INTRODUCTION

Recently, it was proposed to modify the concept and the denomination of NAFLD for metabolic dysfunction-associated fatty liver disease (MAFLD), in which metabolic parameters are used for this liver disease diagnosis, in contrast to the previous situation, in which the diagnosis was made by excluding other etiologies (1,2).

These changes are important, as we are dealing with a growing health problem, that is considered nowadays as the main cause of chronic liver disease in the world (3). The necessary criteria for the MAFLD diagnosis are those considered to be risk factors and which may influence the disease’s natural history, such as obesity/overweight, changes in blood glucose and metabolic syndrome (MS) parameters frequently found in these patients (4,5). Excess weight has been associated with a higher prevalence of NAFLD and progression of liver fibrosis, alone or when associated with changes in glycemic levels, an association that also increases the risk of developing hepatocellular carcinoma (HCC) (6-8).

Regardless of obesity, changes in blood glucose and the presence of type 2 diabetes mellitus (T2DM) are important in relation to the disease’s severity, as they increase the risk of progressing to cirrhosis and the appearance of HCC (7,8). On the other hand, it was shown in a cohort study with 19 years of follow-up that mortality from all causes was 24 times higher in patients with NAFLD with components of MS than individuals with NAFLD without the presence of MS (10).

In addition to these criteria, IR, calculated from the homeostatic model, with HOMA-IR >2.5 was introduced as a risk factor for MAFLD in thin patients or those with normal body mass index...
(BMI), when it is associated with another risk factor, such as one of the criteria of MS or diagnosis of pre-diabetes or even with high plasma high-sensitivity C-reactive protein level(1,2).

In this study, we aimed to assess the importance of a cutoff value of HOMA-IR >2.5 in the differentiation of non-diabetic patients with histological diagnosis of NAFLD in relation to clinical, biochemical and histological parameters, in order to validate the importance of such cutoff value in the characterization of the MAFLD and its severity.

METHODS

Retrospective study, with data collection from medical records of individuals with NAFLD treated at the outpatient clinic of liver disease of the Gastroenterology Section of Universidade Federal de São Paulo (UNIFESP) in the period between 2014 to 2019. The sample consisted of patients of both genders with NAFLD confirmed by histological analysis of liver tissue.

Exclusion criteria were considered: patients with a previous or current diagnosis of T2DM; history of alcohol ingestion >140 g per week for men and >70 g per week for women; debilitated patients with impaired nutritional status or the presence of any type of neoplasia including hepatocellular carcinoma. Those individuals with positive serological markers for hepatitis B or C virus or other recognized etiologies in liver biopsy, using insulin-sensitizing or iron replacement drugs or hepatotoxic drugs and patients with portal hypertension detected by clinical, radiological and/or endoscopic criteria were also excluded(11).

The following demographic and clinical variables were assessed in this study: age, gender, presence of metabolic comorbid conditions, like altered blood glucose (glucose >99 mg/dL and <125 mg/dL), systemic arterial hypertension, dyslipidemia and MS according to ATP III criteria(12). Weight and height were evaluated to determine the BMI obtained using the formula [BMI = weight (kg) / height (m²)] using the World Health Organization classification to assess nutritional status according to BMI(13). Waist circumference (WC) was measured with the patient standing, at the last rib and the iliac crest, at its smallest perimeter and at the end of a normal expiration(14).

The serum activity of the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and serum levels of total cholesterol (TC), HDL-cholesterol, triglycerides (TG), LDL-cholesterol, and blood glucose were obtained using an automated colorimetric method in Cobas Mira, Roche, Switzerland. Serum ferritin was determined by chemiluminescence, with upper normal limits of up to 400 ng/mL for men and 150 ng/mL for women. The insulin values were determined by an immunofluorometric assay (Perkin Elmer BR-CS), which enabled the determination of HOMA-IR index, calculated using the formula: HOMA-IR = [glucose (mmol/L) * insulin (µU/mL)] / 22.5 and the presence of IR was characterized by HOMA-IR >2.5(15).

Histological evaluation of liver tissue fragments obtained by percutaneous biopsy was performed according to the criteria of Kleiner et al.(16).

In the statistical analysis, the categorical variables were described as absolute and relative frequencies. The numerical variables were described by mean and standard deviation. The other non-normal continuous variables were described by quartiles. For univariate analysis, comparing groups with and without IR, we used the chi-square test for categorical variables and the Student’s “t” test for independent samples or Mann-Whitney U test for normal or non-normal continuous variables, respectively. For the identification of variables independently associated with HOMA-IR >2.5, a logistic regression model was designed. The correlation analysis between histological, clinical and biochemical parameters was performed according to Pearson’s relationship coefficient (with normal distribution) or Spearman’s (without normal distribution). All tests were two-tailed and the results with P<0.05 were considered significant. All analyzes were performed using SPSS software (IBM SPSS Statistics for Windows, version 24.0. Armonk, NY: IBM Corp.) This study was approved by the Research Ethics Committee from UNIFESP, under decision number 1.662.400.

RESULTS

The calculation of sample size aimed the obtainment of a 60% prevalence of NAFLD cases with HOMA-IR >2.5, with a sampling error of 7% and a 95% confidence interval, according to previous published data(4). From 202 selected consecutive medical records, 28 ended up being excluded because they did not present values of biochemical tests studied at the time of biopsy that were diagnosed with diabetes mellitus or with detectable portal hypertension. Thus, 174 patients remained, with a mean age of 53.6±11.2 years old, 39.7% male, with an average BMI of 30.3 kg/m². In this same table it is observed that, except for the presence of MS, significantly more prevalent among patients with IR, none of the other demographic and nutritional parameters studied was able to differentiate the group of patients with and without IR, measured by HOMA-IR.

In the analysis of biochemical data, in addition to fasting insulin values and, obviously, HOMA-IR, there were also significant differences between groups in the values of enzymes AST, ALT and GGT and ferritin in absolute and categorized values (TABLE 1).

As to morphological variables observed in liver histology, there was a higher frequency of ballooning, steatohepatitis and advanced fibrosis in patients with elevated HOMA-IR (TABLE 2).

The logistic regression analysis of factors that were significantly associated in the univariate analysis shows that the presence of IR, characterized from the values of HOMA-IR >2.5, was independently associated with elevated serum ferritin levels, presence of NASH in liver biopsy and MS assessed by ATP-III criteria (TABLE 3).

DISCUSSION

IR plays a central role in the installation of steatosis and appears to be important in the progression of NAFLD to more advanced forms of the disease, which makes it an important pathophysiological mechanism of NAFLD(17-19). Although IR is defined based on more complex tests such as a hyperinsulinemic-euglycemic clamp, its evaluation by HOMA-IR has been more used, due to the simplicity of its determination and good correlation with glycemic clamp in non-diabetic patients(20,21), in addition, the intrasubject coefficients of variation of current methods are around 7.7% to 10.3%(20). Another point to be addressed is the cutoff value of HOMA-IR used in the definition of IR. Although the value used here was proposed by a group of experts from the European Association for the Study of the Liver for the characterization of MAFLD(1,2), it must be remembered that there is a huge variation.
TABLE 1. Distribution of demographic, nutritional, metabolic and laboratory variables in the sample, according to the presence (HOMA-IR >2.5) or absence of insulin resistance (HOMA-IR <2.5).

| Variable                          | Total (n=174) | HOMA IR <2.5 (n=61) | HOMA-IR ≥2.5 (n=113) | P-value |
|-----------------------------------|---------------|---------------------|----------------------|---------|
| Age (mean ± SD)                   | 53.6±11.2     | 54.0±11.1           | 53.3±11.3            | 0.719   |
| Male                              |               |                     |                      |         |
| Male                              | 69 (39.7)     | 22 (36.1)           | 47 (41.6)            | 0.477   |
| BMI (Mean ± SD)                   | 30.3±4.5      | 29.8±4.6            | 30.6±4.5             | 0.273   |
| BMI ≥25                           | 153 (87.9)    | 51 (83.6)           | 102 (90.3)           | 0.198   |
| Increased WC                      | 132 (75.9)    | 43 (70.5)           | 89 (78.8)            | 0.224   |
| Arterial hypertension             | 119 (68.4)    | 44 (72.1)           | 75 (66.4)            | 0.436   |
| Metabolic syndrome                | 133 (76.4)    | 40 (65.6)           | 93 (82.3)            | 0.013   |
| Hypercholesterolemia              | 69 (39.7)     | 23 (37.7)           | 46 (40.7)            | 0.699   |
| High LDL-c                        | 66 (37.9)     | 21 (34.4)           | 45 (39.8)            | 0.484   |
| Low HDL-c                         | 89 (51.1)     | 27 (44.3)           | 62 (54.9)            | 0.182   |
| Hypertriglyceridemia              | 81 (46.6)     | 26 (42.6)           | 55 (48.7)            | 0.445   |
| Hyperglycemia                     | 90 (51.7)     | 26 (42.6)           | 64 (56.6)            | 0.078   |
| AST                               | 29 (23–42)    | 28 (21–36)          | 33 (24–44)           | 0.008   |
| ALT                               | 38 (27–61)    | 35 (24–46)          | 42 (28–74)           | 0.023   |
| Ferritin                          | 51 (32–92)    | 40 (24–86)          | 57 (36–102)          | 0.009   |
| Ferritin (yes/no)                 | 195 (109–393) | 137 (78–326)        | 264 (119–404)        | 0.010   |
| Elevated ferritin                 | 74 (42.5)     | 17 (27.9)           | 57 (50.4)            | 0.004   |
| Basal insulin                     | 13 (7–20)     | 6 (4–8)             | 17 (14–24)           | <0.001  |
| HOMA-IR                           | 3.3 (1.8–5.0) | 1.5 (1.0–1.9)       | 4.3 (3.4–6.3)        | <0.001  |

Data presented as n (%), unless specified. Values corrected for gender according to ATP-III. SD: standard deviation; BMI: body mass index; WC: waist circumference. LDL: low-density lipoprotein; HDL: high-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; HOMA-IR: homeostasis model assessment-insulin resistance.

TABLE 2. Characterization of studied groups according to the presence (HOMA-IR ≥2.5) or absence of insulin resistance (HOMA-IR <2.5) and histological variables obtained through percutaneous liver biopsy and classified according to Kleiner et al. (16).

| Variables                          | Total (174) | HOMA-IR <2.5 | HOMA-IR >2.5 | P-value |
|-----------------------------------|-------------|--------------|--------------|---------|
| Steatosis (0/1/2/3)                |             |              |              |         |
| Inflammation (0/1/2/3)             |             |              |              |         |
| Ballooning (0/1/2)                 |             |              |              |         |
| NASH                              | 127 (73.0)  | 35 (57.4)    | 92 (81.4)    | 0.001   |
| Fibrosis (any grade)              | 100 (57.5)  | 24 (39.3)    | 76 (67.3)    | <0.001  |
| Degree of fibrosis                |             |              |              |         |
| 0                                 | 74 (42.5)   | 37 (60.7)    | 37 (32.7)    | 0.006   |
| 1                                 | 46 (26.4)   | 13 (21.3)    | 33 (29.2)    |         |
| 2                                 | 26 (14.9)   | 7 (11.5)     | 19 (16.8)    |         |
| 3                                 | 19 (10.9)   | 2 (3.3)      | 17 (15.0)    |         |
| 4                                 | 9 (5.2)     | 2 (3.3)      | 7 (6.2)      |         |
| Advanced fibrosis (grades 3–4)    | 28 (16.1)   | 4 (6.6)      | 24 (21.2)    | 0.012   |
| Siderosis                         | 32 (18.4)   | 7 (11.5)     | 25 (22.1)    | 0.091   |

HOMA-IR: homeostasis model assessment-insulin resistance; NASH: non-alcoholic steatohepatitis.

TABLE 3. Multivariate analysis by logistic regression to identify factors independently associated with insulin resistance (HOMA-IR ≥2.5) in studied patients.

| Variables                          | OR  | 95%CI          | P-value |
|-----------------------------------|-----|----------------|---------|
| Elevated ferritin (yes/no)        | 2.401| 1.238–4.657 | 0.010   |
| NASH (present/absent)             | 2.633| 1.313–5.279 | 0.006   |
| GGT (each unit)                   | 1.004| 1.000–1.008 | 0.076   |
| Metabolic syndrome (present/absent)| 2.221| 1.048–4.704 | 0.037   |
| ALT (each unit)                   | 1.002| 0.994–1.011 | 0.591   |
| Advanced fibrosis (present/absent)| 1.866| 0.739–4.708 | 0.187   |

OR: odds ratio; HOMA-IR: homeostasis model assessment-insulin resistance; NASH: non-alcoholic steatohepatitis; GGT: gamma glutamyl transferase; ALT: alanine aminotransferase. N=174.
in the dependence of the studied population and employed methodology. Although not unanimous, HOMA-IR with a cutoff value of 2.5 has been used in the diagnosis of NAFLD in several previous studies. In addition, this cutoff coincides with or is close to those observed in Brazil in patients with NAFLD and in the characterization of IR in the general population.

Patients with T2DM were excluded from the evaluation because it is already a NAFLD criteria and because different values can be obtained, especially in advanced diabetes due to the interference of functional exhaustion of beta cells. Cirrhotic patients with portal hypertension, in turn, present hyperinsulinemia due to pancreatic hypersecretion, decreased hepatic insulin degradation and increased glucagon levels and, therefore, were also excluded from the sample. Although IR is considered almost universal in NAFLD, about one-third of Brazilian NAFLD patients have HOMA-IR values below the established cutoff. Thus, we decided to study the value of HOMA-IR in our patients diagnosed with NAFLD confirmed by biopsy and its relationship with clinical, metabolic and histological parameters.

From the definitions and criteria, we selected a population mainly composed of women, with an average age of 55 years and an average BMI of 30.5 kg/m². MS and visceral obesity were observed in 80% of cases, and more than three-quarters of patients had NASH and some degree of fibrosis in just over half of the patients. These histological data are comparable to those obtained in the Brazilian multi-center study carried out in reference services, which found almost 60% of NASH and liver fibrosis in 46% of these patients. These data certainly reflect the bias in the referral of patients to the disease’s reference centers and in the selection of patients for liver biopsy.

In this study, when patients were separated according to the presence or absence of IR, the fact that there are no differences in BMI is noteworthy, since in the general population there is a relationship between BMI and HOMA-IR, and in NAFLD patients the degree of steatosis and HOMA-IR decreases. However, in these NAFLD patients BMI seemed not to be directly associated with IR; is relation had been challenged, in contrast to the significant correlation. Corroborating these data, in a study with 138 morbidly obese individuals, only 34% of them had high HOMA-IR values, with no correlation between this parameter and the obesity degree. Also, studies in thin patients with NAFLD demonstrate that these patients have a lower frequency of MS, but show higher levels of HOMA-IR and plasma TG when compared with obese patients with NAFLD.

The mean values of liver enzyme activity were similar to those described in other studies. Elevated levels of GGT activity have been linked to the presence of IR in several studies in NAFLD, associating these changes with excessive deposition of visceral fat, steatosis and fibrosis. Serum hyperferritinemia is frequently seen in patients with NAFLD. In our study, it was possible to verify the presence of hyperferritinemia in 50% of individuals with IR compared to only 28% in NAFLD patients with HOMA-IR <2.5 and this parameter remained present as a factor independently related to the presence of IR. Such a result is consistent with findings of Brudevold et al. that in patients without iron overload, hyperferritinemia was associated with the presence of steatosis and NAFLD. The association between hyperferritinemia and IR was observed by several other authors, having been considered a marker of oxidative stress for NAFLD and related to the presence of IR, glucose intolerance and progression of liver disease. In addition, elevated serum ferritin has been associated with increased histological activity, being an independent predictor for advanced fibrosis in patients with NAFLD. Considering the presence of siderosis as any degree of iron deposition detected in the biopsy, regardless of its location, we observed a significant correlation between ferritinemia and siderosis (rS=0.420, P<0.001, data not shown), but it should be noted that only 18% of patients had detectable iron in liver histology and there was no relationship between IR and iron deposition in liver tissue.

As expected, our study showed the well-known relationship between MS and IR. Studies confirm the role of IR in the pathophysiology of MS. IR is increasingly recognized as a key factor linking MS and NAFLD, being associated with increased circulating levels of free fatty acids (FFA) and excessive accumulation of FFA in liver tissue. The contribution of MS to NAFLD involves different factors such as IR, central obesity, inflammation and oxidative stress. Although most individuals with NAFLD have IR, only a portion of those with NAFLD exhibit complete MS.

The observed relationship between IR and histological signs of progressive liver disease (NASH and fibrosis) was expected since IR is considered a disease progression factor. Experimental data have highlighted high levels of glucose and insulinemia would stimulate the release of connective tissue growth factor and that hyperinsulinemia could directly induce oxidative stress and stimulate the proliferation of hepatic stellate cells, resulting in the progression of fibrosis.

The inclusion of cirrhotic patients can be discussed as a confounding factor in this study, since, regardless of the etiology, there is a high prevalence of IR in cirrhosis when compared to control subjects or with less advanced fibrosis. It is known that the lower glucose uptake in the splanchnic region and, especially, in the skeletal muscle is associated with IR in these patients. In addition, reduced liver function, or port-systemic shunts, could contribute to increasing insulinemia and stimulating fibrogenesis. In addition to the small participation of these patients in the sample (only 5%), the exclusion of patients with clinically detectable portal hypertension, as previously mentioned, would minimize the impact of the presence of these cirrhotic patients in the analysis.

The analyzed histological parameter that was independently associated with IR was steatohepatitis. Indeed, IR tends to be more frequent in patients with NASH and with elevated enzymes, it was observed that disease progression was associated with IR and more pronounced steatosis and weight gain above 5 kg during follow-up. In another study in individuals with severe obesity (BMI ≥40 kg/m²) with NAFLD it was observed that HOMA-IR was an independent factor in predicting NASH in this population. Besides interrelated in NAFLD, individually, the three factors that were related to the presence of IR are also factors associated with the progression of NAFLD, as observed in meta-analyses and logistic regression studies.

CONCLUSION

Thus HOMA-IR values >2.5 identify patients with NAFLD with distinct clinical and metabolic characteristics and with a greater potential for progression of liver disease, which validates its use in identifying patients with NAFLD.
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Authors’ contribution

Barreto BFM: conceptualization; data curation; funding acquisition; methodology; project administration; writing original draft; writing review and editing. Punaro GR: writing; review and editing.

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