Practical Predictors of Fibrosis in Non-alcoholic Fatty Liver Disease: Immunoglobulin-A and HOMA-IR

S Boga[1], AR Koksal[1], H Alkim[1], MB YilmazOzguven[2], M Bayram[1], İ Sen[1], C Alkim[1]

ABSTRACT

Objective: Elevated immunoglobulin (Ig)-A levels and its relationship with fibrosis in alcoholic liver disease (ALD) were known. Non-alcoholic fatty liver disease (NAFLD) shows similar histology and pathophysiology with ALD. The potential relationship of serum Ig (IgA, IgG, and IgM) levels and other routinely used biochemical tests with the histological stage of liver damage in biopsy-proven NAFLD patients was investigated here.

Methods: Seventy patients and 54 volunteers as controls were included.

Results: No statistical difference was found between NAFLD *vs* controls and non-alcoholic steatohepatitis (NASH) [n = 53] *vs* non-NASH (n = 17) in terms of Ig levels. When NAFLD patients with normal and elevated IgA levels were compared NASH and diabetes mellitus ratios were found higher in the latter group.Serum IgA levels were significantly correlated with the stage of fibrosis ($r = 0.636$, $p < 0.001$). When NAFLD patients were compared as patients with no/mild fibrosis and patients with advanced fibrosis IgA, age, gender, homeostasis model of insulin resistance (HOMA-IR), and body-mass indexwere all significantly higher in advanced fibrosis subgroup, furthermore IgA and HOMA-IR were established as the independent predictors of fibrosis by logistic regression analysis. When IgA levels were evaluated by ROC analysis to differentiate advanced fibrosis from mildfibrosis, AUC was 0.874 at the cut-off level of 391.5 mg/dL for IgA with 78.9% sensitivity and 88.2% specificity.

Conclusions: Serum IgA levels showed a stepwise increase with the increasing fibrosis stages in NAFLD patients. HOMA-IR and serum IgA are independent predictors of fibrosis that can be easily accessed in daily practice.

Keywords: Fatty liver, fibrosis, immunoglobulin, insulin resistance

From: [1]Department of Gastroenterologyand [2]Department of Pathology, Sisli Hamidiye Etfal Education and Research Hospital, Istanbul, Turkey.

Correspondence: Dr S Boga Department of Medicine and Surgery, Division of Digestive Diseases and Section of Transplantation and ImmunologymYale University School of Medicine, Yale University Medical Center, 333 Cedar Street, LMP 1080, New Haven, CT 06520, USA. Fax: +1 203-785-6645
Email: salihboga@yahoo.com

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease all over the world and its incidence increases with the increasing obesity and diabetes. While most of the NAFLD patients have fatty liver without inflammation or tissue injury namely "simple steatosis", some of them may develop Non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis (1).

A rapid and accurate assesment of fibrosis in NAFLD is critical for clinical management because these patients have increased risks for complications of cirrhosis and hepatocellular carcinoma and therefore should be followed by screening programmes (2). Moreover, it is recently reported that besides predicting liver-related mortality, advanced fibrosis in NAFLD also predicts increased rate of mortality secondary to cardiovascular diseases (3). That's why, patients with higher risks for forthcoming complications should be detected early enough to enable close follow up and to decrease NAFLD related morbidity and mortality.

Serum immunoglobulins (Igs) are routinely checked to aid diagnosis when a suspected liver disease is investigated. Unique patterns of increases in serum Igs are seen in specific liver diseases such as IgM in primary biliary cholangitis, IgA in alcoholic liver disease (ALD), and IgG in autoimmune hepatitis, whereas polyclonal increase is commonly seen in liver cirrhosis (4, 5). The ALD is regarded as an IgA-related disease. Moreover, IgA deposition along hepatic sinusoids and relation of serum IgA concentrations with the severity of hepaticfibrosis were reported in ALD (6, 7). The likeliness between histopathological characteristics and natural histories of ALD and NAFLD gives the impression that both of these diseases may share common pathogenic mechanisms (8). NAFLD as the hepatic manifestation of metabolic syndrome is known to be related to insulin resistance and diabetes mellitus (DM). Previous studies showed high serum IgAlevels in cases with metabolic syndrome and DM. Moreover

metabolic abnormalities of these patients, hypertriglyceridemia and diabetic complications, associated positively with IgA levels (9, 10).

Because of the similarities of NAFLD and ALD and close association of NAFLD with the metabolic syndrome, we hypothesized that serum IgA may be associated with hepatic fibrosis and/or degree of hepatic damage in NAFLD. Therefore we aimed to search the potential relation of serum Ig levels with the histological stage of liver injury in biopsy-proven NAFLD patients to assess any possible clinical role that can be attributed to serum Igs or other routinely used biochemical tests for diagnosing and/or staging NAFLD.

MATERIALS AND METHODS

This observational case-control study consisted of 70 NAFLD patients and 54 healthy controls. The NAFLD patients were consecutively examined at the Gastroenterology Department of Sisli Hamidiye Etfal Education and Research Hospital between January 2015 and September 2015. In addition to confirmation of steatosis by abdominal ultrasonography (US), all had elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels at least for six months. They had no history of drinking alcohol more than 20 g/day, did not use any hepatotoxic drugs, hormone replacement therapy or herbal products, or had no diagnosis of viral or autoimmune hepatitis, alpha-1 antitrypsin deficiency, disorder of mineral metabolism, anemia, ischemic cardiac or cerebrovascular disease, impaired renal function and malignancies. The estimate of insulin resistance was computed by the homeostasis model of insulin resistance (HOMA-IR) index, with the formula: Insulin resistance = fasting plasma insulin (in microunits per milliliter) x fasting plasma glucose (in millimoles per liter) / 22.5. The DM diagnosis was established by using the criteria of American Diabetes Association (11).

Venous blood samples of all participants were drawn after an overnight fasting, at the same time with the liver biopsy. Routine blood chemistry analyses including serum Ig concentrations were done at thebiochemistry laboratory of our center. The normal ranges for serum IgA, IgG, and IgM were 70-400 mg/dL, 740–1600 mg/dL, and 45–230 mg/dL respectively. Ig levels were considered elevated in this study if the value of Ig is higher than the upper limit of the normal range (ULN).

A 16-gauge Hepafix needle was used to perform US guided liver biopsies. An expert hepatopathologist blinded to participants' data appraised biopsy specimens and divided NAFLD patients into two subgroups as NASH and non-NASH with respect to the presence and grade of steatosis, ballooning degeneration and lobular inflammation evaluated according to National Institute of Diabetes and Digestive and Kidney Diseases Non-alcoholic Steatohepatitis (NIDDK NASH) Clinical Research Network scoring system (12). Additionally fibrosis was staged with a five-grade scoring system where no fibrosis was scored as Stage 0, perisinusoidal or periportal fibrosis was scored as Stage 1, perisinusoidal and portal/periportal fibrosis was scored as Stage 2, bridging fibrosis was scored as Stage 3 and cirrhosis was scored as Stage 4.

Ethical aspect

The study was conducted in accordance with the "Declaration of Helsinki" and was approved by local Ethics Committee of Sisli Hamidiye Etfal Education and Research Hospital. All participants of the study provided verbal and written informed consents

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows (IBM Corp, Armonk, NY, USA) was used to perform all analyses. Visual (histograms, probability plots) and analytical methods (Shapiro-Wilk test) were used to determine if the variables are

distributed normally or not. Mann-Whitney U test was used to compare ordinal variables and continous variables that were not normally distributed. The differences between two study groups with normally distributed continuous variables such as age, BMI, albumin, IgA, IgG and IgM were assessed by the Student *t*-test.Serum IgA levels of five different fibrosis stage groups were evaluated by Kruskal-Wallis test. The *p*-value that was calculated by Bonferroni correction and considered to be statistically significant in the post-hoc comparisons was < 0.01. Correlations between the variables were analysed by the Pearson and Spearman's tests depending on the normality of variables. A value of $p < 0.05$ (2-sided) was considered statistically significant. The statistically significantly different variables between advanced fibrosis and no/mild fibrosis groups (age, gender, BMI, HOMA-IR and serum IgA levels) were further analysed by multiple logistic regression analysis to identify independent predictors of fibrosis. The capacity of serum IgA and HOMA-IR levels in predicting presence of advanced fibrosis were assessed by performing ROC (receiver operating characteristics) curve analysis. The point on ROC curve that is closest to the upper left corner was defined as the optimal cut-off value and sensitivity, specificity, positivepredictive value (PPV) and negative predictive value (NPV) were calculated accordingly. When investigating the changes in IgA levels by diabetes status, the effect of fibrosis was adjusted using two-way Analysis of Variance test.

RESULTS

The main clinical and biochemical features of NAFLD patients and controls are shown in Table 1. Age, gender distribution and albumin levels were similar between two groups. NAFLD group BMI, ALT, AST, gamma-glutamyl transpeptidase (GGT), total and low density lipoprotein (LDL) cholesterol, triglyceride, HOMA-IR and high sensitive C-reactive protein (hs-CRP) levels were significantly higher; and high density lipoprotein (HDL) cholesterol levels were significantly lower than controls. NAFLD patients did not show any statistically

significant difference in terms of IgA, IgG and IgM levels when compared to controls (324.8 ± 101.2 *vs* 282.9 ± 85.1 mg/dL $p = 0.180$, 1236.4 ± 262.6 *vs* 1160.7 ± 176.9 mg/dL $p = 0.340$ and 95.1 ± 41.5 *vs* 104.7 ± 34.5 mg/dL $p = 0.441$, respectively).

Table 1: Clinical and biochemical features of the study population

	NAFLD (n = 70)	**Controls (n = 54)**	***p*-value**
Age	45.8±11.4	41.5±10.0	0.096
Gender, F/M	45/25	32/22	0.163
BMI, kg/m^2	31.4±4.4	21.9±2.8	<0.001
ALT, IU/L	74 [49.5-95.5]	18.0 [10.0-27.0]	<0.001
AST, IU/L	54.5 [40.0-71.5]	18.5 [15.3-21.0]	<0.001
GGT, IU/L	43.5 [28.3-67.0]	11.0 [7.3-18.8]	<0.001
Albumin, g/L	4.55±0.4	4.7±0.3	0.191
HOMA-IR	3.9 [2.7-6.3]	1.8 [1.1-2.9]	<0.001
hs-CRP, mg/dL	4.2 [2.2-6.8]	1.1 [1.0-1.2]	<0.001
Total cholesterol, mg/dL	196.5 [167.5-231.3]	163.0 [147.7-186.0]	0.002
LDL-C, mg/dL	115.9 [97.8-148.2]	101.0 [84.3-114.9]	0.028
HDL-C, mg/dL	43.0 [36.8-50.8]	54.5 [49.3-59.5]	0.011
TG, mg/dL	146.0 [98.5-205.0]	83.0 [47.8-134.8]	0.002
IgA, mg/dL	324.8±101.2	282.9±85.1	0.180
IgG, mg/dL	1236.4±262.6	1160.7±176.9	0.340
IgM, mg/dL	95.1±41.5	104.7±34.5	0.441

Values are presented using means ± standard deviations for normally distributed and medians and first and third quartiles in the brackets for the non-normally distributed variables. BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high-sensitivity c-reactive protein LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglyceride, Ig: immunoglobulin

Non-alcoholic fatty liver disease (n = 53) and non-NASH (n = 17) patients were also compared in terms of clinical and biochemical features as shown in Table 2; and HOMA-IR level wasfound statistically significantly higher in NASH group.

Table 2: Clinical and biochemical features of NASH and non-NASH groups

	NASH (n = 53)	**Non-NASH (n = 17)**	***p*-value**
Age	45.4±11.8	47.0±10.5	0.507
Gender, F/M	33/20	12/5	0.533
BMI, kg/m^2	31.9±4.5	29.7±3.5	0.065
ALT, IU/L	77.0 [57.5-105.5]	65.0 [48.5-128.0]	0.247
AST, IU/L	55.0 [40.0-73.5]	54.0 [41.5-70]	0.706
GGT, IU/L	56.0 [24.5-71.5]	41.0 [29.5-66.0]	0.737
Albumin, g/L	4.57±0.4	4.48±0.4	0.398
HOMA-IR	4.5 [2.8-6.4]	2.9 [2.1-4.0]	0.021
hs-CRP, mg/dL	4.7 [2.1-6.9]	3.6 [2.2-5.9]	0.584
Total cholesterol, mg/dL	197.0 [165.0-219.5]	191.0 [173.5-238.0]	0.538
LDL-C, mg/dL	121.0 [98.2-149.4]	106.0 [94.4-139.5]	0.158
HDL-C, mg/dL	43.0 [36.5-48.5]	47.0 [37.0-55.0]	0.415
TG, mg/dL	155.0 [94.5-237.0]	134.0 [98.0-188.5]	0.524
IgA, mg/dL	337.7±91.7	284.6±120.6	0.058
IgG, mg/dL	1255.4±265.1	1177.0±253.0	0.288
IgM, mg/dL	96.5±42.0	90.2±40.7	0.588

Values are presented using means ± standard deviations for normally distributed and medians and first and third quartiles in the brackets for the non-normally distributed variables. BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high-sensitivity c-reactive protein LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglyceride, Ig: immunoglobulin

Although Serum IgA level was higher in NASH compared to non-NASH, this difference could not reach statistical significance (337.7 ± 91.7 *vs* 284.6 ± 120.6 mg/dL p = 0.058). There was no statistically significant difference in terms of IgG and IgM levels between NASH and non-NASH groups as well (1255.4 ± 265.1 *vs* 1177.0 ± 253.0 mg/dL $p = 0.288$ and 96.5 ± 42.0 *vs* 90.2 ± 40.7 mg/dL $p = 0.588$, respectively).

Overall, 21 (30%) patients had elevated serum IgA levels (> 400 mg/dL). Serum IgG was elevated (> 1600 mg/dL) in seven (10%) patients and serum IgM was elevated (> 230 mg/dL) in three patients (4%). NAFLD patients were divided into two subgroups as Elevated IgA (n = 21) and Normal IgA (n = 49). A comparison of the clinical and biochemical features of NAFLD patients with normal and elevated serum IgA levels is shown in Table 3. BMI, ALT, AST, GGT, albumin, total, HDL and LDL-cholesterol levels, triglyceride and hs-CRP levels were similar but age and HOMA-IR levels were statistically significantly higher in elevated IgA subgroup than the normal IgA subgroup. Additionally the ratio of NASH and DM patients were statistically significantly higher in the elevated IgA subgroup in comparison to normal IgA subgroup (52.9% *vs* 47.1% $p = 0.018$ and 71.4% *vs* 42.9% $p = 0.028$, respectively).

Relationship between serum Ig levels and liver histology:

When the correlations between serum IgA, IgG, IgM levels and steatosis grade, lobular inflammation stage, ballooning degeneration score and fibrosis stage were investigated, the only significant correlation found was between fibrosis and IgA levels ($r = 0.636$, $p < 0.001$).

While the relation between serum IgA levels and NAFLD patients' fibrosis stages were further analysed, a gradual increase in serum IgA levels with the increasing stage of liver fibrosis was found [F0:220.4 ± 57.2 mg/dL, n = 6; F1:281.4 ± 83.4 mg/dL, n = 24; F2:313.9 ± 69.5 mg/dL, n = 21; F3:408.6 ± 72.1 mg/dL, n = 17; F4:562.0 ± 73.5 mg/dL, n=2] ($p < 0.001$) (Fig.1).

Table 3: Clinical and biochemical features of NAFLD patients with elevated and normal IgA levels

	Elevated IgA (n = 21)	**Normal IgA (n = 49)**	**p value**
Age	49.6±8.7	44.1±12.1	0.037
Gender, F/M	13/8	32/17	0.267
BMI, kg/m^2	31.4±5.0	31.3±4.1	0.971
ALT, IU/L	74 [49.5-95.5]	78.0 [54.5-117.0]	0.533
AST, IU/L	48.0 [41.0-64.5]	56.0 [40.0-78.5]	0.298
GGT, IU/L	45.0 [25.5-67.0]	42.0 [29.5-66.5]	0.907
Albumin, g/L	4.4±0.4	4.6±0.4	0.053
HOMA-IR	5.6 [3.3-7.0]	3.4 [2.6-5.1]	0.007
hs-CRP, mg/dL	4.5 [2.5-5.9]	4.1 [1.8-6.9]	0.976
Total cholesterol, mg/dL	201.0 [161-216.5]	192.0 [170.0-227.5]	0.844
LDL-C, mg/dL	128.0 [98.8-147.5]	115.0 [97.2-149.4]	0.918
HDL-C, mg/dL	41.0 [36.5-45]	46.0 [36.5-53.0]	0.084
TG, mg/dL	127.0 [90.5-170.0]	154.0 [99.5-220.0]	0.714
IgA, mg/dL	453.3±52.0	269.7±57.2	<0.001
IgG, mg/dL	1246.5±206.4	1232.0±285.1	0.838
IgM, mg/dL	100.1±52.2	92.8±36.4	0.511
NASH ratio, %	%52.9	%47.1	0.018
DM ratio, %	%71.4	%42.9	0.028

Values are presented using means ± standard deviations for normally distributed and medians and first and third quartiles in the brackets for the non-normally distributed variables. BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high-sensitivity c-reactive protein LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglyceride, Ig: immunoglobulin, NASH: Non-alcoholic steatohepatitis, DM: Diabetes Mellitus

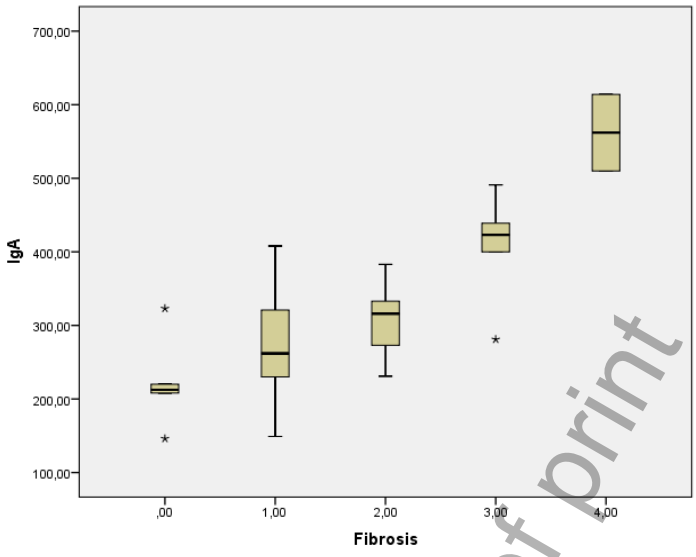

Fig. 1:Serum IgA level in each stage of liver fibrosis of NAFLD patients. A stepwise increase in serum IgA level was observed with the increasing severity of hepatic fibrosis ($p < 0.001$).

Significant differences were observed between F0-F2, F1-F3, F2-F3, F2-F4 and F3-F4 fibrosis stages ($p = 0.006$, $p < 0.001$, $p < 0.001$, $p < 0.001$ and p = 0.01, respectively). Moreover, serum IgA levels were more frequently elevated in NASH patients in comparison to non-NASH ones (52.9% *vs* 22.9%, $p = 0.018$).

Non-alcoholic fatty liver disease patients were further classified to two subgroups with respect to hepatic fibrosis as patients with no/mild fibrosis (stage 0–2, n = 51) and patients with advanced fibrosis [Stage 3–4, n = 19] (Table 4). When these subgroups were compared statistically significantly higher age, female gender frequency, BMI, HOMA-IR and serum IgA levels were found in advanced fibrosis subgroup. In order to identify which of these variables were independently associated with advancedfibrosis a logistic regression analysis was performed adjusting for age, gender, BMI, HOMA-IR and serum IgA, yielding HOMA-IR and serum IgA as the independent predictors of advanced fibrosis (OR=1.78 [1.15-2.78], $p = 0.01$ and OR = 1.02 [1.01-1.03], $p < 0.001$, respectively).

Table 4: Clinical and biochemical features of NAFLD patients with no/mild and advancedfibrosis

	No/Mild Fibrosis (Stage 0-2, n = 51)	**AdvancedFibrosis (Stage 3-4, n = 19)**	***p*-value**
Age	44.2±12.2	49.9±7.7	0.026
Gender, F/M	14/37	11/8	0.018
BMI, kg/m^2	30.7±4.1	33.2±4.6	0.031
ALT, IU/L	76.0 [53.0-116.0]	82.0 [49.0-102.0]	0.958
AST, IU/L	55.0 [40.0-71.0]	54.0 [40.0-89.0]	0.979
GGT, IU/L	48.0 [29.0-67.0]	37.0 [26.0-67.0]	0.579
Albumin, g/L	4.6±0.3	4.5±0.5	0.200
HOMA-IR	3.4 [2.6-4.8]	6.3 [5.8-6.8]	0.001
CRP, mg/dL	4.5 [2.2-6.9]	3.6 [2.1-5.9]	0.456
Total cholesterol, mg/dL	201.0 [172.0-234.0]	189.0 [157.0-217.0]	0.127
LDL-C, mg/dL	116.8 [98.8-150.0]	104.8 [94.0-147.0]	0.273
HDL-C, mg/dL	44.0 [36.0-53.0]	42.0 [38.0-47.0]	0.341
TG, mg/dL	151.0 [100.0-205.0]	127.0 [85.0-168.0]	0.210
IgA, mg/dL	287.6±79.5	424.8±85.2	<0.001
IgG, mg/dL	1245.5±280.0	1211.9±213.8	0.638
IgM, mg/dL	94.1±37.7	97.6±51.6	0.754

Values are presented using means ± standard deviations for normally distributed and medians and first and third quartiles in the brackets for the non-normally distributed variables. BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high-sensitivity c-reactive protein LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglyceride, Ig: immunoglobulin

While we aimed to search differentiation between cases with advancedfibrosis and with no/mildfibrosis, the area under the curve (AUC) for HOMA-IR obtained by ROC analysis was 0.717 with a $p = 0.005$ (Fig. 2).

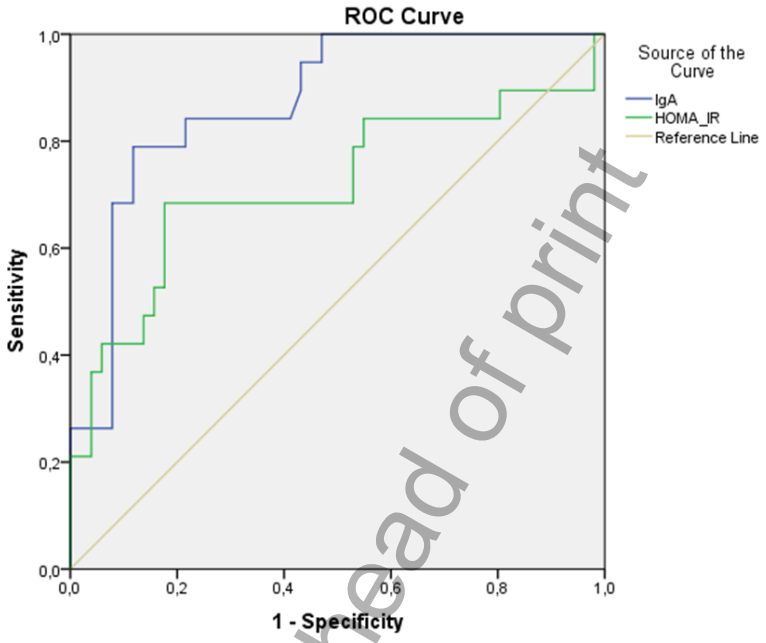

Fig. 2: Receiver operating characteristic curve to differentiate NAFLD patients with advancedfibrosis from NAFLD patients with no/mildfibrosis according to serum IgA level and HOMA-IR (Area under the curve 0.874 and 0.717, respectively).

The optimum HOMA-IR cut-off value was 5.39 with sensitivity, specificity, PPV and NPV values of 65.4%, 82.4%, 79.5%, and 72.3%, respectively. When IgA levels were evaluated by ROC analysis to differentiate advanced fibrosis from no/mild fibrosis AUC was 0.874 with a $p < 0.001$ (Fig. 2). At the cut-off level of 391.5 mg/dL for IgA, sensitivity was 78.9%, specificity was 88.2%, PPV was 87.0% and NPV was 80.7%.

Relationship between serum Igs and presence of DM:

Serum IgA levels were significantly higher in patients with DM (n = 36) in comparison to patients without diagnosis of DM (n = 34) (350.2 ± 98.0 mg/dL *vs* 297.9 ± 98.9 mg/dL, $p = 0.03$). To check if the statistically significantly high serum IgA levels in DM compared to non-DM is due to the effect of hepatic fibrosis of DM patients or not, analysis of variance was

performed by adding fibrosis as a confounding factor and statistical significance was found to be no longer existing (p = 0.914), revealing that this difference was actually because of high fibrosis in DM patients. There was no relationship between serum IgG and IgM levels and presence of DM in NAFLD patients (p = 0.265 and p = 0.628, respectively).

DISCUSSION

The clinical spectrum of NAFLD includes a relatively mild form, named simple steatosis and a more severe progressive form, namely NASH, which is characterized by fat accumulation in companion with inflammation and hepatocyte injury (1). For a long while, patients with simple steatosis were considered to have a benign course with no/little progression, whilst progression to cirrhosis was seen solely in steatohepatitis cases. However, emerging data suggest that fibrosis progression may be seen not only in NASH but also in simple steatosis (13–15). Moreover, fibrosis stage is reported to be the most potent predictor for disease spesific mortality in patients with NAFLD (3). Therefore, it is important to predict fibrosis non-invasively and to take appropriate measures in the management of NAFLD patients accordingly.

ALD shares similar pathogenic mechanisms with NAFLD and severity of hepatic fibrosis in ALD is found in relation with serum IgA levels (6, 8). NAFLD also has a close relationship with insulin resistance and metabolic syndrome and cases with metabolic syndrome and Type 2 DM are reported to have higher serum IgA levels (9, 10).

In this study, we evaluated the potential relationships between serum Ig levels and clinical and histological features of NAFLD patients and demonstrated a gradual significant increase in serum IgA levels with the increasing severity of liver fibrosis. Moreover, we found HOMA-IR and serum IgA as independent predictors of fibrosis. Because diagnosis of NAFLD

usually involves exclusion of other liver diseases such as primary biliary cholangitis and autoimmune hepatitis by checking serum IgM and IgG levels, a couple of studies also investigated the possible clinical utility of measuring serum Ig levels in NAFLD. In the study of Tomita *et al* (16) serum IgA levels were found significanlty increased in advanced NASH compared to milder NASH. They also investigated the relation of serum IgA levels with fibrosis but differently from our study they did not include simple steatosis patients to this analysis and found serum IgA concentration as a fibrosis predictor solely in NASH.

In the retrospective study of McPherson *et al* (17), NAFLD patients who had serum Igs measured within six months of liver biopsy were included in analysis and serum levels of IgA were found significantly higher in NASH patients in comparison to patients with simple steatosis. Although the difference of serum IgA levels between NASH and non-NASH groups could not reach statistical significance in our prospective study, compatible with study mentioned above we found more frequently elevated (> 1ULN) serum IgA levels in NASH group compared to non-NASH group. Even though they found elevated serum IgA levels in almost half of the NAFLD cohort (46%) and this ratio was only 30% in our study, they reported a significant positive relationship between serum IgA levels and fibrosis stages of NAFLD patients concordantly with our findings.

Rodriguez-Segade *et al* (18) investigatedserum IgA concentrations in a total of 3475 diabetic patients and found significantly higher IgA concentrations in diabetic patients compared to controls. Besides diabetic complications were found associated with significant increases in serum IgA concentrations in their study. In the present study we found higher IgA levels in diabetics compared to non-diabetics coherently. McPherson *et al* (17) also found high serum IgA levels in subjects with Type 2 DM in their study and because this relationship persisted when patients only with milder fibrosis were considered, they suggested that this relationship was independent of fibrosis. In our study although we found significantly higher

IgA levels in overall DM patients, different from their study our further analysis by adding fibrosis as a confounding factor showed that this difference was in fact depending on fibrosis.

A large body of studies reveals that several metabolic conditions, such as obesity, insulin resistance,hyperlipidemia, and hypertension are strongly associated with NAFLD (19–21) suggesting that NAFLD is the hepatic manifestation of metabolic syndrome. Insulin resistance, the key element of metabolic syndrome, is one of the main pathogenic mechanisms for the onset and progression of NAFLD (22, 23). Its pathophysiology includes the aggregation of lipids in the liver, in which insulin resistance plays an important role by enabling the transfer of fatty acids into the hepatocytes (24). Elevated inflammation markers have also been reported in NAFLD reflecting a low grade chronic inflammatory state related to insulin resistance (25, 26).

Moreover, insulin resistance is accompanied by hyperinsulinemia and insulin itself has profibrogenic properties. Paradis *et al* (27) reported that incubation of hepatic stellate cells with glucose or insulin leads to overexpression of connective tissue growth factor that is related to hepatic fibrosis. Despite the amount of available information regarding direct contribution of insulin resistance or hyperinsulinemia to fibrosis there is discrepancy between different studies. While most of them establishes DM (28, 29) and insulin resistance (30, 31) as predictors of fibrosis, few studies yielded no relationship between insulin resistance and estimation of fibrosis (32). When the results of bulk of recent data that puts insulin resistance forward as a significant risk factor for liver fibrosis in NAFLD patients is kept in mind (20, 29, 33) it is not surprising that the results of this study sets insulin resistance as an independent predictor of liver fibrosis.

There are several limitations in this study. First, the relatively small sample size especially in the most severe fibrosis subgroup (Stage 4) limits the generalizability of our conclusions. Second,we were unable to determine hepatic IgA by using immunohistochemical analysis. The cause of elevated IgA in NAFLD is unknown. Because the gut is a major source

of IgA and presence of a gut microbiome with unique features was shown in NAFLD patients (34) such a finding would give more clues about the source of increased serum IgA. Finally because this article presents the preliminary data of an ongoing study, follow-up biopsies to check potential fibrosis progression rate are not included here.

In conclusion the results of the present study demonstrated a gradual increase in serum IgA levels with the increasing severity of liver fibrosis and showed that HOMA-IR and serum IgA may be used as independent predictors offibrosis. These clinical parameters that can be easily assessed during daily routine of practicing clinicians can have important practical implications. Given that hepatic fibrosis is a surrogate marker for progressive liver disease, early estimation of NAFLD patients that possibly have advanced fibrosis can lead to a strategy to choose the most appropriate candidate for liver biopsy and can increase the yield and decrease unnecessary biopsies with associated costs and morbidity. Moreover these patients may more urgently be considered for clinical trials of new medications for NAFLD. In this setting, NAFLD patients with high HOMA-IR and IgA levels are at the highest risk for progressive fibrosis and should be managed attentively and included in closer monitoring programmes.

AUTHORS' NOTE

We confirm that there are no financial or other relations that could lead to a conflict to interest. We also verify that all authors had access to the data and a role in writing the manuscript.

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