AMERICAN JOURNAL OF BIOMEDICINE AND PHARMACY
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Article

Biochemical Alterations Associated with Non-Alcoholic Fatty Liver Disease in Iraqi Patients Attending Private Clinics in Baghdad

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Citation: Al-Akelie O. T., Al-Bayati A. M., Mustafa N. M., Mohammed R. A. K. Biochemical Alterations Associated with Non-Alcoholic Fatty Liver Disease in Iraqi Patients Attending Private Clinics in Baghdad. American Journal of Biomedicine and Pharmacy 2026, 3(6), 11-21.

Received: 10th Mar 2026
Revised: 11th Apr 2026
Accepted: 24th May 2026
Published: 10th Jun 2026

Abstract: Background: NAFLD is a prevalent metabolic disorder throughout the Middle East; however, the biochemical profile related to NAFLD has not been adequately defined in Iraqi populations. This cross-sectional study provides an overview of liver, metabolic, inflammatory, and oxidative stress biomarkers of NAFLD patients seen in private gastroenterology practices in Baghdad, Iraq. Methods. 120 patients with confirmed NAFLD (diagnosed by abdominal ultrasound based on clinical criteria) and 60 matched healthy control subjects (matched for age and gender) were recruited from March 2023 to September 2024. Fasting serum samples were analyzed for liver function tests (ALT, AST, ALP, GGT, total bilirubin), lipid profile (total cholesterol, triglycerides, LDL and HDL, fasting glucose), insulin resistance (HOMA-IR), complete blood count, C-reactive protein (CRP), ferritin, and malondialdehyde (MDA), and total antioxidant capacity (TAC). Results. Compared to healthy controls, patients with NAFLD had statistically significant higher levels of ALT (62.4 ± 18.3 vs. 22.1 ± 6.4 U/L), AST (48.7 ± 14.9 vs. 19.8 ± 5.7 U/L), GGT (55.3 ± 20.1 vs. 21.4 ± 7.3 U/L), triglycerides (2.31 ± 0.74 vs. 1.12 ±0.31 mmol/L), HOMA-IR (3.87 ± 1.24 vs. 1.43 ± 0.52), CRP (8.6 ± 3.2 vs. 2.1 ± 0.9 mg/L), ferritin (186.4 ± 67.3 vs. 74.2 ± 22.1 μg/L), and MDA (4.18 ± 1.02 vs. 1.76 ± 0.48 nmol/mL) (all $p < 0.001$). Patients also had significantly lower levels of HDL and TAC. According to multivariate logistic regression analysis, HOMA-IR (OR 4.12, 95% CI 2.34-7.26), MDA (OR 3.67, 95% CI 1.98-6.81), and GGT (OR 2.88, 95% CI 1.54-5.38) were identified as independent predictors of NAFLD severity. Conclusions. Biochemical data support that Iraqi patients with NAFLD have an identifiable biochemical signature indicating hepatocellular injury, atherogenic dyslipidemia, insulin resistance, systemic inflammation, and oxidative stress. These findings propose specialized integrated biochemical screening protocols for Iraqi clinical practices.

Keywords: Non-alcoholic fatty liver disease; NAFLD; biochemical markers; insulin resistance; oxidative stress; dyslipidaemia; Iraq; Baghdad; HOMA-IR; liver enzymes.

1. INTRODUCTION

NAFLD, or non-alcoholic fatty liver disease, is the most prevalent chronic liver problem globally and covers a variety of pathologies: from simple (fatty) liver to non-alcoholic (fatty liver with inflammation) with fibrosis and cirrhosis to liver cancer. Approximately 25% of the world's population has NAFLD, with rates as high as 32-37% among the Middle Eastern populations.

The causes of NAFLD are complex, a "multi-hit" process of insulin resistance, lipotoxicity, oxidative stress, mitochondrial injury, and changes in gut bacteria that contribute to liver cell injury, inflammation of the liver and scarring of the liver.[1],[2]

Similar to other countries in the Arab World, the nutritional and lifestyle transition in Iraq has led to calorie-dense diets from the West, decreased physical activity and rapid increases in obesity, Type II diabetes, and metabolic syndrome providing favourable conditions for the development of NAFLD. In Baghdad and other major Iraqi cities, population-based surveys have shown that over 60% of urban adults are overweight (BMI ≥ 25 kg/m²) and/or obese (BMI ≥ 30 kg/m²). [3],[4]

NAFLD is further characterised by a number of related biochemical abnormalities. The elevated level of serum aminotransferases in the blood (particularly alanine aminotransferase or ALT and aspartate aminotransferase or AST) has been a marker of liver damage for many years; however, the sensitivity and specificity of ALT and AST for assessing the level of severity in Non-Alcoholic Fatty Liver Disease (NAFLD) are relatively poor [5]. Gamma-Glutamyl Transferase (GGT) has been studied and identified on its own as an independent predictor of insulin resistance and fatty liver due to its association with oxidative stress and altered glutathione metabolism in the liver [6].

Dyslipidemia is another primary metabolic sign of NAFLD, which is commonly viewed to be a component of the metabolic syndrome. Dyslipidemia can be defined as high triglycerides, low high-density lipoprotein cholesterol (HDL-C), high low-density lipoprotein cholesterol (LDL-C), and elevated number of small, dense LDL particles, all of which make up an atherogenic lipid pattern, increasing the individual's cardiovascular risk tremendously [7],[8].

Insulin resistance plays a critical role in the pathology of NAFLD. Hyperinsulinemia causes increased production of lipids in the liver de novo, inhibited beta -oxidation of fatty acids, and promotes the accumulation of triglyceride within the liver. The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), measured from fasting blood glucose and insulin levels, has been verified as a valid, noninvasive measure of insulin resistance for use in research studies [9].

Oxidation stress is a major mechanism for the transition from fatty liver to non-alcoholic steatohepatitis (NASH). Reactive oxygen species (ROS) generated by defective mitochondria, endoplasmic reticulum (ER) stress, and cytochrome P450 2E1 (CYP2E1) activity initiate lipid peroxidation, with malondialdehyde (MDA) as a well-recognized biomarker of lipid peroxidation.Additionally, the decline in natural antioxidants within the body results in the continuation of oxidative injury to the body.[10],[11]

Higher levels of systemic inflammation (higher serum hs-crp levels and higher memory levels of pro-inflammatory cytokines) increase the amount of damage to the liver as well as promote accelerated fibrotic liver growth. Patients that have an elevated serum ferritin level (which is considered a marker of the exogenous acute phase and also considered to be an indirect measure of how much iron is stored in the liver) are also found to carry a greater burden of disease when compared anatomically (through a histological examination) will also demonstrate a correlation between the finding of nonalcoholic steatohepatitis (nash) and the finding of elevated serum ferritin through histological analysis that is independent of excess iron deposition.[12],[13]

There is a significant lack of information regarding the biochemical profile of non-alcoholic fatty liver disease (NAFLD) found in Iraq. There is a strong likelihood of having a skewed representation of the prevalence or degree of NAFLD by relying solely on patient health data that have been collected through tertiary care institutions.[14]

There are potentially many factors unique to the country of Iraq that can potentially alter the biochemical expression of NAFLD. There are many socioeconomic and environmental factors that may fall into this category. Some examples of these factors include (but, are not limited to): an extensive history of pervasive socioeconomic dislocation, a lack of access to preventive healthcare, high rates of

micronutrient deficiencies, and the level of stress experienced by the populations of Iraq.[15] In addition, we do not have sufficient data regarding differences in the genetic variation of specific lipids and hepatic steatosis (for example, PNPLA3 I148M) between the different ethnicities present in the Iraqi population.[16]

By defining the biochemical signature associated with non-alcoholic fatty liver disease (NAFLD) in the Iraqi population we would be able to support the development of non-invasive scoring systems that will allow for:

To assure that the most appropriate and effective biomarkers are used to determine the severity and progression of NAFLD, To demonstrate the value of establishing therapeutic priorities among the NAFLD patient population, and T identifying those patients with a higher risk for developing cardiovascular or hepatic complications.

Validation of non-invasive biochemical indices is essential due to the limited number of liver biopsies being performed outside of high-level care.[17],[18]

Studies that have been done in countries around Iraq have demonstrated that elevated transaminases, atherogenic dyslipidaemia and insulin resistance are consistent and reliable biochemical markers of NAFLD in the Arab and Middle Eastern patient population.[19],[20] However, it cannot necessarily be assumed that these studies have been performed with accuracy for patients in Iraq due to differing dietary habits, background genetic differences, the presence of different co-morbidities and long-term environmental exposures to various types of agents.[21]

The objective of the current study on NAFLD patients in Iraq is to more accurately describe the hepatic, metabolic, inflammatory and oxidative damage to the liver in patients with NAFLD, through the completion of a multi-dimensional analysis of the following:

i. The comparison of a multi-dimensional panel of biochemical markers associated with NAFLD to an equivalent matched control group

ii. The degree of ultrasonographically assessed liver damage (attributable to NAFLD) and its association with the biochemical parameters assessed above

iii. The biochemical parameters identified in the above analyses that independently correlate with NAFLD, when analysed using multivariate statistical techniques that analyse these same parameters throughout each of the dimensional panel analyses.[22]

The study will serve to support additional evidence-based biochemical screening guidelines developed for use in the NAFLD patient population in Iraq, with the ultimate goal being to assist the health care system in making clinical decisions regarding patient care within the private health care delivery systems in Iraq.

2. METHODOLOGY

2.1 Study Design and Setting

The design of this study was cross-sectional due to the large quantity of data collected from three independent private gastroenterology and internal medicine clinics in Baghdad, Iraq (Al-Mansour, Karrada and Al-Jadriya districts), between March 2023 and September 2024. In addition, informed consent was obtained from all participants in accordance with the Declaration of Helsinki.[23]

2.2 Study Population

Consecutive sampling was carried out to recruit 120 patients with a diagnosis of NAFLD and 60 age- and sex-matched healthy control subjects. Patients were diagnosed as having NAFLD according to the European Association for the Study of the Liver (EASL) Clinical Practice Guidelines, as confirmed by an observed having abdominal ultrasound imaging; hepatic steatosis, and the absence of any secondary causes of hepatic fat accumulation (i.e., alcohol consumption greater than 10 grams/day in women or 20 grams/day in men; medications known to cause steatosis; active viral hepatitis type B or C; autoimmune hepatitis; Wilson's disease; and hemochromatosis).[24]

To be eligible for inclusion, adult patients aged from 18 to 65 years old, with ultrasound proven liver steatosis, must have 2 of the following metabolic risk factors: body mass index (BMI) greater than 25 kg/m^2; waist circumference values that exceeded 94 cm in males or 80 cm in female; and with a fasting blood glucose or known diabetic (T2DM) of more than 5.6 mmol/L; triglyceride values were

greater than 1.7; HDL-cholesterol levels were below 1.0 (males) or 1.3 (females); and hypertension (>130/85 mmHg or taking antihypertensive medications).

Exclusion criteria consisted of confirmed positive viral hepatitis (Hepatitis B surface antibody or anti-HIV antibody), alcohol dependency (assessed with the AUDIT-C instrument), autoimmune or cholestatic liver disease, current corticosteroid use, use of amiodarone or methotrexate, pregnancy and lactation, or severe systemic illness (i.e., renal insufficiency, malignancy, heart failure, etc.). Healthy control subjects were selected from the visitors accompanying non-trauma patients or from clinic staff members who did not have hepatic steatosis by imaging, and had no metabolic comorbidities, and have normal laboratory values. [25]

2.3 Anthropometric and Clinical Assessment

Measures of body weight and height were taken using calibrated electronic scales and a fixed stature measuring instruments following standardised methods. The calculation of BMI (kg) is determined by the weight divided by the height squared (m^2). The waist circumference was determined as measured at the midpoint between the lowest rib and the iliac crest in a standing, relaxed position. Average blood pressure was defined as the average of two readings taken five minutes apart with a mercury sphygmomanometer. A qualified internist facilitated the collection of complete medical history, medication history and the internist then provided a comprehensive clinical examination.

2.4 Abdominal Ultrasonography

An abdominal ultrasound was performed by a skilled radiologist with the Toshiba Aplio 300 ultrasound machine using a 3.5 MHz curved transducer (probe). The grading of hepatic steatosis was done according to the standard grade scoring system:(1) Grade 1 (mild) will have a slight increase in echogenicity while still being able to visualize the diaphragm and intrahepatic vessels; (2) Grade 2 (moderate) will have a moderate increase in echogenicity and no or minimal impairment in visualizing the border of intrahepatic vessels; (3) Grade 3 (severe) will have an extremely high degree of echogenicity with very little or no ability to visualize the diaphragm or posterior portion of the liver. [26]

2.5 Biochemical Analysis

Venous blood samples are obtained while an individual is at least 12 hours fasted (overnight) before the actual collection of those samples. Serum is obtained through centrifugation (3000 rpm for 10 minutes) of collected samples no more than 1 hour after they were collected. Routine samples will be run fresh and non-routine samples (MDA and TAC assay) will be aliquotted and frozen at −80 degrees C for later analysis. All analysis will occur at a private accredited diagnostic lab certified by the Iraqi Health Ministry. [27]

Liver function tests (including ALT, AST, alkaline phosphatase (ALP), GGT and total bilirubin) were conducted using a Beckman Coulter AU680 Automated Chemistry Analyser (Beckman Coulter, Inc., Brea, CA, USA) with manufacturer validated reagent kits. Serum lipid panel (total cholesterol, triglycerides, LDL-C, and HDL-C) were quantified using Roche Diagnostics Reagent Kits and were done using Enzymatic Colorimetric methods.

Fasting blood glucose and serum insulin were evaluated by a hexokinase method and Electrochemiluminescence Immunoassay (ECLIA) on the Cobas e411 analyser (Roche Diagnostics, Switzerland). HOMA IR = (fasting insulin (μU/mL) × fasting glucose (mmol/L)) / 22.5. High-Sensitivity C-Reactive Protein (hs-CRP) was quantified using a Particle-Enhanced Immunoturbidimetric Assay. Serum Ferritin was evaluated by Electrochemiluminescence Immunoassay. MDA was quantified using the Thiobarbituric Acid Reactive Substances (TBARS) Assay using a commercially available kit (Cayman Chemical, Ann Arbor, MI, USA) and a spectrophotometer reading at 532nm. Total Antioxidant Capacity (TAC) was quantified using the Ferric Reducing Ability of Plasma (FRAP) method.[28],[29]

2.6 Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics version 27.0 (IBM Corporation) (Armonk, NY, USA). Continuous variables were evaluated for normality using the Shapiro-Wilk test, and using QQ plots. Variables that were normally distributed are presented as the mean ± Standard deviation (SD); and variables that are not normally distributed are presented as the

median (inter-qartile range [IQR]). Comparisons between NAFLD patients and controls were performed using either an independent samples t test (parametric), or Mann Whitney U test (non-parametric), as appropriate. For comparison of categorical variables, chi-square tests were applied.[30]

Comparison of biochemical parameters across severity of NAFLD (gradients) was performed using analysis of variance (ANOVA) with post-hoc correction using Bonferroni. Correlation coefficients were calculated using either Pearson or Spearman to examine the correlative relationship between continuous biochemistry variables and HOMA-IR. Multiple binary logistic regression models were evaluated with the outcome being the diagnosis of NAFLD, including covariates that were statistically significant by univariate analysis ($p < 0.05$). Odds ratios (OR) and 95% confidence intervals (CI) are reported. A two-tailed p-value of < 0.05 was considered statistically significant for all tests.[31]

3. RESULTS

3.1 Demographic and Clinical Characteristics

Demographic information of NAFLD and control groups is displayed in table 1. The ages and sexes of subjects in the control and NAFLD groups were well-matched. NAFLD patients also exhibited significantly higher metrics than controls in all measured variables with the exception of their diastolic blood pressure. Of the NAFLD patients, most had Grade II steatosis (58.3%).

Table 1. Demographic and Clinical Characteristics of Study Participants.

Variable	NAFLD (n=120)	Controls (n=60)	p-value
Age (years)	42.6 ± 11.3	41.8 ± 10.9	0.624
Sex (Male/Female)	68/52	34/26	0.981
BMI (kg/m²)	31.4 ± 4.7	23.8 ± 2.9	<0.001
Waist circumference (cm)	98.7 ± 10.2	82.1 ± 7.6	<0.001
Systolic BP (mmHg)	134.2 ± 14.8	118.6 ± 9.3	<0.001
Diastolic BP (mmHg)	87.4 ± 9.1	76.3 ± 7.2	<0.001
T2DM, n (%)	48 (40.0%)	5 (8.3%)	<0.001
Hypertension, n (%)	54 (45.0%)	6 (10.0%)	<0.001
Steatosis Grade I / II / III	26/70/24 (21.7/58.3/20.0%)	N/A	—

Data are mean ± SD or n (%). BP: blood pressure; T2DM: type 2 diabetes mellitus; BMI: body mass index; N/A: not applicable.

3.2 Liver Enzymes and Lipid Profile

Liver function tests (including all hepatic enzymes) and lipid profile (Table 2) were significantly higher in NAFLD than in control group for all hepatic enzymes. However, ALT had the greatest elevation among the hepatic enzyme. HDL-C was significantly lower in NAFLD patients than in control group; however, the total cholesterol and triglycerides (TG) and LDL-C were significantly higher (increased) in NAFLD group than in control group.

Table 2. Liver Function Tests and Lipid Profile in NAFLD Patients and Controls.

Parameter	NAFLD (n=120)	Controls (n=60)	p-value
ALT (U/L)	62.4 ± 18.3	22.1 ± 6.4	<0.001
AST (U/L)	48.7 ± 14.9	19.8 ± 5.7	<0.001

Parameter	NAFLD (n=120)	Controls (n=60)	p-value
ALP (U/L)	112.6 ± 34.8	72.4 ± 18.2	<0.001
GGT (U/L)	55.3 ± 20.1	21.4 ± 7.3	<0.001
Total bilirubin (μmol/L)	14.8 ± 5.6	11.2 ± 3.4	0.032
Total cholesterol (mmol/L)	5.84 ± 1.12	4.61 ± 0.78	<0.001
Triglycerides (mmol/L)	2.31 ± 0.74	1.12 ± 0.31	<0.001
LDL-C (mmol/L)	3.62 ± 0.94	2.84 ± 0.62	<0.001
HDL-C (mmol/L)	0.98 ± 0.22	1.48 ± 0.31	<0.001

Data are mean ± SD. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.

3.3 Metabolic, Inflammatory, and Oxidative Stress Markers

The information related to fasting glucose levels, insulin resistance indices, inflammatory markers, and oxidative stress biomarkers are presented in Table 3. Individuals with NAFLD have been shown to have significantly elevated HOMA-IR, CRP, ferritin, and MDA; they also had significantly lower TAC than individuals without NAFLD. The differences were consistently significant in all three grades of steatosis; there was a clear gradient of increasing biochemical derangements from Grade I to Grade III.

Table 3. Metabolic, Inflammatory, and Oxidative Stress Parameters by Steatosis Grade.

Parameter	Controls (n=60)	Grade I (n=26)	Grade II (n=70)	Grade III (n=24)	p
Fasting glucose (mmol/L)	4.81 ± 0.43	5.64 ± 0.62	6.24 ± 0.88	7.12 ± 1.14	<.001
Fasting insulin (μU/mL)	7.2 ± 2.1	12.4 ± 3.6	17.8 ± 5.2	24.3 ± 7.4	<.001
HOMA-IR	1.43 ± 0.52	2.76 ± 0.84	3.94 ± 1.18	5.62 ± 1.76	<.001
hs-CRP (mg/L)	2.1 ± 0.9	5.4 ± 1.8	8.8 ± 3.1	13.2 ± 4.6	<.001
Ferritin (μg/L)	74.2 ± 22.1	124.8 ± 38.4	192.4 ± 62.7	268.6 ± 84.3	<.001
MDA (nmol/mL)	1.76 ± 0.48	2.84 ± 0.62	4.32 ± 0.98	6.14 ± 1.32	<.001
TAC (mmol/L)	1.84 ± 0.34	1.42 ± 0.28	1.08 ± 0.22	0.74 ± 0.18	<.001

Data are mean ± SD. HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; hs-CRP: high-sensitivity C-reactive protein; MDA: malondialdehyde; TAC: total antioxidant capacity. p-values derived from one-way ANOVA with Bonferroni post-hoc correction.

3.4 Multivariate Predictors of NAFLD

Variables HOMA-IR, MDA, and GGT were recognized as the three best separate predictors of NAFLD when adjusted for age, gender, and BMI ($p < 0.001$). After controlling for these variables, HOMA-IR also exhibited the highest degree of correlation with steatosis grading among the three variable groups ($r = 0.68$; $p < 0.001$), followed by MDA ($r = 0.62$; $p < 0.001$) and ferritin ($r = 0.54$; $p < 0.001$).[32]

4. DISCUSSION

This study offers a systematic description of biochemical aspects related to nonalcoholic fatty liver disease (NAFLD) in a selected group of patients from Iraq using private health system settings based in Baghdad. It highlights both clinical detail and context for these results within Iraq's local epidemiology. As such, these results verify and add to those seen in other countries across the region of the Middle East for these individuals and also show some of the features unique to NAFLD in the Iraqi population.[33]

The elevations of liver enzymes observed among the patients in this study are consistent with established patterns of injury to the liver with NAFLD. The modest elevation in ALT for these subjects when compared to individuals with acute hepatitis at the time of diagnosis was more significant than the elevation of AST, resulting in a mean AST/ALT ratio being less than 1.0 for most cases and therefore showing no evidence of alcohol being the cause for the development of NAFLD, which is again consistent with other studies performed in the Middle East.5,6 The significantly increased serum levels of GGT in these patients are particularly noteworthy because GGT has been suggested to be a multifunctional biomarker for NAFLD reflecting not only the possibility for biliary or hepatocellular injury, but also potentially serving as a surrogate marker for hepatic oxidative stress and systemic insulin resistance in NAFLD research. According to our regression model, GGT was determined to be an independent predictor of NAFLD, providing additional evidence for its use as a clinically useful non-invasive test for patients in low resource settings.[6]

The atherogenic dyslipidaemia profile exhibited by the cohort studied, consisting of high triglyceride levels, low levels of HDL-C, and elevated levels of LDL-C, closely matches the hepatic dysregulation of lipid export that forms the basis for the pathophysiology of NAFLD.Individuals with insulin resistance have decreased lipoprotein lipase activity in the liver while upregulating sterol regulatory element-binding protein 1c (SREBP-1c) mediated de novo lipogenesis, as well as decreased apolipoprotein B-100 synthesis — all of which contribute to an increased risk for atherogenesis through the development of dyslipidemia. These findings underscore the need for lipid-reducing strategies to be incorporated into the overall management of NAFLD individuals within Baghdad since the prevalence of dyslipidemia amongst the cohort studied represents a significant cardiovascular risk burden.

HOMA-IR was the strongest independent biochemical predictor of NAFLD in our statistical analysis, with an odds ratio of 4.12. This finding represents a biologically rational and clinically important conclusion. The characteristics of insulin resistance induce hepatocellular lipogenesis by hyperactivating SREBP-1c and ChREBP transcription factors, impair mitochondrial oxidation of fatty acids, and induce the release of pro-inflammatory cytokines from adipose tissue (i.e., TNF-α, IL-6), which will create a pro-inflammatory and hepatotoxic environment. In addition, the increases of HOMA-IR values across the increasing grades of steatosis (grade I: 1.43, grade II: 2.76, grade III: 3.94, and grade IV: 5.62) provide strong evidence that worsening insulin resistance correlates with worsening steatosis (e.g., fibrosis prediction models incorporate HOMA-IR as a core variable).

Increased levels of oxidative stress, in particular MDA — the primary product of lipid peroxidation — in conjunction with the depletion of total antioxidant capacity (TAC), are consistent with the multi-hit and parallel hit models of NAFLD progression.These models indicate that hepatocytes with fat (lipids) are more likely to experience damage from oxidants due to dysfunction of the mitochondrial electron transport system, the induction of CYP2E1 and the activation of Kupffer cells. The amount of MDA is significantly increased in Grade III steatosis (6.14 ± 1.32 nmol/mL; mean ± sd) compared with control subjects (1.76 ± 0.48 nmol/mL), which is similar to values obtained from NASH-confirmed biopsy subjects in both the European and East Asian literature. This indicates that at least a subset of our Grade III patient population may have histologic evidence of ongoing inflammation of steatohepatitis.

The apparent decrease in antioxidant capacity (TAC) in our subjects indicates a deficiency in total antioxidant capability on a systemic level; this includes decreased levels of glutathione, superoxide dismutase, catalase, and vitamin E, all of which are consumed during the neutralisation of reactive

oxygen species produced during lipid peroxidation cascade. Antioxidant deficiency has been demonstrated to occur in several middle-eastern cohorts of NAFLD and provides the basis (mechanism) by which to investigate the supplementation of antioxidants. However, the clinical benefit of antioxidant intervention within the Iraqi context has not yet been established.

Elevated serum ferritin levels in our NAFLD subjects may be due to many overlapping mechanisms including: acute phase response related to inflammation in the liver; oxidative stress from iron contributing to injury to hepatocytes; or, hepatocytes that are damaged, and release of stores of iron.Control and Steatosis Grades exhibit a progressive increase (74.2, 124.8, 192.4, 268.6 μg/L) in Ferritin levels, which correlates with literature documenting Ferritin as being an independent risk factor for advanced fibrosis in NAFLD, in addition to being a non-specific inflammatory marker.[12],[13] The study controlled for hereditary hemochromatosis and iron overload syndromes; therefore, the hyperferritinaemia observed in our NAFLD cohort can be attributed most parsimoniously to inflammatory and steatohepatitic mechanisms rather than from iron dysregulation.[34][35][36][37][38]

Elevated hs-CRP levels in our NAFLD cohort demonstrate the systemic inflammation associated with and probably exacerbating hepatic injury in this condition. CRP, produced mainly by hepatocytes due to IL-6 signaling, is a downstream effector of the innate immune system activated by the translocation of lipopolysaccharide from a dysbiotic gut microbiome, activation of TLR4 in Kupffer cells via free fatty acids, and dysregulation of adipokines. The progressive increase in hs-CRP levels across Steatosis Grades in our data is consistent with other studies proposing CRP-based, non-invasive scoring systems to measure NAFLD severity.[39]

From a clinical and public health standpoint, our findings have multiple ramifications for the Iraqi healthcare system. Most especially, the high relative prevalence of metabolic co-morbidities (40% T2DM, 45% hypertension) found in our NAFLD cohort provide compelling rationale for instituting integrated metabolic care pathways to simultaneously address hepatic and cardiometabolic risk.The second conclusion is that HOMA-IR, MDA, and GGT each have a strong independent predictive ability, so combining these three easily accessible biomarkers into an easily obtainable and effective way to screen and stratify patients without the need for liver biopsies represents a low-cost option to be used in private clinic settings [40].

In comparing our results with those of similar studies from the Arabic and Persian regions of the Middle East, such as Iran, Saudi Arabia, and Jordan, we found both similarities in and unique cultural differences between the Arabian and Persian study populations. In all three regions of the Middle East there were also many similarities with regard to hypertriglyceridaemia, increased ALT levels, and insulin resistance as being the most important biochemical features of NAFLD in both the Arabic and Persian study populations. Conversely, our study cohort in Iraq showed higher average levels of MDA and ferritin concentration than those previously published for similarly sized groups of patients in Iran. This is likely due to the different types of fatty foods consumed in Iraq compared to Iran due to cultural dietary differences, including but not limited to the amount of red meat and refined carbohydrates consumed in the Iraqi diet or potentially due to the differing prevalence of smoking and psychosocial stress within the two regions.

Variations in the biochemical, pathological, and demographic characteristics of study participants across different studies highlight the necessity for locally generated data for the purposes of developing appropriate diagnostic and prognostic cut-off values.

Certain limitations exist in this study. First, the cross-sectional design prohibits us from determining any cause-and-effect relationships among the different biochemical markers and the degree of steatosis in our patients; therefore, we cannot definitively establish whether changes in the biochemical markers preceded the degree of steatosis or vice-versa. Second, NAFLD was defined by ultrasound criteria, which are considered appropriate and widely used, but they do not allow for differentiation between simple steatosis and non-alcoholic steatohepatitis (NASH) unless there is histological confirmation via liver biopsy. Third, because no liver biopsy data were available, we were unable to correlate the biochemical results of the study with the stage of fibrosis in our patients. Fourth,

our study sample was a convenience sample from a single clinic in Baghdad, so it is possible that the clinic-based sample is not representative of all patients in the larger population of Baghdad, especially those patients who are receiving care in the public health sector and those with undiagnosed NAFLD. Fifth, because we did not measure cytokine profiles (TNF-α, IL-6, adiponectin) and genetic polymorphisms (such as PNPLA3 and TM6SF2), we cannot make any mechanistic conclusions regarding the intersubject variability of NAFLD.

We recommend that additional studies be performed in the Iraqi population using a prospective study design that includes liver biopsy along with longitudinal data collection, genetic testing, and microbiome analysis to better describe and understand NAFLD in this population. Study of validation of non-invasive scoring systems based on the three biomarker panel identified in this study should also be an important area of future research.

5. CONCLUSION

Patients with NAFLD in Baghdad's private clinics show a multi-domain biochemical phenotype (henceforth referred to as a biochemical phenotype) that is comprised of evidence of hepatocellular injury, atherogenic dyslipidaemia, insulin resistance, systemic inflammation, iron dysregulation, and oxidative stress. The biochemical severity of these phenotypes otherwise termed biochemical derangements is similarly proportional to the degree of ultrasonic steatosis. HOMA-IR, MDA, and GGT are the best independent predictors of NAFLD in this group, indicating that they should be incorporated into locally adapted, non-invasive diagnostic protocols for NAFLD in Iraq. The data collected provides an evidence-based approach for developing biochemical screening guidelines and Clinical Management Strategies for NAFLD in a Private Healthcare environment in Iraq.

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