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# Association Between Serum Hemoglobin Levels and Non Alcoholic Fatty Liver Disease in a Mexican Population

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## ABSTRACT

**Introduction and aim.** Nonalcoholic fatty liver disease (NAFLD) is closely associated with overweight and obesity, becoming one of the most prevalent hepatic diseases nowadays. Circulating hemoglobin (Hb) concentration is significantly higher in people with NAFLD, compared to healthy patients. While liver biopsy remains the gold standard for NAFLD diagnosis, it is not the best technique due to adverse events that may occur. Therefore it is important to find less invasive and more sensitive markers. This study aimed to determine the association of serum Hb levels in patients with steatosis and fibrosis as a noninvasive marker. **Material and methods.** A 1,186 patient cross-sectional study nested in a randomized clinical trial (NCT01874249) was conducted. Patients were diagnosed by ultrasound for hepatic steatosis and fibroscan for fibrosis; blood test and anthropometric measurements were also assessed. **Results.** Serum Hb increased proportionally related to the steatosis level, being significantly higher in patients with severe steatosis than in patients with moderate and mild steatosis. **Conclusion.** Patients with non-alcoholic fatty liver disease showed elevated levels of circulating Hb, evidence that suggests that Hb exerts a protective role, as it may act as an antioxidant and may counteract the adverse effects of this disease.

**Key words.** Antioxidant. Hepatic disease. Non-invasive markers. Steatosis.

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease and its development is a complex multifactorial process that involves multiple genetic and protein regulation alterations that are associated globally with overweight and obesity, becoming one of the most common hepatic diseases in adults and children.[1,2] The spectrum of this illness ranges from steatosis to nonalcoholic steatohepatitis (NASH), evolving through cirrhosis and liver failure.[3-5] The second cause of hepatic transplantation in the United States is related to the evolution of NASH to cirrhosis and is also increasing in Europe. Several serotransferases, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) are strongly associated with steatosis and may act as serum biomarkers of liver damage.

The usefulness of various diagnostic-imaging tools has been assessed to identify liver fat levels. Hepatic ultrasound (USG) is the most simple and economical method to identify steatosis. In the general population the sensitivity and specificity of USG to detect steatohepatitis is 60-94% and 84-95% respectively, however it is not useful for fibrosis detection which is the main prognostic factor for NAFLD progression.

Transient elastography has demonstrated its utility detecting liver fibrosis, especially in advanced stages. To improve the elastography performance is recommended the combination with other non-invasive markers. However, the clinical practice guidelines suggest a moderate level of

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evidence in conjunction with other non-invasive liver fibrosis markers.[10]

Moreover elevated levels of free serum hemoglobin (Hb) have been associated with increased steatosis prevalence, this relation was independent of body mass index (BMI), type 2 diabetes, and other metabolic diseases, suggesting that Hb could be a biomarker for the disease. Also, the knowledge that erythrocytes specifically expressed Hb has been questioned since it has been found in different cell types including neurons, retinal cells, alveolar cells, kidney mesangial cells, macrophages, and hepatocytes, facilitating reactive oxygen and nitrogen species detoxification.[4,5,11-13]

## EXPERIMENTAL PROCEDURES

### Study design

A cross-sectional study nested in a randomized clinical trial (NCT01874249) (https://clinicaltrials.gov/show/NCT01874249) was performed in Medica Sur Clinic and Foundation Preventive Medicine Unit. The ethics committee approved the protocol and all participants received an informed consent.

The sample was selected from a consecutive number of patients who attended a preventive medical examination between June 2013 and June 2015. Participating subjects were between 18 and 69 years old, had overweight or obesity (BMI ≥ 27 kg/m$^2$), and hepatic steatosis, diagnosed by ultrasound. Patients were randomized into five groups of intervention according to random control trial's (RCT) methodology, which is electronic educational information about causes and consequences of NAFLD. Only two of these groups were evaluated by transient elastography to assess the presence of liver fibrosis. Exclusion criteria: liver disease other than NAFLD; tamoxifen, methotrexate, amiodarone, diltiazem or any antiretroviral; alcohol > 140 g per week, viral or autoimmune liver diseases and blood transfusion before 1990. To compare serum Hb levels, a control group of 289 patients apparently healthy, without liver steatosis and body mass index < 25 kg/m$^2$ was included. The control group was extracted from another cross-sectional study. Patients with BMI < 25 kg/m$^2$ were selected to compare serum hemoglobin levels with the study group.

Abdominal ultrasound was performed with a six-hour fasting; a 3.5 MHz transducer (Elegra; Siemens Medical Systems, Mountain Grove CA) was used to obtain a sagittal view of the right lobe of the liver, a transverse view of the lateral segment of the liver and any focal areas of altered echotexture. Liver steatosis was defined as increased parenchymal echogenicity regarding kidney cortex, without portal and hepatic vein echogenicity and poor visualization of the diaphragm and the liver. The degree of liver steatosis was classified according to ultrasound imaging characteristics, mild steatosis with increased hepatic echogenicity, visible periportal and diaphragmatic echogenicity; moderate steatosis, with increased hepatic echogenicity and imperceptible periportal echogenicity, without diaphragm shadowing; and severe steatosis with increased hepatic echogenicity with subtle periportal echogenicity and shadowing of the diaphragm.[14] Body fat percentage was assessed by bioelectrical impedance (Tanita Corporation, Tokyo, Japan).

Transient elastography was performed with both probes (M and XL) in all patients, considered accurate if they fulfilled the following characteristics:

- At least ten valid shots.
- ≥ 60% success rate and
- Interquartile/median range (IQR/M) ≤ 30%.

To decide the probe size, a cut probe of ≥ 35 mm thick in the abdominal ultrasound (distance from the skin to the liver) was used. The fibrosis degree was determined according to the Wong, *et al.* cutoffs; F2 > 7 kPa, F3 > 8.7 kPa and F4 >10.3 kPa.[15]

Complete blood count, blood glucose, lipid panel (total cholesterol, HDL, triglycerides and LDL), C-reactive protein, total bilirubin (TB), direct bilirubin (DB), indirect bilirubin (IB), blood urea nitrogen,, creatinine, Hb, liver function test and anthropometric measurements (weight, height, waist and hip circumference, waist-hip index ratio, BMI and body composition by bioelectrical impedance) were evaluated in fasting. Demographic and clinical characteristics were evaluated in all patients.

### Statistical analysis

Variables distribution was determined using the Kolmogorov-Smirnov test. Continuous variables are presented as medians and interquartile range; categorical variables are presented as numbers and percentages. The difference of serum Hb concentrations in steatosis was performed by a Kruskal-Wallis test with adjustments for body mass index and smoking. To compare the differences between serum Hb levels and clinical characteristics at each steatosis level and liver fibrosis, patients were classified into two groups according to the mean Hb concentration in the population. This association was analyzed by Mann-Whitney U. the p-value ≤ 0.05 was considered statistically significant. Univariate and multivariate analyses were performed by logistic regression, to identify the independent associations of serum hemoglobin with NAFLD presence and severity. Statistical analysis was performed using SPSS v.20.0 (SPSS, Chicago, IL, USA).

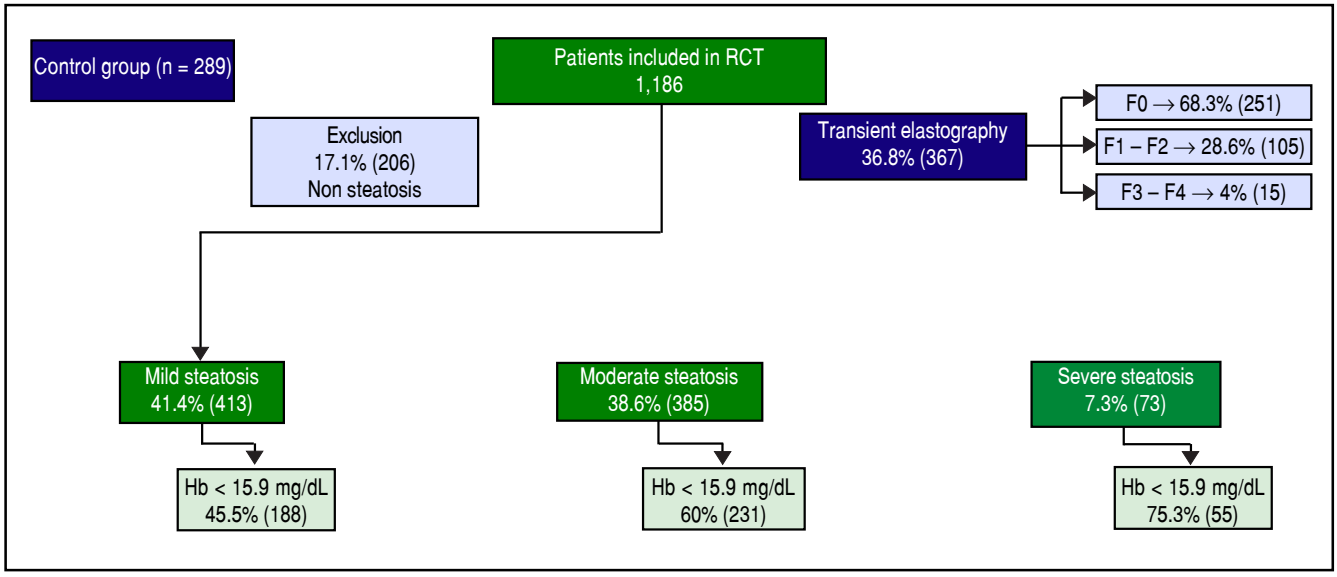


***Figure 1.*** *Patient's distribution according steatosis stage and serum Hb levels. RCT: randomized clinical trial. Hb: hemoglobin.*

## RESULTS

The clinical trial included 1186 patients, of whom 206 were excluded for not having the diagnosis of hepatic steatosis; therefore only 997 patients were included (Figure 1). Most of the population on study group were men (82.4%) with an average age of 47 ± 8 years and a mean BMI of 29.8 kg/m$^2$ [28.4-32.3], 26.8% of the population had a smoking history. The general characteristics of the control group and patients with steatosis are shown in table 1.

According to abdominal ultrasound, most patients 41.4% (n = 413) had a slight degree of hepatic steatosis, 38.6% (n = 385) moderate steatosis and 7% (n = 73) severe steatosis. As part of the study, transient elastography was performed in 40.9% (n = 367) patients, the mean success rate for transient elastography evaluation was a 100% [IQR 88-100]. Fibrosis prevalence was 12% (n = 120), of which 3.6% (n = 36) had advanced fibrosis (F3-F4) according to the Metavir fibrosis scale. Since only 40% of patients had transient elastography, we evaluated liver fibrosis according to NAFLD Score and FIB4 in a study group. Serum hemoglobin was not associated with presence or severity of liver fibrosis.

The median serum Hb concentration in study group was 14.9 mg/dL [IQR 14.2-15.9] for the control group; for the mild steatosis group 15.7 mg/dL [IQR 14.8-16.4]; for the moderate steatosis group 16.1 mg/dL [IQR 15.4-16.8] and for the severe steatosis group 16.3 mg/dL [IQR 15.8-16.8]. In the comparison analysis, serum Hb concentration increased proportional to the steatosis level ($p \leq 0.001$), being significantly higher in patients with severe steatosis than in patients with moderate and mild steatosis (Figure 2).

Patients with steatosis were divided into two groups regarding the Hb concentration median (15.9 mg/dL), the proportion of patients with moderate and severe steatosis that show higher levels above the median were 58.5% (n = 233) and 75.7% (n = 56) respectively ($p \leq 0.001$). When comparing Hb levels in the presence of fibrosis, no significant associations were found. However, these patients showed significantly lower serum HDL levels and higher weight, BMI, waist circumference, body fat mass, as well as higher PCR and AST serum levels.

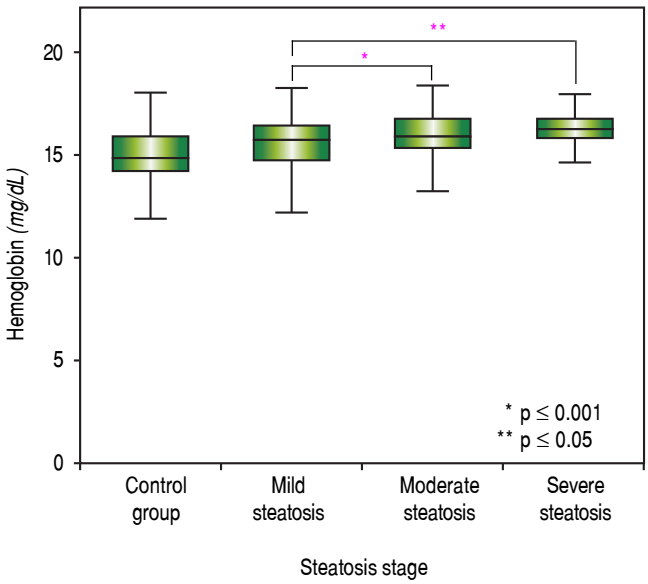


***Figure 2.*** *Serum Hb differences between control group and patients with liver steatosis.*

**Table 1.** Patient's baseline characteristics

| Characteristic | Study group (n=997) % (n) /Median [IQR] | | | | Control group (n=289) % (n) /Median [IQR] | p |
|---|---|---|---|---|---|---|
| | Non steatosis (n = 126) | Mild steatosis (n = 413) | Moderate steatosis (n = 385) | Severe steatosis (n = 73) | | |
| Male | 66.7% (84) | 77.2% (319) | 89.6% (345) | 94.5% (69) | 47.1% (136) | ≤ 0.0001 |
| Age (years) | 44 (40-52) | 47 (41-53) | 46 (40-53) | 47 (41-53) | 40 (36-45) | ≤ 0.0001 |
| Weight (kg) | 84.4 (76.6-92) | 86 (78.8-93.6) | 90.4 (83.1-100.9) | 95.2 (88.2-106.2) | 62.7 (56.5-70.6) | ≤ 0.0001 |
| Height (m) | 1.70 (1.60-1.76) | 1.70 (1.63-1.76) | 1.72 (1.67-1.77) | 1.72 (1.69-1.77) | 1.67 (1.60-1.73) | ≤ 0.0001 |
| BMI (kg/m$^2$) | 28.8 (27.9-30.6) | 29.4 (28.2-31.5) | 30.4 (28.6-33.4) | 31.6 (29.8-35.3) | 22.9 (21.7-24) | ≤ 0.0001 |
| WC (cm) | 100 (95-104) | 102 (97-107) | 105 (100-112) | 110 (102.7-117) | 81.5 (78-86) | ≤ 0.0001 |
| Body fat mass (%) | 31.6 (27-37.7) | 31.3 (27.9-36.7) | 31.6 (28.6-35.6) | 33.5 (30.5 -39.6) | 23.1 (20.5-29.2) | ≤ 0.0001 |
| Diabetes mellitus | 4.0% (5) | 5.1% (21) | 7.8% (30) | 12.3% (9) | NA | |
| Hypertension | 9.5% (12) | 17.7% (73) | 19.2% (74) | 16.4% (12) | NA | |
| Metabolic syndrome | 24.6% (31) | 40% (165) | 50.1% (193) | 45.2% (33) | NA | |
| Smoking | 34.1% (43) | 24.7% (102) | 26.8% (103) | 26% (19) | 8.3% (24) | ≤ 0.0001 |
| Glucose (mg/dL) | 93.4 (89-98) | 95.2 (90-102.6) | 98.2 (92-108) | 103.6 (93.2-119.2) | 88.3 (84-93) | ≤ 0.0001 |
| Creatinine (mg(dL) | 0.90 (0.75-1.01) | 0.92 (0.81-1.02) | 0.93 (0.85-1.02) | 0.96 (0.88-1.06) | 0.84 (0.7-0.9) | ≤ 0.0001 |
| Uric acid (mg/dL) | 6.0 (5.1-7.1) | 6.2 (5.4-7.2) | 6.6 (5.9-7.5) | 7.03 (6.2-7.9) | NA | |
| CRP (mg/L) | 2.3 (0.99-3.8) | 2.2 (1.1-3.8) | 2.4 (1.3-4.7) | 3 (1.7-5) | 0.9 (0.4-1.5) | ≤ 0.0001 |
| Albumin (mg/dL) | 4.1 (3.9-4.2) | 4.1 (4.-4.3) | 4.2 (4.0-4.4) | 4.2 (4.0-4.4) | 4.2 (4.1-4.4) | 0.02 |
| Alkaline phosphatase (U/L) | 68 (56-77) | 67 (59-80) | 70 (60-83) | 69 (61-85.5) | 58.6 (50.5-68) | ≤ 0.0001 |
| Total bilirubin (mg/dL) | 0.74 (0.63-0.95) | 0.81 (0.66-1.01) | 0.84 (0.68-1.05) | 0.81 (0.70-1.07) | 0.87 (0.7-1.06) | ≤ 0.0001 |
| Direct bilirubin (mg/dL) | 0.11 (0.0-0.14) | 0.11 (0.0-0.14) | 0.12 (0.0-0.15) | 0.12 (0.0-0.15) | 0.11 (0.10-0.13) | ≤ 0.0001 |
| AST (U/L) | 24.5 (21.7-30) | 26 (23-33) | 30 (25-37) | 33 (26.5-42) | 22.2 (19-25) | ≤ 0.0001 |
| ALT (U/L) | 25 (19-36) | 30 (23-41) | 39 (30-54) | 46 (35-62.5) | 20.6 (15.5-27) | ≤ 0.0001 |
| Hb (g(dL) | 15.4 (14.4-16.1) | 15.7 (14.8 -16.4) | 16.1 (15.4-16.8) | 16.3 (15.8 -16.8) | 14.9 (14.2-15.9) | ≤ 0.0001 |
| Platelets (x 10$^3$/µL) | 221 (192-252) | 224 (193.5-260.5) | 213 (182.5-245.5) | 219 (192-251.5) | 219.2 (200-260) | 0.118 |
| Triglycerides (mg/dL) | 151.5 (110-207.2) | 172.7 (128.5-237) | 197 (149.1-280.9) | 212 (149.6-268.8) | 112.3 (85-149.5) | ≤ 0.0001 |
| Cholesterol (mg/dL) | 204.5 (174.7-230.2) | 204 (179-232) | 205 (178.5-229.1) | 211 (172.6-232.7) | 190.9 (175-214.5) | 0.001 |
| HDL (mg/dL) | 45 (38-53) | 42 (36-47) | 39 (35-44.1) | 39 (36-44.1) | 48.7 (42-56) | ≤ 0.0001 |
| LDL (mg/dL) | 124 (98.7-145) | 124 (101-147) | 120.2 (99-143) | 123 (93-148.5) | 116.2 (103-135.5) | 0.06 |

*IQR: interquartile range. BMI: Body Mass Index. WC: waist circumference. CRP: C reactive protein. AST: aspartate amino transferase. ALT: alanine aminotransferase. Hb: hemoglobin. HDL: high-density lipoprotein. LDL: low-density lipoprotein. NA: non-available.*

**Table 2.** Patient's baseline characteristics by group.

| Characteristic | Mild steatosis | | | Moderate steatosis | | | Severe steatosis | | |
|---|---|---|---|---|---|---|---|---|---|
| | Hb ≥ 15.9 mg/dL | Hb < 15.9 mg/dL | P | Hb ≥ 15.7 mg/dL | Hb < 15.9 mg/dL | P | Hb ≥ 15.9 mg/dL | Hb < 15.9 mg/dL | p |
| Age (years) | 47 (42-54) | 46 (40-53) | NS | 49 (42-55) | 45 (40-52) | ** | 50 (47-56) | 44 (40-51) | * |
| Weight (kg) | 82.4 (75.2-91) | 88.8 (83-96.6) | ** | 90.1 (81.7-101.7) | 90.1 (84-99) | NS | 90.4 (84.4-99.4) | 95.4 (88.5-109.5) | NS |
| Height (m) | 1.67 (1.59-1.73) | 1.72 (1.67-1.77) | ** | 1.71 (1.64-1.77) | 1.72 (1.68-1.77) | NS | 1.70 (1.68-1.74) | 1.73 (1.69-1.78) | NS |
| WC (cm) | 102 (95-105.3) | 103.8 (99-107) | ** | 106 (100-112) | 104 (100.1-112) | NS | 109 (101-115) | 110 (103-117) | NS |
| BFM (%) | 32.4 (28.6-38.1) | 31.05 (27.9-32.5) | ** | 32 (29.3-38.9) | 31 (28.1-34.6) | * | 32 (28.7-39.6) | 34.6 (30.9-39.5) | NS |
| Creatinine (mg/dL) | 0.8 (0.7-0.9) | 0.9 (0.8-1.06) | ** | 0.9 (0.8-1.0) | 0.9 (0.8-1.02) | * | 0.9 (0.7-1.02) | 0.97 (0.9-1.08) | NS |
| Uric Acid (mg/dL) | 5.9 (5.1-6.9) | 6.5 (5.8-7.3) | ** | 6.6 (5.7-7.7) | 6.6 (5.9-7.5) | NS | 6.5 (5.3-6.8) | 7.2 (6.6-8.1) | * |
| CRP (mg/L) | 2.4 (1.2-4.5) | 2.0 (0.9-3.7) | * | 2.6 (1.3-5.0) | 2.2 (1.2-3.9) | NS | 3.4 (2.2-4.8) | 3.2 (1.6-5.2) | NS |
| Albumin (mg/dL) | 4.1 (3.9-4.2) | 4.2 (4.0-4.4) | ** | 4.1 (4.0-4.3) | 4.2 (4.1-4.4) | ** | 4.0 (3.8-4.2) | 4.2 (4.0-4.4) | * |
| TB | 0.76 (0.63-0.92) | 0.92 (0.74-1.11) | ** | 0.8 (0.6-0.9) | 0.8 (0.7-1.1) | ** | 0.61 (0.69-0.9) | 0.8 (0.7-1.0) | NS |
| DB | 0.10 (0.0-0.13) | 0.13 (0.0-0.16) | ** | 0.10 (0.0-0.13) | 0.13 (0.0-1.17) | ** | 0.12 (0.0-0.16) | 0.12 (0.02-0.15) | NS |
| AST (U/L) | 26 (22-30) | 28 (23-36) | ** | 27 (24-33) | 31 (26-38) | ** | 27 (24.7-38.7) | 35 (29.2-43.7) | * |
| ALT (U/L) | 28 (21-40) | 35 (26-48) | ** | 35 (24-33) | 42 (32-59) | ** | 32.5 (25.7-43.7) | 51 (38.2-64.5) | * |
| Platelets (x$10^3$ / µL) | 234 (199-269) | 212.5 (186-247.7) | ** | 224 (188.5-260) | 207 (181-231) | * | 207.5 (184.5-272) | 221.5 (193.7-249.5) | NS |
| Triglycerides (mg/dL) | 162.2 (121-225) | 188 (138-254) | * | 199 (142-287) | 198 (152-272) | NS | 190.2 (153-294) | 217 (148-270) | NS |
| HDL (mg/dL) | 44 (37.5-50.3) | 40 (36-45) | ** | 41 (35-47.05) | 38.5 (34.9-43) | * | 39 (34.8-48.2) | 39 (36-43) | NS |
| LDL (mg/dL) | 124 (100-147) | 129.5 (103-152) | * | 124.6 (100-143.5) | 120 (98-144) | NS | 96 (82-134) | 128 (97.7-150.7) | NS |

*Data shows a<s median [IQR]. *p ≤ 0.05; **p ≤ 0.001. NS: non-significant. WC: waist circumference. BFM: body fat mass. CRP: C reactive protein. TB: total bilirubin. DB: direct bilirubin. AST: aspartate transaminase. ALT: alanine aminotransferase. HDL: high density lipoprotein. LDL: low density lipoprotein.*

It was observed that patients with mild and moderate steatosis with high Hb levels increased their creatinine, total and direct bilirubin concentrations; nevertheless, there was a decrease in the fat percentage, platelets and HDL, ($P \leq 0.05$). However patients with elevated Hb levels in the three different steatosis degrees, compared to those with low Hb levels, shown a significant increase in albumin, AST and ALT levels ($P \leq 0.05$). The uric acid concentration was found to be increased in patients with mild and severe steatosis, coupled with increased Hb levels ($P \leq 0.05$) (Table 2).

In multivariate analysis, gender, waist circumference, serum lipids and hemoglobin were an independent factor for the presence of NAFLD. (Table 3) Independent relationship of serum hemoglobin was also for steatosis severity (Table 4).

## DISCUSSION

Hb is the most common component in erythrocytes and acts as a carbon dioxide and oxygen carrier through the vascular network to body cells,[4,13] which is relatively a recent adaptation during evolution. Also, Hb performs other cellular activities, including oxygen sensing, intracellular oxygen transport, iron metabolism regulation, nitric oxide (NO) and hydrogen peroxide scavenging.[16]

Patients with NAFLD have been found to have significantly higher serum Hb concentrations than the healthy controls,[17-21] indeed they are at an increased risk of generating abnormal liver function, as well is also the primary independent predictor of histologic severity in liver biopsy.[17,22]

In this study, the serum Hb concentration increased proportionally about the steatosis level, being significantly higher in patients with severe steatosis than in patients with moderate and mild steatosis (Figure 2). However, when comparing Hb concentrations in the presence of fibrosis, no significant associations were found. Patients with high Hb levels showed alterations in other metabolic syndrome indicators, such as lipid profile, liver function tests, as well as indicators of renal function and albumin.

We have observed the association between Hb levels and the degree of hepatic steatosis in a group of patients with homogeneous clinical and demographic characteristics; confounding variables involved in elevation of Hb levels, such as smoking and BMI were adjusted in the statistical analysis, however since it is a nested study, Hb association with liver fibrosis was not significant because the insufficient sample size. Moreover, Xu, *et al.* mentioned that NAFLD patients had higher serum Hb concentrations than the healthy controls, suggesting a protective role for Hb in the presence of steatosis, but when inflammation and necrosis appear in the liver, the protective effect disappears.

**Table 3.** Univariate and multivariate analysis of presence of liver steatosis in control and study group.

| Variable | Univariate | | Multivariate | |
|---|---|---|---|---|
| | OR (95% CI) | P | OR (95% CI) | P |
| Male | 4.9 (3.7-6.4) | < 0.0001 | 11.7 (6.7-20.4) - 7.1) | < 0.0001 |
| Smoking | 1.7 (1.3-2.4) | < 0.0001 | | |
| Waist circumference[a] | 8.7 (6.5-11.15) | < 0.0001 | 5.8 (4.1-8.3) | < 0.0001 |
| HDL[b] | 1.9 (1.5-2.4) | < 0.0001 | 1.4 (1.0-1.9) | 0.02 |
| Triglycerides ≥ 150 mg/dL | 4.5 (3.5-5.8 | < 0.0001 | 2.3 (1.6-3.2) | < 0.0001 |
| Glucose ≥ 110 mg/dL | 9.8 (4.9-19.4) | < 0.0001 | 5.4 (2.5-11.4) | < 0.0001 |
| % BFM ≥ 35% | 2.7 (1.9-3.8) | < 0.0001 | 6.3 (3.3-11.7) | < 0.0001 |
| Hb ≥ 15.9 mg/dL | 2.9 (2.3-3.8) | < 0.0001 | 1.5 (1.0-2.1) | 0.01 |
| Cholesterol ≥ 227 mg/dL | 1.6 (1.2-2.1) | 0.001 | | |

*[a] > 88 cm in women, > 102 cm in men; [b] < 50 mg/dL in women, < 40 mg/dL in men. HDL: high density lipoprotein. BFM: body fat mass.*

**Table 4.** Univariate and multivariate analysis of severe steatosis in study group.

| Variable | Univariate | | Multivariate | |
|---|---|---|---|---|
| | OR (95% CI) | P | OR (95% CI) | P |
| Male | 3.4 (2.3-5.0) | < 0.0001 | 3.3 (2.1-5.2) | < 0.0001 |
| Smoking | 1.0 (0.8-1.1) | 0.943 | | |
| Waist circumference[a] | 1.6 (1.2-2.1) | < 0.0001 | 1.9 (1.4-2.5) | < 0.0001 |
| HDL[b] | 1.5 (1.1-1.9) | 0.001 | 1.4 (1.0-1.9) | 0.009 |
| Triglycerides ≥ 150 mg/dL | 1.9 (1.5-2.6) | < 0.0001 | 1.4 (1.0-1.9) | 0.025 |
| Glucose ≥ 110 mg/dL | 2.8 (2.0-4.0) | < 0.0001 | 2.7 (1.8-3.9) | < 0.0001 |
| % BFM ≥ 35% | 1.0 (0.9-1.2) | 0.322 | | |
| Hb ≥ 15.9 mg/dL | 2.2 (1.7-2.8) | < 0.0001 | 1.6 (1.2-2.2) | 0.001 |
| Cholesterol ≥ 227 mg/dL | 1.0 (0.9-1.2) | 0.573 | | |

*[a] > 88 cm in women, > 102 cm in men. [b] < 50 mg/dL in women, < 40 mg/dL in men. HDL: high density lipoprotein. BFM: body fat mass.*

As well, it was observed that patients with mild and moderate steatosis who have high Hb levels increase their creatinine, which is associated with an elevated chronic kidney disease (CKD) prevalence and incidence, leading to the elevation of proinflammatory molecules and oxidative stress, promoting kidney injury.[23-25] The damage can be minimized by the uric acid rise (UA) due to the increase in Hb since it is one of the most important antioxidants in the human body. Nevertheless, there is evidence that UA elevation may cause insulin resistance, which is closely related to the metabolic syndrome and NAFLD development.[26]

Moreover, elevated total and direct bilirubin concentrations regard to controls may contribute to decreasing the risk of NAFLD, probably based on its potential to antagonize oxidative stress and its anti-inflammatory effects that play vital roles in reducing pro-inflammatory cytokines production, such as interleukin-6 and interleukin-1. These findings support that bilirubin protects against NAFLD through impaired glucose metabolism, insulin resistance inhibition, inflammation suppression or complement activation, and lipid accumulation. Additionally, there has been growing evidence suggesting that bilirubin may have a differential influence on lipogenesis and lipolysis of adipose tissue and the free fatty acid metabolism.[27]

Hb uptake in mononuclear cells through CD163 reduces circulating levels. Thus intracellular upregulation implies a protective response under adverse conditions in critical illness. Hb has been localized in kidney mesangial cells, mesencephalic dopaminergic neurons, glial cells, and also in hepatocytes,[16] which confer resistance to oxidative stress by detoxification of highly oxidizing radicals and oxygen homeostasis modulation, suggesting that Hb functions as an antioxidant. Another possible explanation for Hb elevation could be the mobilization of stem cells into the circulation during the NAFLD, as these cells express significant levels of Hb.

Hb is the most important potential source of heme (the functional group of Hb), when oxidized it may propagate inflammatory reactions and enhance cellular susceptibility to lipid peroxidation and oxidative stress;[30] the potential explanation for the associations observed between increased Hb and NAFLD may be related to the excessive iron accumulation regarding physiologic requirements,[17,20,29] in fact, NAFLD can be strongly predicted by high body iron levels.[20] Hepatic iron metabolism is com-

plicated, however, its protective effect can be attributed to an increase in a protein called hepcidin, which is responsible for retaining iron excess, thereby reducing oxidative stress.[31] Iron reduction by phlebotomy treatment, improve NAFLD activity score, possibly due to reactive oxygen species reduction.[18,32]

Heme has also been identified as a Toll-like receptor 4 (TLR4) ligand, which might induce or ameliorate an inflammatory response in some cell types.[30] On the other hand, heme transport to the liver under physiological conditions performed in part by HDL and since HDL levels are decreased in NAFLD when standardized by Hb, serum Hb might not be properly transported to the liver for further processing. Therefore, it seems important to obtain desirable HDL concentrations for the prevention of fatty liver disease.[33]

Some limitations of our study are the lack of liver steatosis measurement in the control group; hepatic steatosis was evaluated by ultrasound, which has precision limitations as a dependent operator method. The ratio of hepatic steatosis in each fraction of serum hemoglobin (REF) has been observed; however, only the association of hemoglobin with liver damage was analyzed with the total concentration of serum hemoglobin.

## CONCLUSION

In this study, NAFLD patients showed high circulating Hb levels, related to hepatic steatosis degree, which could be proposed as an antioxidant since may counteract the adverse effects of this disease.

It is important to mention that Hb could emerge as a serum marker for an early and accurate NAFLD detection.

## ABBREVIATIONS

- **ALT:** alanine aminotransferase.
- **AST:** aspartate aminotransferase.
- **BMI:** body mass index.
- **CKD:** chronic kidney disease
- **DB:** direct bilirubin
- **GGT:** γ-glutamyltransferase.
- **Hb:** hemoglobin.
- **HDL:** high-density lipoprotein.
- **IB:** indirect bilirubin.
- **IQR/M:** interquartile / median range.
- **LDL:** low-density lipoprotein.
- **NAFLD:** nonalcoholic fatty liver disease.
- **NASH:** nonalcoholic steatohepatitis.
- **NO:** nitric oxide.
- **RCT:** random control trial's.
- **TB:** total bilirubin.
- **TLR4:** Toll-like receptor 4.
- **UA:** uric acid.
- **USG:** Hepatic ultrasound.

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## CONFLICT OF INTEREST

Eva Juárez-Hernández, Norberto C. Chávez-Tapia, Diana Brizuela-Alcántara, Misael Uribe, Martha H. Ramos-Ostos and Natalia Nuño-Lámbarri declare not to have any conflict of interest.

## ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of Medica Sur ethics research committee in accordance with the Regulation of the General Law of Health in Mexico and with the 1964 Helsinki declaration.

## INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

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Juárez-Hernández and Nuño-Lámbarri wrote the article. Uribe, Ramos-Ostos and Chávez-Tapia revised and corrected the final version of the manuscript. Brizuela-Alcántara provided the data of control patients included in the analysis. It is it appreciated the assistance received by Beatriz Sanchez-Jimenez and Sofia Lopez-Gil in patient recruitment and to Varenka J. Barbero-Becerra in the correction and improvement of this article.

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