Name of Journal: World Journal of Hepatology
Manuscript NO: 75785
Manuscript Type: ORIGINAL ARTICLE

Observational Study
Higher cardiovascular risk scores and liver fibrosis risk estimated by biomarkers in patients with MAFLD


Abstract
BACKGROUND
The definition of Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) allows identifying metabolically complicated patients. Fibrosis risk scores are related to cardiovascular risk scores and could be useful for the identification of patients at risk of systemic complications.

AIM
To evaluate the relationship between MAFLD and CVR in a group of Mexican patients using the Framingham risk score.

METHODS
Cross-sectional, observational and descriptive study carried out in a cohort of 585 volunteers in the state of Veracruz with MAFLD criteria. The risk of liver fibrosis was
calculated with APRI, NAFLD score and FIB-4, as well as with transient hepatic elastography (TE) with Fibroscan®. The CVR was determined by the Framingham system.

RESULTS
125 participants (21.4%) with MAFLD criteria were evaluated, average age 54.4 years old, 63.2% were women, BMI 32.3 kg / m². The Framingham CVR was high in 43 patients (33.9%). TE was performed in 55.2% volunteers, 39.1% with high CVR and predominance in advanced fibrosis (F3-F4). The logistic regression analysis showed that liver fibrosis, diabetes and hypertension independently increase CVR.

CONCLUSION
In our population, 1 of every 3 patients with MAFLD has a high CVR, in those with high fibrosis risk, the CVR risk is even greater.

Key Words: fatty liver; cardiovascular risk; hepatic steatosis; fibrosis; liver disease


Core Tip: The Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) allows identifying metabolically complicated patients. The evaluate the relationship between hepatic fibrosis and CVR in patients with MAFLD using the Framingham risk score allows us to identify that patients with MAFLD and liver fibrosis by biomarkers have a higher cardiovascular risk than patients without fibrosis.
INTRODUCTION
Nonalcoholic fatty liver disease (NAFLD) is characterized by steatosis greater than 5% in the absence of alcohol consumption and other causes of liver disease. (1) Due to its close relationship with the components of the metabolic syndrome, a consensus of international experts proposed a change of name, resulting in the concept of "metabolic dysfunction-associated fatty liver disease" (MAFLD) as the new terminology. (2-4)

The presence of diabetes mellitus (DM), obesity and metabolic dysregulation to establish the diagnosis of MAFLD can help identify patients with metabolically complicated fatty liver disease and consequently with higher cardiovascular risk (CVR). We consider that the Framingham score can be a useful tool in the evaluation of CVR in patients with MAFLD because it independently assesses the presence of diabetes as a cardiovascular risk factor. (5, 6)

The Hepatic Fibrosis Scoring System is related to CVR scores in patients with MAFLD and can be useful for identifying the risk of systemic complications. (7) However, we do not have Mexican cohorts that consider the new definition of MAFLD and cardiovascular risk. (8) Therefore, the objective of our work was to evaluate the relationship between MAFLD and CVR in a group of Mexican volunteers using the Framingham scale.

MATERIALS AND METHODS
Cross-sectional, observational and descriptive study carried out in the population of a cross-sectional sample evaluated at the Instituto de Investigaciones Medico Biologicas and Centro de Servicios en Salud of the Universidad Veracruzana during February to March 2020. Residents of the State of Veracruz over 18 years-old were invited to participate. After signature informed consent, a medical evaluation was performed, which consisted of anthropometry measurements (weight, height, body mass index
(BMI), waist and hip circumference, waist-hip index), biochemical studies (hematologic biometry, glucose, creatinine, uric acid, lipids, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), bilirubin's, albumin and insulin) and liver ultrasound. In addition, blood pressure, personal history of diabetes mellitus (DM), systemic arterial hypertension, dyslipidemia, cardiovascular events, and tobacco use were recorded.

From the studied population, patients older than 40 years with diagnostic criteria for MAFLD were included. Patients with cancer, terminal disease, history of cardiovascular events and pregnant women were excluded.

The risk of liver fibrosis was determined with APRI, NAFLD and FIB-4 scores. The APRI was at high risk of significant fibrosis with a score of >1.5, indeterminate 0.5-1.5, and unlikely or absent <0.5; NAFLD score was considered high risk with >0.675, indeterminate -1.455 to 0.675, and absent <-1.455; FIB-4 score was considered high risk with >3.25, indeterminate 1.45-3.25 and absent <1.45. (9-11) Transient elastography (TE) with Fibroscan® was performed in patients with undetermined and high risk of liver fibrosis. (12, 13) The CVR was calculated with the Framingham system which evaluates: age, sex, total cholesterol, HDL cholesterol, blood pressure, use of antihypertensive drugs, tobacco consumption, DM, history of vascular disease; classifying patients as low, moderate, and high CVR. (14, 15)

The analysis of the results, elaboration of figures and tables was carried out with the IBM SPSS® Statistics 22.0 version program. Nominal and ordinal variables were described with frequencies and percentages, continuous and discrete variables with measures of central tendency and dispersion according to their distribution. The comparison between groups was carried out with the chi / square test and ANOVA. Non-parametric statistics with Spearman's correlation test were used in the relationship
between CVR and fibrosis. Statistical significance was considered when the p value was <0.05.

The project was carried out in accordance with the principles of Good Clinical Practices and prior approval of the Ethics Committee with number IIMB-UV 2020/03.

RESULTS
Population characteristics
Of the 585 volunteers, 125 participants (21.4%) who met the inclusion criteria were studied, 79 (63.2%) were women, average age 54.4 ± 8.8 years, BMI 32.3 ± 5.3 kg/m².

Cardiovascular risk assessment
According to the Framingham score, 46 patients (36.2%) had mild CVR, 36 (28.3%) moderate and 43 (33.9%) high. No differences were found by sex or BMI between the CVR categories. The patients’ age with high CVR was 59 ± 8.4 years, higher than in mild and moderate CVR (P = 0.028). The presence of DM, hypertension, and tobacco use was significantly higher in patients with high CVR. The determination of glucose and insulin was higher in patients with high CVR; therefore, the HOMA-IR index showed a value greater than 3.0 compatible with insulin resistance (P = 0.000) compared to patients with low and moderate CVR (HOMA-IR 1.96 to 3). The rest of the biochemical parameters evaluated did not show significant differences (table 1). Fibrosis scores showed an increasing trend in patients with high CVR; however, this difference was not significant (0.094).

Evaluation of liver fibrosis
The distribution between the fibrosis risk stages by FIB-4, NAFLD and APRI scores are shown in figure 1. 52 patients (44.8%) with indeterminate and high risk of fibrosis were identified according to FIB-4, 79 (62.2 %) according to NAFLD score and 21 (15.6%) with APRI.
was performed in 69 patients (55.2%) with indeterminate or high risk of fibrosis. Patients were identified as follows, F0: 19 (27.5%), F1: 12 (17.4%), F2: 11 (15.9%), F3: 13 (18.8%) and F4: 14 (20.3%). In the evaluation of hepatic steatosis by controlled attenuation parameter (CAP) the results were the following, S0: 13 patients (18.8%), S1: 7 (5.5%), S2: 3 (4.2%) and S3: 46 (36.2%).

The age distribution showed a significant difference between the risk of fibrosis due to FIB-4, as it was higher in the group with indeterminate fibrosis and lower in patients with absence of fibrosis ($P = 0.000$). The BMI and the comorbidities evaluated did not show significant differences between the risk groups. Patients with high risk of fibrosis had decreased platelet and albumin counts, as well as significantly elevated levels of TB, AST, and phase angle compared to patients without fibrosis or indeterminate risk of fibrosis ($P = 0.000$).

**Liver fibrosis and cardiovascular risk**

The correlation of liver fibrosis risk by FIB-4 and CVR according to the Framingham system showed that 33.4% of patients with MAFLD have a high CVR, predominantly in patients with indeterminate risk of fibrosis (18.2%). 14.4% of the patients with no fibrosis had a high CVR vs 19.8% of the patients with an indeterminate or high risk of fibrosis. The risk of fibrosis did not show a significant correlation with the severity of CVR ($P = 0.257$). The categories of CVR and risk of fibrosis by FIB-4 can be observed in Figure 2.

In 69 patients evaluated with TE, 18 patients (26.1%) were found with mild CVR according to the Framingham system, 24 (34.7%) with moderate and 27 (39.1%) with high risk. It was observed that the group with the greatest number of patients with high CVR were those with advanced fibrosis. A 39.1% of patients with MAFLD have a high CVR at the time of diagnosis with a predominance of advanced fibrosis (F3-F4). In
relation to fibrosis severity, it was noted that in advanced fibrosis, 34.8% have moderate
to high CVR, predominantly observing the correlation between advanced fibrosis and
high CVR. A statistically significant relationship was reported between the presence of
fibrosis and the severity of the CVR \( (P = 0.026) \). The distribution of the different risk
categories in patients with absent or mild-moderate fibrosis was heterogeneous as
shown in Figure 3.

**Hepatic steatosis and cardiovascular risk**

The correlation between CAP and CVR showed that 28.9% of the patients with S3 had a
high CVR. Although most of our patients were found in S3, the severity of the CAP did
not show a significant relationship with the severity of the CVR \( (P = 0.254) \). Table 2
shows the correlation between CVR and CAP.

In the logistic regression analysis, the presence of fibrosis \( P = 0.007 \) (CI 0.157-35.376),
DM \( P = 0.000 \) (CI 0.791-43.555) and hypertension \( P = 0.035 \) (CI 0.085-5.228) were
independently and significantly associated with the CVR, but not with the presence of
steatosis \( P = 0.220 \) (IC 0.144-22.921).

**DISCUSSION**

Metabolic Dysfunction-Associated Fatty Liver Disease is currently the most common
chronic liver disease worldwide, present in 25 to 30% of the population. Although the
severity of the disease criteria has not been established, it is described that the presence
of fibrosis is the most important prognostic marker of mortality, independent of the
severity of the fatty infiltration. (16, 17) Our study, carried out in a Mexican population,
shows that one out of every three patients with MAFLD have a high CVR, and the
higher the fibrosis the greater the cardiovascular risk. We show novel results as it is one
of the first studies to use the new definition of MAFLD in association with CVR. (18)

The change in diagnostic criteria from NAFLD to MAFLD was made recently. Therefore, in Mexico we have few prevalence studies with the new definition. The
study carried out by Bernal et al in 585 healthy volunteers published as a summary showed a prevalence of MAFLD of 42.1%, high if we compare it with the prevalence of NAFLD estimated worldwide.(19) In our cohort, we found that 21.4% of the population over 40 years of age has a MAFLD diagnosis, like the prevalence of NAFLD reported in the adult population of various Western countries. Although the prevalence of DM in patients with NAFLD is 50% to 70%; in our population, it was lower (22.4%). On the other hand, we found a high prevalence of overweight and obesity (97.6%), like that observed in other populations where between 80 and 90% are reported. We consider that the differences may be because previously conducted studies consider the NAFLD criteria. (20, 21)

Liver biopsy is the gold standard in the evaluation of fibrosis, but it carries the risk of complications coupled with high cost. For this reason, the use of non-invasive systems such as serum markers, transient elastography or magnetic resonance imaging is recommended. TE with FibroScan® is the most widely used and validated elastography worldwide with good sensitivity and specificity to diagnose F4 stage (92% and 92%). The FIB-4 index has been shown to be superior to other non-invasive markers in the identification of advanced fibrosis in patients with MAFLD, therefore it is the marker of choice in the two-step algorithms. (22) Shah et al compared the diagnostic performance of non-invasive fibrosis markers and concluded that the FIB-4 index is better at identifying advanced fibrosis in patients with MAFLD. The diagnostic performance of the markers used in our study showed similar results, with a higher correlation of advanced fibrosis by FIB-4 with TE. (23)

The main cause of death in patients with MAFLD is cardiovascular disease; the secondary causes are those related to the liver. Cardiovascular diseases most frequently observed in patients with MAFLD are left ventricular dysfunction, atherosclerotic disease, disturbances in the cardiac conduction system, and cerebral ischemic events. These observations establish the close relationship between the severity of liver disease
and the risk of fatal and non-fatal cardiovascular events. (24) Despite current evidence, in daily clinical practice, MAFLD is considered a benign entity. For this reason, our study focused on the identification of patients with MAFLD and a high risk of cardiovascular events and its relationship with hepatic fibrosis markers. Our purpose was to recommend early identification mechanisms to prevent complications and decrease mortality.

Fatty liver is associated with increased CVR regardless of the presence of DM, dyslipidemia, and hypertension. Therefore, early identification is important to reduce cardiovascular mortality. (25) Our results show that the majority of the population with MAFLD has mild CVR according to the Framingham system. However, more patients with higher CVR were identified at indeterminate risk of fibrosis due to FIB-4 (18.2%). Our results showed that most of the patients with high CVR have advanced fibrosis (F3-F4), in addition to this, these patients had a higher frequency of DM and hypertension. These results reflect that the higher the CVR, the greater the risk of liver fibrosis, which allows the early identification of patients with compensated advanced liver disease.

Cardiovascular disease and arteriosclerosis are the result of endothelial damage, dyslipidemia, and oxidative stress reported more frequently in patients with MAFLD. However, as reported in literature, the severity of hepatic fatty infiltration did not demonstrate a relationship with cardiovascular risk. (26, 27)

Various studies have reported that the FIB-4 index ≥ 2.67 is independently associated with coronary atherosclerosis and cardiovascular events, therefore with an increase in CVR. (28, 29) In our study, results similar to those published in previous clinical trials were observed, exhibiting a correlation between FIB-4 with the CVR systems compared to NAFLD and APRI (p <0.05). Another limitation is that it is not a national representative population, as it only includes volunteers from the State of Veracruz.
It is important to mention and recognize that our study has limitations that must be considered. One of them is that the prevalence of MAFLD was calculated in volunteers older than 40 years and in different clinical trials the population older than 18 years is included, therefore prevalence could be underestimated in our cohort. Finally, it is recognized that the gold standard for evaluating fibrosis and steatosis in patients with MAFLD is liver biopsy. However, due to the risks of this procedure, we performed the evaluation of fibrosis and steatosis with only biochemical markers and transient elastography.

**CONCLUSION**

In conclusion our population, one of every three patients with MAFLD has a high CVR and the greater severity of fibrosis correlates with a greater CVR. According to our results, the early identification of CVR in patients with MAFLD will allow establishing preventive actions and timely treatment to reduce the risk of mortality in this population.
<table>
<thead>
<tr>
<th>#</th>
<th>Source</th>
<th>Words</th>
<th>Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&quot;Posters&quot;, Hepatology, 2020</td>
<td>24</td>
<td>1%</td>
</tr>
<tr>
<td>2</td>
<td>wjgnet.com</td>
<td>23</td>
<td>1%</td>
</tr>
<tr>
<td>3</td>
<td><a href="http://www.elsevier.es">www.elsevier.es</a></td>
<td>19</td>
<td>1%</td>
</tr>
</tbody>
</table>

**Exclusion Settings**

- Exclude Quotes: ON
- Exclude Bibliography: ON
- Exclude Sources: < 1%
- Exclude Matches: < 10 WORDS