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Usefulness of the SAF score to characterize NAFLD/NASH in non-cirrhotic HCV patients

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Abstract

Background: The SAF score (steatosis, activity, and fibrosis) has been developed for the assessment of the histological severity of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). The aim of this study was to assess the usefulness of the SAF score in a homogenous cohort of Egyptian patients with chronic HCV infection (CHC) without any alcohol consumption and without cirrhosis. We performed a prospective cross-sectional study including 70 consecutive Egyptian patients with chronic HCV infection to assess the usefulness of the SAF score to characterize NAFLD/NASH in non-cirrhotic HCV patients. The inclusion criteria included positive serum anti-HCV IgG antibody and positive HCVRNA, absence of treatment, and absence of cirrhosis (fibrosis score < F4). Patients were divided into two groups: with metabolic syndrome (MS) and without metabolic syndrome (non-MS). All patients were exposed to thorough history taking, full clinical examination, and laboratory and ultrasound assessment. Histopathologic evaluation of the liver biopsy for the assessment of steatosis, activity, grade, and fibrosis stage was assessed by 2 pathologists with experience in liver diseases.

Results: We found that the degree of fibrosis increases with aging. Liver biopsies from CHC patients with metabolic syndrome (MS) exhibited a significantly higher stage of fibrosis than biopsies from those without MS; however, the grade of inflammation did not differ significantly between the two groups. No significant correlation was found between the SAF score and the body mass index (BMI) or serum HCV RNA. No significant relation between SAF score, fibrosis, and MS. No significant relation was found between the MS and the level of HCV viremia.

Conclusion: We concluded that steatosis was associated with the fibrosis stage, independently of MS. This suggests that in this population, steatosis might be more related to HCV infection than to NAFLD and that fibrosis progression might be related, at least in part, to the steatosis process, i.e., virus-associated fatty liver disease (VAFLD).

Keywords: SAF, Steatosis, Activity, Fibrosis, NAFLD, NASH, VAFLD, Insulin resistance, Chronic HCV, Metabolic syndrome

Introduction and aim of the work

HCV is a major cause of chronic liver disease with about 170 million patients infected worldwide, and the severity of the disease varies widely from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma [1]. Egypt has the highest level of HCV prevalence in the world, and the percentage of adults (aging from 15 to 59 years) testing positive on the HCVRNA test is 7% of the Egyptian population [2]. The introduction of direct-acting antiviral drugs (DAAs) has revolutionized the treatment of HCV. Much of the decline in the HCV prevalence reflects the effect of treatment with DAAs.

Nonalcoholic fatty liver disease (NAFLD) is seen worldwide and is the most common liver disorder in Western industrialized countries, where the major risk factors for NAFLD, central obesity, type 2 diabetes mellitus, dyslipidemia, and metabolic syndrome are common

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[3]. With the advent of effective treatment for hepatitis C and effective viral control in chronic hepatitis B, hepatologists have turned their attention to NAFLD as the next major liver disease to be conquered. NAFLD and nonalcoholic steatohepatitis (NASH) are increasingly identified as causes of hepatic morbidity and as precursors to hepatocellular carcinoma [4, 5].

Several systems of histological grading have been employed for predicting the response to therapy and selecting the proper line of treatment for chronic viral hepatitis. The most widely used is the Metavir classification [6]. In line with Metavir, which dissociates evaluation of activity (grading) from fibrosis (staging), the SAF score has been proposed for NAFLD, providing a separate semiquantitative assessment of steatosis (S), activity (A) including hepatocellular ballooning and lobular inflammation, and fibrosis (F) [7].

The algorithm has been developed from patients with morbid obesity undergoing bariatric surgery [7]. However, most patients seen in hepatology clinics with suspected NAFLD/NASH are not morbidly obese but rather have one or more features of the metabolic syndrome and, arguably, a liver injury that might differ from that of morbidly obese patients [8]. In a recent paper, the usefulness of the SAF score in the evaluation of biopsies of NAFLD has been validated in a cohort of non-obese French patients with NAFLD and without HCV infection [9]. Also, fatty liver can be influenced by alcohol consumption (even moderate or occasional) or ethnicity. Previously, there are reports of significant differences in the prevalence of NAFLD between ethnicities. Hispanics were at a greater risk for NAFLD than were African-Americans despite a similar prevalence of risk factors between these groups [10].

The aim of this study was to assess the usefulness of the SAF score to characterize NAFLD/NASH in a homogenous cohort of Egyptian patients with chronic HCV infection without any alcohol consumption and without cirrhosis.

Patients and methods

We performed a prospective cross-sectional study including 70 consecutive adult Egyptian patients with chronic HCV infection recruited from the Hepato-Gastroenterology Department at Theodor Bilharz Research Institute (TBRI).

Inclusion criteria:

- Positive serum anti-HCV IgG antibody and detectable HCV-RNA
- Absence of anti-viral treatment (never subjected to interferon or DAA therapy)
- Chronic hepatitis (fibrosis score < F4)

Exclusion criteria:

- The presence of chronic liver disease with different etiologies such as autoimmune hepatitis, hemochromatosis, post-HBV infection, or alcohol consumption (even occasional or minimal)
- Cirrhosis

All patients were subjected to full history taking, thorough clinical examination, ultrasound, laboratory assessment, and liver biopsy for histopathological evaluation. Demographic and clinical data were collected preceding liver biopsy: gender, age (years), weight (kg), height (m), waist circumference (cm), blood pressure (mmHg), and concomitant medications. BMI was calculated as weight divided by the square of the height (kg/m^2). Overweight was considered when a patient had a BMI of 25–30 kg/m^2 and obesity when a patient had a BMI of more than 30 kg/m^2. The metabolic syndrome (MS) was diagnosed according to the National Cholesterol Education Program's Adult Treatment Panel (NCEP ATP) III definition [11, 12] by the presence of three or more of the following criteria: central obesity (waist circumference > 102 cm in men and > 88 cm in women), hypertension (blood pressure ≥ 135/85 mmHg), and fasting plasma glucose at least 110 mg/dl, triglycerides at least 150 mg/dl, and high-density lipoprotein cholesterol less than 40 mg/dl in men or less than 50 mg/dl in women.

Laboratory Methods: After a 12-h fasting, 10-ml venous blood was withdrawn from all patients, divided into appropriate vacutainer tubes: citrated for PT assay, EDTA tubes for CBC and HbA1C estimation, and plain tubes are centrifuged and the serum was used for the assessment of routine laboratory investigations and remote insulin, alfa fetoprotei. Serum separated after centrifugation was aliquoted for hepatic function tests: aspartate and alanine aminotransferases (AST and ALT), alkaline phosphatase (ALP), total protein, albumin and total bilirubin, lipid profile (total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides), and fasting glucose using Beckman AU480 (Beckman Coulter, Ireland). Serum insulin, thyroid-stimulating hormone, and alfa-fetoprotein were assayed using enzyme-linked immunosorbent immunoassay. HCV-RNA was determined quantitatively by real-time PCR. Baseline HCV genotyping was not performed. Glycated hemoglobin (HbA1C) was assayed using turbidimetric immuno-inhibition. All these tests were performed at the time of liver biopsy. Diabetes was diagnosed using the 1997 American Diabetes Association fasting criteria (fasting glucose ≥ 126 mg/dl).

Insulin resistance was assessed by calculation of homeostatic assessment of insulin resistance (HOMA-IR) using the following equation: HOMA-IR= fasting insulin × fasting glucose/22.5 [13] and was defined as HOMA-IR greater than 3.

Abdominal ultrasound was performed by the Equipment: Hitachi, EUB-5500.

Measurements were performed after overnight fasting and the patient in a supine position with emphasis on the following:

- Liver size (normal, enlarged both lobes, dysmorphic liver)
- Liver parenchyma (normal, bright, coarse)
- Presence of portal hypertension
- Spleen size

Patients were divided into two groups: with metabolic syndrome (MS) and without metabolic syndrome (non-MS).

Histopathologic evaluation of the liver biopsy for the assessment of steatosis, activity grade, and fibrosis stage was assessed by 2 pathologists with experience in liver diseases (A.M. and V.P.). Liver biopsies were formalin-fixed and paraffin-embedded. Three-micrometer sections were stained with hematoxylin-eosin and sirius red for fibrosis evaluation. Semi-quantitative assessment of steatosis, hepatocellular ballooning, lobular inflammation (for activity grade), and extent of fibrosis was performed according to the SAF score [9]. Briefly, steatosis was graded as follows: [0 < 5%; 1: 6–33%; 2: 34–66%; 3: > 66%). Activity grade is based on the presence of (1) ballooned cells [(0) absence; (1) round and clear hepatocytes non enlarged; (2) clear and enlarged (2 times normal hepatocytes)] and lobular inflammation [(0) absence; (1) 1–2 foci/lobule; (2) > 2 foci /lobule at × 200 magnification]. All cases were reviewed independently, and in cases of discordances, a consensus reading was performed.

The ethical guidelines of the 1975 Declaration of Helsinki were put into consideration, and patients' written consents have been obtained. The approval for the study was obtained from the institute ethical committee.

Statistical analysis

The sample size was calculated using the G* power program 3.1.9 for a one-tailed test. The sample size calculation based on 1 test, type 1 error (a) = 0.05, power (1-a error probability) = 0.90, and effect size (d) = 0.370. The appropriate minimum sample size for this study group is 67 patients as a minimum. The statistical analysis was conducted by using the statistical SPSS Package program version 25 for Windows (SPSS, Inc., Chicago, IL). Demographic and laboratory numerical data are expressed as mean and standard deviation, but categorical data are expressed as a number and percentage. Diagnostic parameters of subjects were compared using the Wilcoxon-signed rank test for numerical variables but the chi-square test to compare between categorical variables. Reliability test (Cronbach's alpha) was used to compute SAF score applicability in MS and non-MS patients. All statistical analyses were significant at the level of probability less than or equal to 0.05 ($P \leq 0.05$) with 95% confidence interval.

Results

Tables 1 and 2 show the demographic, clinical, and laboratory results of included patients.

-Relation between ALT and AST and degree of fibrosis:

The ALT and/or AST levels were found to increase crescendo from F1 to F3 ($P = 0.0001$) but not the AST/ALT ratio ($P = 0.454$).

-Relation between fibrosis degree and aging:

The degree of fibrosis increases with aging ($P = 0.0001$).

-Relation between degree of fibrosis and presence of metabolic syndrome:

Table 1 Demographic and clinical data

Variables	*N* (%)	Variables	*N* (%)
Gender		Major surgery	
Female	40 (57.1)	No	60 (85.7)
Male	30 (42.9)	Yes	10 (14.3)
Habits		Minor surgery	
Smokers	18 (25.7)	No	40 (57.1)
Non-smokers	52 (74.3)	Yes	30 (42.9)
History of bilharziasis		Hazardous injection	
No	37 (52.9)	No	56 (80)
Yes	33 (47.1)	Yes	14 (20)
Oral treatment (*bilharziasis*)		Diabetes	
No	44 (62.9)	No	50 (71.4)
Yes	26 (37.1)	Yes	20 (28.6)
Parenteral treatment (*bilharziasis*)		HTN	
No	61 (87.1)	No	48 (68.6)
Yes	9 (12.9)	Yes	22 (31.4)
Dental manipulations		Dyslipidemia	
No	30 (42.9)	No	60 (85.7)
Yes	40 (57.1)	Yes	10 (14.3)
Blood transfusion		Hepatomegaly	
No	59 (84.3)	No	36 (51.4)
Yes	11 (15.7)	Yes	34 (48.6)
Metabolic syndrome			
No	45 (64.3)		
Yes	25 (35.7)		

Table 2 Laboratory results

Laboratory tests	Mean ± SD	Normal values
ALT (U/l)	62.00 ± 5.92	ALT < 50 U/l
AST (U/l)	57.73 ± 4.26	AST < 50 U/l
TLC	4397.06 ± 313.40	4–11,000/mm^3
PLT	160,645 ± 126,97	150–400,000/mm^3
Hb (g/dl)	12.17 ± 1.80	12–14 g/dl
Iron (µg/dl)	63.04 ± 11.68	70–180 µg/dl
TIBC (µg/dl)	359.51 ± 62.61	150–300 µg/dl
Bilirubin (mg/dl)	0.83 ± 0.31	Up to 1 mg/dl
ALP (IU/l)	97.85 ± 35.30	80–300 IU/l
Ptn (g/dl)	7.90 ± 0.55	6.6–8.3 g/dl
Alb (g/dl)	4.02 ± 0.58	3.5–5.2g/dl
PT (s)	13.83 ± 1.07	13.83 ±1.07 s
INR	1.17 ± 0.19	1.17 ± 0.19
PC (%)	62.20 ± 4.72	70–100%
FBS (mg/dl)	122.47 ± 45.62	70–100 mg/dl
HBA1c (%)	5.89 ± 1.59	4.5–5.7%
Insulin (mIU/l)	13.59 ± 6.88	< 25 mIU/l
Cholesterol (mg/dl)	172.15 ± 31.47	150–200 mg/dl
HDL-C (mg/dl)	38.02 ± 7.87	> 60 mg/dl
LDL-C (mg/dl)	109.38 ± 26.79	< 40 mg/dl
TG (mg/dl)	128.45 ± 54.12	< 150 mg/dl
HCV-RNA	157,491.08 ± 42,111.74	IU/ml
AFP (ng/ml)	11.52 ± 4.01	0–8.7 ng/ml
TSH (uIU/ml)	2.41 ± 0.60	0.5–4.5 uIU/ml
HOMA	4.13 ± 1.50	Up to 2.5

AST aspartate amino-transferase, *ALT* alanineamino-transferase, *TIBC* total iron-binding capacity, *ptn* total protein, *alb* albumin, *TLC* total leucocytic count, *PT* prothrombin time, *PC* prothrombin concentration, *FBS* fasting blood glucose, *HBA1C* glycosylated hemoglobin, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglycerides, *AFP* alfa fetoprotein, *TSH* thyroid-stimulating hormone, *HOMA* homeostasis model assessment

Table 3 shows that liver biopsies from CHC patients with MS exhibited a significantly higher grade of fibrosis than biopsies from those without MS. However, the grade of inflammation did not differ significantly between the two groups.

Photo 1 and photo 2 illustrate the liver biopsies of 2 patients in the study with a description of the SAF score.

-Relation between steatosis and degree of fibrosis:

Table 4 shows that the presence of steatosis per se, whether related to MS or not, is associated with an advanced stage of fibrosis.

-Relation between SAF score and both BMI, MS, and viremia:

No significant correlation was found between the SAF score and the BMI (< 30 and 30 or more) or serum HCV RNA. BMI for all patients was 30.04 ± 6.43. No significant relation between SAF score fibrosis and MS (P = 0.791). No significant relation was found between the MS and the degree of hepatitis C viremia as shown in Table 5.

Table 3 Relation between metabolic syndrome and grade of steatosis, activity, and fibrosis stage according to SAF score

SAF	Grade	Non-MS (n = 45)	MS (n = 25)	*P* value
Steatosis	S0	25 (35.7%)	10 (14.3%)	0.654
	S1	14 (20.0%)	10 (14.3%)	
	S2	5 (7.1%)	4 (5.7%)	
	S3	1 (1.4%)	1 (1.4%)	
Activity	A0	30 (66.7%)	15 (60.0%)	0.791
	A1	4 (8.9%)	3 (12.0%)	
	A2	10 (22.2%)	7 (28.0%)	
	A3	1 (2.2%)	0 (0.0%)	
Fibrosis	F0	24 (34.3%)	7 (10.0%)	0.036*
	F1	5 (7.1%)	9 (12.9%)	
	F2	7 (10.0%)	4 (5.7%)	
	F3	9 (12.90%)	5 (7.10%)	

Data are expressed by number (percentage)

P value probability value

* Significant (P < 0.05)

Ultrasound results: Forty-five patients had bright liver and 25 patients had bright hepatomegaly. Thirty patients had portal hypertension with dilated PV while the rest had normal PV diameter. Splenomegaly was found in 35 patients.

Multivariate regression analysis:

Multivariate linear regression analysis for SAF score associated with independent risk factors is as follows: BMI (P = 0.276), MS (P = 0.515), IR (P = 0.420), and HCV-RNA (P = 0.284).

Discussion

One-quarter of the global population is estimated to have nonalcoholic fatty liver disease (NAFLD). Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in the Western world, with a prevalence of 20% [14]. The incidence of nonalcoholic steatohepatitis (NASH) is projected to increase by up to 56% in the next 10 years [10]. Recently, the SAF score emerged as a new algorithm to assess NAFLD and diagnose NASH. Initially, this score was developed for classifying NAFLD in morbidly obese patients [12], but it has now been validated in a cohort of patients with NAFLD and metabolic syndrome [9]. The SAF score separates steatosis from parenchymal necro-inflammation, two features that may have distinct prognostic potential.

Interestingly, independent from the classification of whether NASH is present, the overall histological severity of disease is scored separately as mild disease (A < 2, F < 2) or significant disease ($A \geq 2$, $F \geq 2$), also considering fibrosis staging. Therefore, NAFLD patients with less fat but still advanced fibrosis, and without steato-hepatitis,

Table 4 Relationship between steatosis (S) and fibrosis (F) according to SAF score

Items		S				*P* value
		S0	S1	S2	S3	
F	F0	29 (41.4%)	2 (2.9%)	0 (0.0%)	0 (0.0%)	0.0001*
	F1	3 (4.3%)	8 (11.4%)	3 (4.3%)	0 (0.0%)	
	F2	0 (0.0%)	5 (10.0%)	4 (5.7%)	2 (2.9%)	
	F3	3 (4.3%)	7 (10.0%)	3 (4.3%)	1 (1.4%)	

Data are expressed as number (percentage)

* Significant (*P* value <0.05)

Table 5 Relation between metabolic syndrome and level of HCV viremia

Metabolic syndrome (MS)	Quantitative HCVRNA PCR, mean ± SD	*P*
MS present	993,21.20 ± 165,856.571	$p > 0.417$ NS
MS absent	156,001.32 ± 483,402.995	$p > 0.417$ NS

would be classified as having "significant disease," even though they did not fulfill the criteria of NASH. Thus, the fibrosis component has an impact on the SAF score that may be relevant for long-term prognostication, although the association between the SAF score and long-term liver-related mortality has not yet been evaluated.

In our prospective cross-sectional study, the multivariate analysis showed that steatosis is an independent and significant factor associated with the degree of liver fibrosis. The more is the steatosis degree, the worse is the fibrosis. Interestingly, steatosis in HCV patients has also been associated with more severe histological injury and higher fibrosis scores, suggesting that fat in the liver is a biologically active tissue [15]. Steatosis can be significantly and independently associated with fibrosis in chronic hepatitis C. Insulin resistance (IR) is the possible link between the metabolic abnormalities in HCV patients with steatosis and the progression of hepatic fibrosis. Hyperinsulinemia in HCV patients may potentially promote fibrogenesis through either altered cytokine production, including TNF α, or by its direct effect on hepatic stellate cells [16, 17].

Although the mechanisms underlying the development of parenchymal steatosis in HCV infection are not exactly known, there are some findings to describe the mechanism of fat accumulation. The first series of mechanisms basically focuses on HCV proteins as the first step of the damage sequence. At least two HCV proteins (core protein and NS5A) are suspected to interact with the cell machinery involved in lipid metabolism [15].

Both HCV and steatosis are two synchronized factors causing fibrosis. The conflicting results of the previous studies that discuss the relation between steatosis and fibrosis may be due to the variation of the studied population and the abundance of risk factors [18–21].

In our study, baseline HCV genotyping was not performed, as it is assumed that more than 90% of HCV-infected patients in Egypt are infected with HCV-G4, and the remaining patients are infected with HCV-G1, with genotypes 2, 3, 5, and 6 almost non-existent and this analysis assumes that almost all patients are infected with HCV-G4 [22, 23]. HCV-G4 is known to induce less steatosis than genotype 3. However, it was found in an Egyptian study that HCV genotype 4 was associated with steatosis in more than half the patients, even in the absence of risk factors for metabolic steatosis. This was independent of the viral load but positively correlated with fibrosis and inflammation in these patients. This type of steatosis is possibly viral-induced (VAFLD) [24].

We found in our results that the degree of fibrosis increased with the progress of age. In agreement with our results, in a recent study, 25 patients with NAFL and 45 patients with NASH and/or advanced fibrosis were followed with repeated liver biopsy for an average of 3.7 years. Among the patients with NAFL, 16 patients (64%) developed NASH, eight of which had severe ballooning and six showing bridging fibrosis. Mild lobular inflammation or any degree of fibrosis conveyed a higher risk of progression than simple steatosis alone. Older age and deterioration of metabolic risk factors were associated with a more rapid progression [25].

A recent meta-analysis evaluated 411 patients with biopsy-proven NAFLD from 11 cohort studies (150 patients with NAFL and 261 patients with NASH). In the whole cohort, 33.6% of patients had fibrosis progression. This result was also observed in patients with NAFL but at a slower rate. In those with NAFL, it took an average of 14.3 years to progress one stage in fibrosis score;

however, in those with NASH, the time to progress with one stage was halved to 7.1 years [26]. Taken together, the data indicate that fibrosis progression is also observed in patients with NAFL, particularly in those with mild inflammatory changes, delicate fibrosis, older age, or deterioration of metabolic risk factors. However, patients with NASH have a more rapid course, with a significant risk for liver-related mortality [27].

In our study, both ALT and/or AST were found to progressively increase from F1 to F3. ALT showed a significant correlation with activity degree of SAF scoring system. However, AST/ALT ratio showed no significant relation to the activity or fibrosis degree of SAF score. In a recent study, they found that clinical predictors of advanced fibrosis in NAFLD are male sex, Caucasian ethnicity, diabetes mellitus, obesity, and increased AST or ALT levels [8, 28]. However, there was a poor correlation between ALT levels and NASH, or the stage of fibrosis [29]. In a study of 222 patients with NAFLD, 23% had normal ALT. The proportion of patients with advanced fibrosis was similar among those with normal and elevated ALT [26]. Contrary to our results, another study found that AST was a better predictor for advanced fibrosis than ALT. In early studies on NAFLD, an AST/ALT ratio > 1 was found to be associated with advanced fibrosis [8]. Another test that includes AST is the AST/platelet ratio index (APRI) [27] and had a negative predictive value of 94% to exclude advanced fibrosis (F3–4) in NAFLD. In a Chinese study, Zou et al. reported that AST/ALT ratio is often less than 1 in NASH, whereas a ratio above 1 would suggest an alcoholic steatohepatitis or evolution toward liver cirrhosis. In this respect, the higher AST levels would reflect more extensive mitochondrial damages [30].

We found that there is no significant correlation between SAF score and BMI (< 30 and 30 or more). Variation in body fat distribution in relation to histology is evident. As the overall fat mass measured by DXA decreased, both hepatic steatosis grade and disease severity worsened. In contrast, an increased trunk: limb ratio of fat was associated with increased steatosis grade. Finally, adipocyte size and volume from the abdominal wall biopsies showed a linear correlation with increased severity of histological diagnosis of NAFLD, but only in women. For unclear reasons, this did not hold true in the men and is an area worth further evaluation [31].

Our study showed no significant correlation between insulin resistance (IR) and BMI. Vadukoot et al. in 2016 [32] found that although IR was significantly higher in obese patients with NASH as compared to lean NASH (BMI < 23 kg/m^2), there was no significant difference in the correlation of IR with histology between these 2 groups. No significant difference was observed with regard to IR level with SAF. Fasting insulin level was comparable among patients with lean and obese NASH. No significant correlation was found between fibrosis of SAF score and between other risk factors increasing fibrosis such as HCV-RNA-PCR, age, MS, or IR.

Our study had been done on homogenous genotype 4 HCV non-cirrhotic, nonalcoholic Egyptian patients; however, the study had limitations including the relatively small number of patients, being conducted at the era of starting the use of DAA with difficulty to enrol new patients who agree to undergo liver biopsy. Also, most of our patients had early hepatic affection. Only 20% of our patients had advanced fibrosis (F3). Also, 60% of our patients had low BMI (below 30) and half showed no steatosis, which could affect our results.

Conclusion

In this prospective cohort study performed on Egyptian patients with chronic HCV infection without alcohol consumption and without cirrhosis, we found that steatosis was associated with the fibrosis stage independently of the metabolic syndrome. This suggests that in this population, steatosis might be more related to HCV infection than to NAFLD and that fibrosis progression might be related, at least in part, to the steatosis process, i.e., virus-associated fatty liver disease (VAFLD).

Abbreviations

SAF: Steatosis, activity, and fibrosis; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; MS: Metabolic syndrome; BMI: Body mass index; CHC: Chronic HCV infection; VAFLD: Virus-associated fatty liver disease; DAAs: Direct-acting antiviral drugs; WHO: World Health Organization; APRI: AST/platelet ratio index; IR: Insulin resistance; DXA: Dual-energy X-ray absorptiometry.

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Authors' contributions

All authors contribute equally like the corresponding author in creating the idea of the article, gathering the information, drafting and writing, and reviewing and editing the manuscript in the final shape. The material preparation, data collection, and analysis were performed by AER, MEG, AM, VP, IN, MS, AM, NP, and MA. The first draft of the manuscript was written by AER, MEG, AM, VP, PM, and TA, and all authors commented on the previous versions of the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

Datasets are available on request. This research involved animal participants. This was not an animal research.

Declarations

Ethics approval and consent to participate
The ethical committee of Theodor Bilharz Research Institute approved and registered the research, and all patients signed an informed consent.

Consent for publication
All the authors consented to publish the work.

Competing interests
The authors declare that they have no competing interests.

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