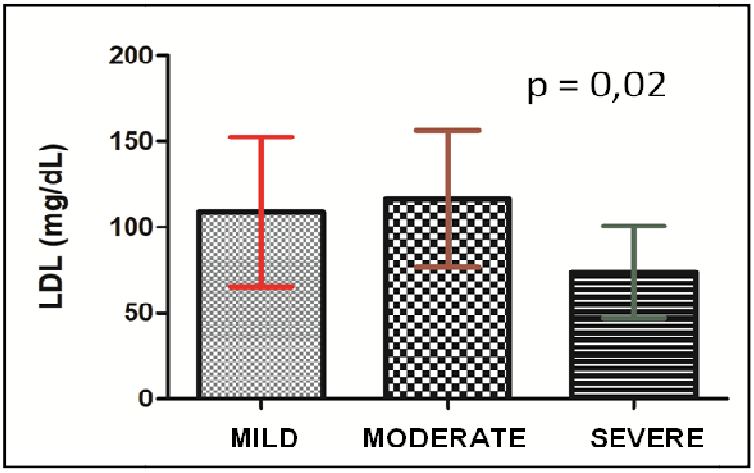

Figure 1. Serum low density lipoprotein (LDL) levels in patients with non-alcoholic hepatitis

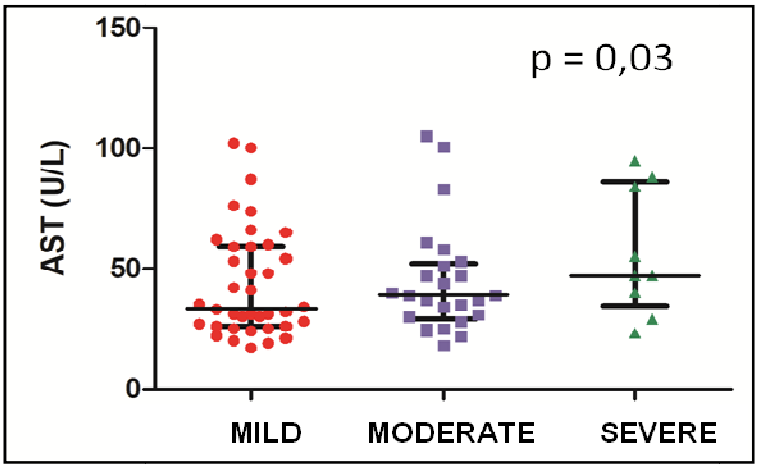

Figure 2. Serum aspartate aminotransferases (AST) levels in patients with non-alcoholic hepatitis

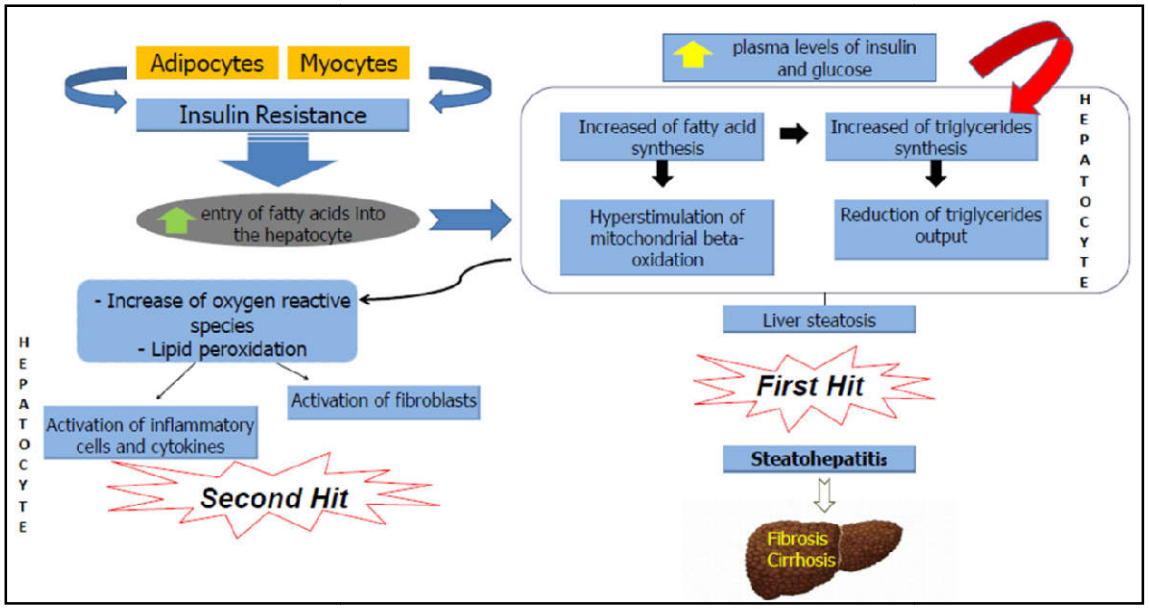

Figure 3. Schematic diagram of the development of non-alcoholic steatohepatitis

DISCUSSION

Although the pathophysiology of NASH is not completely understood, it is believed that it develops in two steps. The first one, called first hit, is related to the increase of plasma insulin levels, generating oxidative stress in the hepatocytes, with increased fatty acid synthesis, mitochondrial beta-oxidation, and triglyceride deposition. Associated with all this, there is a difficult for the hepatocyte to export these triglycerides; therefore, the liver, previously healthy, increasingly becomes steatotic and prone to progressive damage (Perlemuter *et al*., 2007). This is the NAFLD group. A minority of these patients present with increased production of reactive oxygen species and lipid peroxidation, thus, activating proinflammatory cells, cytokines, and fibroblasts. This picture characterizes the second hit, thus, the development of steatohepatitis, which tends to progress to hepatic fibrosis and cirrhosis. Insulin resistance, therefore, constitutes the key point for the development of NASH, (Perlemuter *et al*., 2007; Begriche *et al*., 2006; Khan *et al*., 2018) justifying the strong relation between the disease and type 2 DM (Figure 2). Cardiovascular diseases are also quite prevalent in these patients, and the most frequent association is with SAH. Epidemiological data are not sufficient to support the causal association of NAFLD and SAH, but in clinical practice this relationship is seen frequently (Ryoo *et al*., 2014). In the present study, SAH was the most frequent clinical parameter associated with NASH (65.7% of all, 60% of men and 68.8% of women). The frequency of SAH was seen to be proportional to the degree of hepatic steatosis, occurring in 88.8% of patients with severe steatosis and in 58.9% with mild steatosis (p = 0.03). These results are in agreement with previously published literature, since cardiovascular diseases, in addition to being more frequent, are associated with higher mortality in patients with NASH. When comparing prospectively for 13.7 years, between patients with NASH and the general population, Ekstedt *et al*. (2006) found that the risk of death from cardiovascular diseases is 15.5% vs 7.5% (p = 0.04), respectively. Subsequently Angle *et al*. (2015) retrospectively analysed 619 patients diagnosed with NAFLD. In this study, with a median of 12.6 years of follow-up, cardiovascular diseases were the main cause of death (38.3%), followed by non-hepatic cancer (18.7%), complications of cirrhosis (7.8%), and hepatocellular carcinoma (1%). In addition, Ryoo et. al. (2014) showed a significant association between SAH and the degree of NAFLD. When 22,090 men were prospectively evaluated, this association was independent of age, BMI, triglycerides, serum creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), regular exercise and DM (Ryoo *et al*., 2014).

The prevalence of hypertension has been reported to be higher with increasing degree of hepatic steatosis. Despite this strong association, studies are still lacking that can explain pathophysiological relationship of SAH with NAFLD. Insulin resistance is the main risk factor for the development of MS, followed by genetic factors and lifestyle related factors (Reaven, 2005; Gallagher *et al*., 2011). The central event in the pathophysiological development of MS is the activity of adipokines and deposition of free fatty acids in adipose tissue, leading to visceral obesity. When this accumulation occurs in the liver, there is a series of inflammatory and oxidative processes that damage the hepatocyte; this explains the significant clinical association between NAFLD, NASH, and MS (Feldstein *et al*., 2004; Pessayre and Fromenty, 2005).

In the present study, this relationship was also relevant, with MS seen in 39 patients (53.4%), 14 men (56%) and 25 women (52%). The association of MS with severe NASH, revealed in our study is in concordance with previous studies. Besides that, patients with NASH present a higher prevalence of the risk factors of MS, such as hypertension, DM and low levels of HDL (Marchesini *et al*., 2003). An Australian study with several obese individuals showed that insulin resistance, increased ALT, and SAH are independent predictive factors of NASH.[24] Corroborating these data, a Chinese study compared 95 patients with NAFLD (divided into NASH and non-NASH), showing that patients with steatohepatitis had significantly higher levels of triglycerides, BMI and waist-to-hip ratio (Dixon *et al*., 2001). The major serum abnormalities associated directly with liver injury are elevation of AST and ALT, usually no more than five times the upper limit of normal (Purnomo *et al*., 2015**).** In the present study, increased levels of AST were proportional to the degree of hepatic steatosis (p = 0.02), which was not observed with ALT levels. Another parameter that may suggest the degree of hepatic impairment is serum LDL level, which usually tends to be proportional to the degree of hepatic steatosis (Dietrich and Hellerbrand, 2014). In the current study, although LDL levels showed statistically significant difference among the 3 groups (p = 0.02), we did not find this proportional relationship. Patients with moderate hepatic steatosis had the highest LDL values, followed by those with mild and severe steatosis, respectively. We attribute this discordance to the limited number of patients with severe steatosis (Kleiner *et al*., 2005). The present study has limitations, being a retrospective and observational study. There is also a possibility of selection bias, while using data from a single centre. However, all clinical and diagnostic evaluation was performed by the same group of investigators from the outset, thus reducing probable biases.

Conclusion

NASH is more frequent in women and presents as mild disease in most cases. Systemic arterial hypertension is significantly associated with NASH, males showing a greater degree of association. The association of SAH and MS with NASH shows proportional relationship with the degree of severity of the liver disease.

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Glossary of Abbreviations

ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
BMI: body mass index
DM: type 2 diabetes mellitus
GGT: Gamma-glutamyl transferase
HDL: high density lipoprotein
LDL: low density lipoprotein
MS: metabolic syndrome
NAFLD: non-alcoholic fatty liver disease
NASH: non-alcoholic steatohepatitis
SAH: systemic arterial hypertension

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