Comparative Analysis of Metabolic and Inflammatory Biomarker Profiles in Phenotypes of Metabolic Dysfunction-Associated Fatty Liver Disease

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ABSTRACT

Objective: To investigate the differences in metabolic and inflammatory biomarker profiles across three metabolic dysfunction-associated fatty liver disease (MAFLD) phenotypes.

Study Design: A comparative observational study.

Place and Duration of the Study: This study was conducted at Pakistan Aeronautical Complex Hospital Kamra, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from November 2021 to March 2024.

Methodology: This study included 393 patients (aged 20–70 years) with ultrasound-confirmed hepatic steatosis and a fatty liver index (FLI) ≥30. Patients were categorised into T2D-associated MAFLD (n = 134), obesity-MAFLD (n = 221), and lean-MAFLD (n = 38) groups according to the MAFLD diagnostic criteria. Healthy controls (n = 109) were included for comparison. Anthropometric parameters (body mass index [BMI], waist circumference [WC]), biochemical markers (liver enzymes, lipid profile), inflammatory cytokines (high-sensitivity C-reactive protein, interleukin-6, tumour necrosis factor-alpha, adiponectin, leptin, cytokeratin-18, malondialdehyde), and markers of hepatic fibrosis were measured.

Results: MAFLD patients had higher BMI, WC, metabolic, and inflammatory biomarkers compared to healthy controls ($p < 0.001$). T2D-MAFLD patients had elevated lipid profiles, liver enzymes, inflammatory cytokines, and fibrosis indices as compared to other two groups ($p < 0.001$). The Obese-MAFLD group showed elevated triglycerides, FLI, cytokines, and leptin ($p < 0.001$) compared to the lean-MAFLD group. Lean-MAFLD patients exhibited higher fasting glucose and blood pressure than the obese phenotype ($p < 0.05$).

Conclusion: The MAFLD phenotypes exhibit distinct biomarker profiles. The T2D-MAFLD group had the most severe metabolic and inflammatory dysfunction, while the obese-MAFLD group showed moderate liver and lipid abnormalities. The lean-MAFLD group had mild metabolic disturbances. Identifying phenotype-specific biomarkers may aid early detection, risk assessment, and personalised treatment to improve patient outcomes.

Key Words: *Metabolic dysfunction-associated fatty liver disease, T2D MAFLD, Obese –MAFLD, Lean-MAFLD, Biochemical and inflammatory markers.*

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INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) is identified by the presence of hepatic steatosis alongside one of the following: obesity, Type 2 diabetes mellitus (T2D), or metabolic disturbance in patients whose body mass index (BMI) falls within the normal range. MAFLD, a major contributor to chronic liver disease (CLD), is linked to substantial hepatic, metabolic, and cardiovascular complications, with a global and local prevalence of approximately 30.01% and 29.82%, respectively.[1-3]

Although liver biopsy remains the definitive diagnostic tool, its invasiveness and high cost have promoted the adoption of non-invasive methods, such as ultrasonography and the fatty liver index (FLI).[4] With a sensitivity of 83.4% and specificity of 81.0%, ultrasonography remains a reliable tool for evaluating hepatic steatosis.[5] The FLI, incorporating BMI, waist circumference (WC), triglyceride (TG) levels, and gamma-glutamyl transferase (GGT), achieves an area under the ROC curve (AUROC) of 0.82 at a diagnostic threshold of ≥30.[6]

MAFLD is a heterogeneous condition with three predominant phenotypes: T2D-associated MAFLD (T2D-MAFLD), obesity-associated MAFLD (OB-MAFLD), and lean MAFLD (L-MAFLD).

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The T2D-MAFLD phenotype is characterised by insulin resistance, chronic hyperglycaemia, and an increased risk of fibrosis progression.[7] The OB-MAFLD is primarily linked to excessive adiposity, systemic inflammation, and dyslipidaemia, often exacerbated by poor dietary habits and sedentary lifestyles,[8] while L-MAFLD involves normal BMI but exhibits metabolic dysfunction and visceral adiposity.[9]

Metabolic and inflammatory biomarkers are very important for the pathogenesis and progression of MAFLD. Fasting plasma glucose (FPG), homeostatic model assessment for insulin resistance (HOMA-IR), liver enzymes including alanine transaminase (ALT), aspartate aminotransferase (AST), GGT, and lipid metrics (total cholesterol [TC], low-density lipoprotein cholesterol [LDL-c], TG, and high-density lipoprotein cholesterol [HDL-c]) reflect underlying metabolic dysfunction.[8]

Pro-inflammatory mediators comprising high-sensitivity C-reactive protein (hs-CRP), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), in addition to markers of oxidative stress (e.g., malondialdehyde [MDA]), adipocytokines (adiponectin [AdpN], and leptin), and apoptotic-related markers like cytokeratin-18 (CK-18), are also instrumental in the MAFLD development.[10]

Despite the growing body of research, few studies to date have systematically compared the metabolic and inflammatory biomarker profiles across different MAFLD phenotypes. This study aimed to compare metabolic and inflammatory biomarker profiles among the MAFLD phenotypes to support earlier diagnosis, risk stratification, and phenotype-specific treatment strategies.

METHODOLOGY

This comparative observational study was conducted at Pakistan Aeronautical Complex Hospital Kamra, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from November 2021 to March 2024. The study protocol received ethical clearance from the Institutional Review Boards of both participating institutions (READ-IRB/2021/024 and PACH-IRB/2021/06). All participants gave written consent, consistent with the ethical guidelines of the Declaration of Helsinki.

Participants aged 20–70 years with a history of T2D, pre-diabetes, obesity, hypertension, or persistently elevated ALT were screened using ultrasound and the FLI. MAFLD was diagnosed in 393 patients based on FLI ≥30,[6] ultrasound-confirmed hepatic steatosis, and the presence of either T2D (FPG ≥7.0 mmol/L or HbA1c ≥6.5%), obesity (BMI ≥ 23 kg/m^2), or in lean individuals (BMI <23 kg/m^2), at least two metabolic risk factors per MAFLD.[1,2] Exclusion criteria included viral or autoimmune hepatitis, Wilson's disease, endocrine disorders, alcohol use, hepatosteatogenic drugs, pregnancy, and lactation. Healthy controls (n = 109) had no evidence of hepatic steatosis or metabolic dysfunction.

Demographic data, medical history, anthropometric measurements (BMI and WC), and blood pressure (BP) were recorded. Fasting venous blood (10 mL) was drawn following a 10–12 hour overnight fast. Collected samples were rapidly processed and maintained at –80°C until the analytical procedures were performed. The samples were analysed for FPG, lipid profile, liver enzymes, and hs-CRP using the Cobas® c501 chemistry analyser (Roche Diagnostics, Basel, Switzerland). Serum insulin levels were assessed on the Cobas® e411 (Roche Diagnostics, Basel, Switzerland). Enzyme-linked immunosorbent assays (Elabscience®, Houston, TX, USA) were used to measure IL-6, TNF-α, leptin, adiponectin, MDA, and CK-18. Platelet counts were measured using Sysmex® XP-100 (Sysmex Corporation, Japan).

BMI was calculated using the formula weight in kg/(height in m^2). The FLI,[6] fibrosis index based on four factors (FIB-4), AST to platelet ratio index (APRI), atherogenic index of plasma (AIP), and HOMA-IR were calculated using validated published formulae.[11,12]

Hepatic steatosis was confirmed by an experienced radiologist using B-mode ultrasonography (Canon Xario™ 100G; Canon Medical Systems, USA), based on four established criteria: hepatorenal echo contrast, increased hepatic echogenicity, reduced posterior beam penetration, and obscured vascular architecture.[13] The findings were confirmed by a second radiologist who was blinded to the initial results.

Sample size estimation was performed using G*Power version 3.1.9.2, based on a one-way ANOVA model, assuming an effect size of 0.2, 95% power, and α = 0.05, which required 390 participants (130 per MAFLD phenotype), according to Cohen's criteria.[14] Data were analysed by using the SPSS version 21. Normality was evaluated by the Shapiro-Wilk test. Continuous variables were reported in the form of mean ± standard and deviation (SD) and were compared using t-test or ANOVA with the Bonferroni correction. Categorical variables were summarised as frequencies and percentages, and comparisons were made using the Chi-square test. Biomarker associations were identified using a univariate analysis, followed by multivariate logistic regression. ROC analysis was employed for diagnostic accuracy. A p-value less than 0.05 (two-tailed) was deemed statistically significant.

RESULTS

Of the 502 patients screened, 109 were excluded (28 with viral hepatitis, 9 hypothyroidism, 4 on liver-affecting medicines, 32 without steatosis on ultrasound, 17 with FLI <30, and 19 who declined participation). The remaining 393 patients diagnosed with MAFLD were classified into three phenotypes: T2D-MAFLD (n = 134, 34.0%), OB-MAFLD (n = 221, 56.0%), and L-MAFLD (n = 38, 10.0%) (Figure 1). Despite extended sampling over 2.5 years, the required number of the lean cases could not be achieved due to the low regional prevalence of L-MAFLD (~3.0%).[15]

Table I: Demographic and clinical characteristics of healthy controls *versus* MAFLD patients.

Variables	Healthy controls (n = 109)	MAFLD patients (n = 393)	*p-values
Gender n (%)	-	-	-
Male	61 (56)**	241 (61.3)	0.32
Female	48 (44)	152 (38.7)	
Age (years)	43.3 ± 8.36***	44.7 ± 8.07	0.10
Weight (kg)	65.7 ± 9.85	76.5 ± 13.28	<0.001
Height (m)	1.7 ± 0.12	1.64 ± 0.08	<0.001
BMI (kg/m^2)	21.3 ± 1.08	28.3 ± 4.20	<0.001
WC (cm)	76.7 ± 4.81	99.3 ± 7.84	<0.001
SBP mm of Hg	123.0 ± 3.80	128.0 ± 6.59	<0.001
DBP mm of Hg	72.3 ± 4.11	74.6 ± 5.50	<0.001
TC (mmol/L)	4.8 ± 0.58	5.2 ± 0.89	<0.001
LDL-c (mmol/L)	2.9 ± 0.62	3.1 ± 0.92	0.003
HDL-c (mmol/L)	1.3 ± 0.15	1.1 ± 0.19	<0.001
TG (mmol/L)	1.5 ± 0.22	2.1 ± 0.58	<0.001
ALT (IU/L)	28.8 ± 6.89	42.9 ± 14.22	<0.001
AST (IU/L)	30.4 ± 9.01	41.3 ± 13.06	<0.001
GGT (IU/L)	23.7 ± 11.09	47.0 ± 14.59	<0.001
Albumin (g/L)	43.9 ± 2.26	39.0 ± 3.36	<0.001
FPG (mmol/L)	5.0 ± 0.61	6.3 ± 1.17	<0.001
FSI (IU/L)	8.2 ± 2.03	10.3 ± 3.06	<0.001
hs-CRP (mg/L)	1.8 ± 0.49	3.2 ± 1.02	<0.001
IL-6 (pg/mL)	3.3 ± 1.16	4.8 ± 1.77	<0.001
TNF-α (pg/mL)	3.6 ± 1.19	5.6 ± 1.75	<0.001
AdpN (µg/mL)	12.4 ± 3.53	9.1 ± 2.97	<0.001
Leptin (ng/mL)	8.8 ± 3.13	12.6±5.71	<0.001
CK-18 (mIU/mL)	33.3 ± 12.89	64.2 ± 31.25	<0.001
MDA (ng/mL)	15.0 ± 4.97	25.9 ± 10.84	<0.001
Platelet (x10^9/L)	237.7 ± 46.5	198.7 ± 52.9	<0.001
HOMA-IR	1.8 ± 0.45	2.8 ± 0.73	<0.001
FLI	14.6 ± 5.59	72.7 ± 17.34	<0.001
FIB-4	1.08 ± 0.44	1.55 ± 0.68	<0.001
APRI	0.32 ± 0.10	0.56 ± 0.24	<0.001
AIP	0.05 ± 0.08	0.27 ± 0.15	<0.001

**Group comparisons were conducted using the independent t-test or the Chi-square test, depending on the type of data. **Data are presented as absolute frequency alongside corresponding percentages. ***Results are expressed as mean values with standard deviation (mean ± SD).*
T2DM-MAFLD: Type 2 diabetes-metabolic dysfunction associated fatty liver disease; BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: Total cholesterol; LDL-c: Low density lipoprotein-cholesterol; HDL-c: High density lipoprotein-cholesterol; TG: Triglyceride; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; FPG: Fasting plasma glucose; FSI: Fasting serum insulin; hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-alpha; AdpN: Adiponectin; CK-18: Cytokeratin-18; MDA: Malondialdehyde; HOMA-IR: Homeostatic model assessment of insulin resistance; FLI: Fatty liver index; FIB-4: Fibrosis index based on 4 factors; APRI: AST-to-platelet ratio index; AIP: Atherogenic index of plasma.

Table II: Demographic, anthropometric, and biochemical profiles of the MAFLD phenotypes.

Variables	T2D-MAFLD (n = 134)	OB-MAFLD (n = 221)	L-MAFLD (n = 38)	*p-values	**p-values	***p-values
Age (years)	48.2 ± 6.83****	43.2 ± 8.00	41.2 ± 8.37	<0.001	<0.001	0.93
BMI (Kg/m^2)	27.5 ± 3.58	30.0 ± 3.25	20.8 ± 1.23	<0.001	<0.001	<0.001
WC (cm)	101.8 ± 4.93	99.2 ± 8.24	90.6 ± 7.67	0.004	<0.001	<0.001
SBP mm of Hg	131.3 ± 7.85	125.4 ± 4.37	131.6 ± 5.53	<0.001	>0.99	<0.001
DBP mm of Hg	75.3 ± 6.74	73.8 ± 4.11	76.7 ± 6.79	0.051	0.86	0.009
TC (mmol/L)	5.5 ± 0.74	5.1 ± 0.91	4.4 ± 0.80	<0.001	<0.001	<0.001
LDL-c mmol/L	3.4 ± 0.78	3.0 ± 0.93	2.3 ± 0.79	<0.001	<0.001	<0.001
HDL-c mmol/L	1.0 ± 0.21	1.1 ± 0.17	1.3 ± 0.12	>0.99	<0.001	<0.001
TG (mmol/L)	2.1 ± 0.57	2.2 ± 0.59	1.7 ± 0.40	0.59	0.001	<0.001
ALT (IU/L)	44.0 ± 13.03	43.6 ± 15.29	35.1 ± 8.48	>0.99	0.001	0.001
AST (IU/L)	41.8 ± 13.60	42.2 ± 13.17	34.37 ± 7.33	>0.99	0.005	0.002
GGT (IU/L)	48.5 ± 14.63	45.7 ± 14.63	49.5 ± 13.69	0.41	>0.99	0.70
Albumin (g/L)	36.9 ± 2.24	39.5 ± 2.96	43.8 ± 2.67	<0.001	<0.001	<0.001
FPG (mmol/L)	7.63 ± 0.65	5.56 ± 0.72	6.00 ± 0.52	<0.001	<0.001	0.001
FSI (IU/L)	8.8 ± 1.41	11.3 ± 3.45	9.5 ± 2.71	<0.001	0.96	0.001
hs-CRP (mg/L)	3.16 ± 1.09	3.37 ± 0.99	2.8 ± 0.88	0.23	0.47	0.01
IL-6 (pg/mL)	4.9 ± 1.66	4.9 ± 1.85	3.8 ± 1.32	>0.99	0.003	0.001
TNF-α (pg/mL)	5.8 ± 2.06	5.6 ± 1.51	4.4 ± 1.27	>0.99	<0.001	<0.001
AdpN (µg/mL)	10.8 ± 3.34	8.2 ± 2.34	8.7 ± 2.17	<0.001	<0.001	>0.99
Leptin (ng/mL)	12.3 ± 6.3	13.6 ± 5.18	7.6 ± 3.11	0.09	<0.001	<0.001
CK-18 mIU/mL	65.8 ± 30.6	66.7 ± 32.0	43.7 ± 18.99	>0.99	<0.001	<0.001
MDA (ng/mL)	26.8 ± 10.4	26.7 ± 11.1	18.9 ± 7.47	>0.99	<0.001	<0.001
Platelet x10^9/L	171.4 ± 38.4	210.83 ± 54.8	224.6 ± 47.0	<0.001	<0.001	0.63
HOMA-IR	3.0 ± 0.53	2.8 ± 0.83	2.5 ± 0.61	0.03	0.001	0.15
FLI	74.6 ± 14.8	77.6 ± 12.6	37.8 ± 6.7	0.10	<0.001	<0.001
FIB-4	1.90 ± 0.78	1.41 ± 0.56	1.13 ± 0.40	<0.001	<0.001	0.04
APRI	0.64 ± 0.27	0.53 ± 0.22	0.40 ± 0.13	<0.001	<0.001	0.003
AIP	0.28 ± 0.15	0.29 ± 0.14	0.12 ± 0.11	>0.99	<0.001	<0.001

**Differences between the T2D-MAFLD and OB-MAFLD groups were assessed using the post-hoc Bonferroni test using the ANOVA. **Differences between the T2D-MAFLD and L-MAFLD groups were assessed using the post-hoc Bonferroni test using the ANOVA. ***Differences between the OB-MAFLD and L-MAFLD groups were assessed using the post-hoc Bonferroni test using the ANOVA. ****Results are expressed as mean values with standard deviation (mean ± SD).*
T2D-MAFLD: Type 2 diabetes-associated-metabolic dysfunction associated fatty liver disease; OB-MAFLD: Obesity-associated MAFLD; L-MAFLD: Lean MAFLD; BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: Total cholesterol; LDL-c: Low density lipoprotein-cholesterol; HDL-c: High density lipoprotein-cholesterol; TG: Triglyceride; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; FPG: Fasting plasma glucose; FSI: Fasting serum insulin; hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-alpha; AdpN: Adiponectin; CK-18: Cytokeratin-18; MDA: Malondialdehyde; HOMA-IR: Homeostatic model assessment of insulin resistance; FLI: Fatty liver index; FIB-4: Fibrosis index based on 4 factors; APRI: AST-to-platelet ratio index; AIP: Atherogenic index of plasma.

Table III: Univariate analysis of biomarkers in the MAFLD phenotypes.

Variables	T2D-MAFLD Group n = 134		OB-MAFLD Group n = 221		L-MAFLD Group n = 38	
	OR (95% CI)	p-values	OR (95% CI)	p-values	OR (95% CI)	p-values
Age (years)	1.09 (1.05-1.13)	<0.001	0.99 (0.97-1.02)	0.93	0.97 (0.92-1.01)	0.20
Gender (male)	1.32 (0.79-2.21)	0.28	1.16 (0.73-1.85)	0.51	1.51 (0.70-3.26)	0.29
WC (cm)	2.05 (1.37-3.07)	<0.001	1.74 (1.47-2.07)	<0.001	1.36 (1.23-1.51)	<0.001
SBP (mm of Hg)	1.25 (1.17-1.33)	<0.001	1.14 (1.08-1.21)	<0.001	1.61 (1.35-1.91)	<0.001
DBP (mm of Hg)	1.09 (1.04-1.15)	<0.001	1.09 (1.03-1.15)	0.002	1.19 (1.09-1.30)	<0.001
TC (mmol/L)	3.98 (2.57-6.16)	<0.001	1.55 (1.16-2.08)	0.003	0.40 (0.22-0.73)	0.003
LDL-c (mmol/L)	2.99 (2.00-4.46)	<0.001	1.22 (0.93-1.61)	0.14	0.32 (0.18-0.58)	<0.001
HDL-c (mmol/L)	0.002 (.00-.01)	<0.001	0.001 (.00- .00)	<0.001	0.89 (0.07-11.0)	0.93
TG (mmol/L)	50.1 (17.4-144)	<0.001	53.4 (21.1-135)	<0.001	27.0 (6.19-118)	<0.001
ALT (IU/L)	1.16 (1.11-1.21)	<0.001	1.11 (1.08-1.14)	<0.001	1.12 (1.06-1.19)	<0.001
AST (IU/L)	1.09 (1.06-1.12)	<0.001	1.10 (1.07-1.13)	<0.001	1.05 (1.00-1.10)	0.02
GGT (IU/L)	1.15 (1.11-1.19)	<0.001	1.15 (1.11-1.19)	<0.001	1.14 (1.09-1.18)	<0.001
Albumin (g/L)	0.23 (0.14-0.36)	<0.001	0.53 (0.46-0.61)	<0.001	0.96 (0.85-1.16)	0.96
FPG (mmol/L)	639 (28-14422)	<0.001	2.97 (2.07-4.26)	<0.001	13.4 (5.6-32.0)	<0.001
FSI (IU/L)	1.24 (1.06-1.44)	0.006	1.76 (1.51-2.05)	<0.001	1.29 (1.08-1.52)	0.003
hs-CRP (mg/L)	5.9 (3.7-9.1)	<0.001	7.4 (4.7-11.5)	<0.001	8.2 (4.1-16.6)	<0.001
IL-6 (pg/mL)	2.08 (1.68-2.59)	<0.001	1.75 (1.49-2.05)	<0.001	1.41 (1.04-1.92)	0.02
TNF-α (pg/mL)	2.10 (1.72-2.56)	<0.001	2.42 (1.98-2.94)	<0.001	1.69 (1.23-2.34)	0.001
AdpN (µg/mL)	0.87 (0.81-0.94)	0.001	0.61 (0.54-0.68)	<0.001	0.67 (0.57-0.78)	<0.001
Leptin (ng/mL)	1.21 (1.12-1.31)	<0.001	1.31 (1.21-1.41)	<0.001	0.87 (0.76-0.99)	0.04
CK-18 (mIU/mL)	1.06 (1.04-1.08)	<0.001	1.06 (1.04-1.08)	<0.001	1.04 (1.02-1.07)	0.001
MDA (ng/mL)	1.20 (1.14-1.26)	<0.001	1.17 (1.12-1.22)	<0.001	1.11 (1.04-1.19)	0.001
Platelet x10^9/L	0.96 (0.95-0.97)	<0.001	0.99 (0.98-0.99)	<0.001	0.99 (0.98-1.00)	0.13
HOMA-IR (>2.5)	78.4 (31.9-192)	<0.001	18.2 (8.13-41.3)	<0.001	11.7 (4.3-31.9)	<0.001
FIB-4 (>1.3)	8.02 (4.5-14.3)	<0.001	3.04 (1.85-4.98)	<0.001	1.16 (0.52-2.58)	0.71
APRI (>0.5)	28.8 (11.8-70.5)	<0.001	15.8 (6.7-37.6)	<0.001	4.57 (1.47-14.22)	0.009
AIP (>0.11)	14.7 (7.88-27.5)	<0.001	21.6 (11.9-39.2)	<0.001	3.21 (1.49-6.93)	0.003

T2D-MAFLD: Type 2 diabetes-associated-metabolic dysfunction associated fatty liver disease; OB-MAFLD: Obesity-associated MAFLD; L-MAFLD: Lean MAFLD; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: Total cholesterol; LDL-c: Low density lipoprotein-cholesterol; HDL-c: High density lipoprotein-cholesterol; TG: Triglyceride; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; FPG: Fasting plasma glucose; FSI: Fasting serum insulin; hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-alpha; AdpN: Adiponectin; CK-18: Cytokeratin-18; MDA: Malondialdehyde; HOMA-IR: Homeostatic model assessment of insulin resistance; FLI: Fatty liver index; FIB-4: Fibrosis index based on 4 factors; APRI: AST-to-platelet ratio index; AIP: Atherogenic index of plasma. A p-value was calculated using the univariate binary logistic regression analysis.

Table IV: Multivariate logistic regression analysis of biomarkers in MAFLD phenotypes.

Variables	T2D-MAFLD Group		OB-MAFLD Group		L-MAFLD Group	
	p-values	OR (95% CI)	p-values	OR (95% CI)	p-values	OR (95% CI)
Model 1*	-	-	-	-	-	-
TC (mmol/L)	NS	-	NS	-	NS	-
LDL (mmol/L)	0.014	2.6 (1.2-5.7)	NS	-	NS	-
HDL (mmol/L)	<0.001	0.001 (0.00-0.02)	<0.001	0.001 (0.00- 0.01)	NS	-
TG (mmol/L)	<0.001	66.4 (12.6-349)	<0.001	41.5 (14.6-117)	<0.001	84.9 (8.1-881)
Model 2**	-	-	-	-	-	-
ALT (IU/L)	0.008	1.3 (1.07-1.67)	NS	-	NS	
AST (IU/L)	NS	-	0.004	1.06 (1.02-1.11)	NS	-
GGT (IU/L)	0.012	1.16 (1.03-1.30)	<0.001	1.12 (1.08-1.16)	<0.001	1.14 (1.09-1.18)
Albumin (g/L)	<0.001	0.12 (0.04-0.36)	<0.001	0.51 (0.42-0.62)	NS	-
Model 3***	-	-	-	-	-	-
hs-CRP (mg/L)	<0.001	7.9 (3.0-20.7)	0.001	51.7 (5.2-511)	<0.001	8.26 (3.5-19.3)
IL-6 (pg/mL)	<0.001	2.5 (1.5-4.2)	0.045	2 (1.0-4.2)	NS	-
TNF-α (pg/mL)	<0.001	3.6 (1.8-7.2)	0.005	5.8 (1.7-19.7)	NS	-
AdpN (µg/mL)	NS	-	0.002	0.4 (0.2-0.7)	0.001	0.65 (0.52-0.82)
Leptin (ng/mL)	0.139	1.1 (0.9-1.4)	0.004	1.5 (1.1-1.9)	NS	-
CK-18 mIU/mL	0.004	1.0 (1.0-1.1)	0.008	1.1 (1.0 -1.3)	0.012	1.05 (1.01-1.09)
MDA (ng/mL)	0.016	1.1 (1.0-1.2)	0.029	1.1 (1.0-1.3)	0.030	1.11 (1.01-1.22)
Model 4***						
HOMA (>2.5)	<0.001	79.3 (23-272)	<0.001	15.1 (5.8-39.1)	<0.001	9.8 (3.5-27.3)
APRI (>0.5)	<0.001	14.5 (4.0-51)	<0.001	9.8 (3.6-26.9)	NS	-
AIP (>0.11)	<0.001	11.1 (3.4-35)	<0.001	14.8 (7.2-30.7)	0.05	2.3 (0.99-5.5)

*T2DM-MAFLD: Type 2 diabetes-associated-metabolic dysfunction associated fatty liver disease; OB-MAFLD: Obesity-associated MAFLD; L-MAFLD: Lean MAFLD; TC: Total cholesterol; LDL-c: Low density lipoprotein-cholesterol; HDL-c: High density lipoprotein-cholesterol; TG: Triglyceride; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-alpha; AdpN: Adiponectin; CK-18: Cytokeratin-18; MDA: Malondialdehyde; HOMA: Homeostatic model assessment of insulin resistance; APRI: AST-to-platelet ratio index; AIP: Atherogenic index of plasma; NS = insignificant p-value. *Model adjusted for age, gender, and blood pressure;**Model adjusted for age; ***Model adjusted for age and gender. A p-value was calculated through the multivariate binary logistic regression analysis.*

Table I presents the comparative data between MAFLD patients and healthy controls. Age and gender distributions were comparable between the groups, with no statistically significant variation (p = 0.32). In contrast, MAFLD patients demonstrated elevated BMI, WC, BP, lipid profiles, hepatic enzymes, FPG, and pro-inflammatory markers such as hs-CRP, IL-6, TNF-α, leptin, CK-18, and MDA (p <0.001). Patients with MAFLD had higher HOMA-IR, FLI, FIB-4, APRI, and AIP scores than healthy controls (p <0.001).

Table II provides a comparative analysis of the MAFLD phenotypes. T2D-MAFLD showed the most pronounced metabolic abnormalities. Although BMI was lower than in the OB-MAFLD group (p <0.001), T2D-MAFLD patients had significantly higher central obesity, as reflected by WC (p = 0.004). This group demonstrated the highest levels of FPG and HOMA-IR among all phenotypes, accompanied by significant elevations in TC and LDL-c (p <0.001), however, although ALT and AST levels were also elevated, they did not differ significantly from those seen in the OB-MAFLD group.

Table V: Diagnostic accuracy of biomarkers among the three phenotypes of MAFLD.

Variables	Cut-off	T2D-MAFLD Group			OB-MAFLD Group			L-MAFLD Group		
		AUC	Sen%	Spec%	AUC	Sen%	Spec%	AUC	Sen%	Spec%
TC	5.40	0.75	61.2	82.6	0.60	39.8	82.6	0.35	10.5	82.6
LDL-c	3.49	0.72	52.2	82.6	0.54	31.2	82.6	0.30	10.5	82.6
HDL-c	1.19	0.79	59.7	83.5	0.82	68.8	83.5	0.51	15.8	83.5
ALT	35.5	0.83	70.1	80.7	0.78	66.5	80.7	0.71	52.6	80.7
AST	38.5	0.74	56.7	84.4	0.76	54.8	84.4	0.64	23.7	84.4
FSI	10.27	0.58	15.7	80.7	0.78	51.1	80.7	0.63	36.8	80.7
Hs-CRP	2.34	0.84	75.4	86.2	0.89	78.3	86.2	0.81	78.9	86.7
IL-6	4.42	0.77	63.4	80.7	0.76	65.2	78.9	0.62	34.2	78.9
TNF-α	4.70	0.81	68.7	80.7	0.85	76.9	80.7	0.67	39.5	80.7
Leptin	10.2	0.70	60.4	73.4	0.78	69.7	73.4	0.40	15.8	73.4
CK-18	44.6	0.81	67.9	80.7	0.82	72.9	80.7	0.65	50.0	79.8
MDA	18.7	0.83	71.6	80.7	0.81	72.4	80.7	0.66	44.7	80.7
HOMA	2.58	0.96	83.6	97.2	0.85	53.4	97.2	0.80	44.7	96.3
FIB-4	1.48	0.82	66.4	80.7	0.68	37.1	80.7	0.55	23.7	80.7
APRI	0.4	0.87	71.6	87.2	0.80	57	87.2	0.67	44.7	77.1
AIP	0.15	0.90	80.6	89.0	0.91	81.4	89	0.69	39.5	89.0

T2DM-MAFLD: Type 2 diabetes-associated-metabolic dysfunction associated fatty liver disease; OB-MAFLD: Obesity-associated MAFLD; L-MAFLD: Lean MAFLD; AUC: Area under the curve; Sen: Sensitivity; Spec: Specificity; TC: Total cholesterol; LDL-c: Low density lipoprotein-cholesterol; HDL-c: High density lipoprotein-cholesterol; ALT: Alanine amino-transferase; AST: Aspartate aminotransferase; FSI: Fasting serum insulin; hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-alpha; CK-18: Cytokeratin-18; MDA: Malondialdehyde; HOMA: Homeostatic model assessment of insulin resistance; FIB-4: Fibrosis index based on 4 factors; APRI: AST-to-platelet ratio index; AIP: Atherogenic index of plasma.

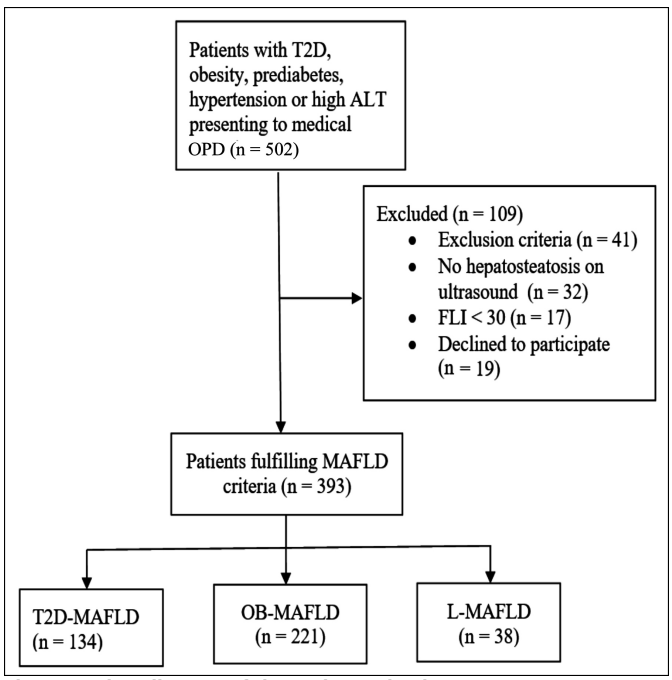

Figure 1: Flow diagram of the patient selection process.

The T2D-MAFLD group exhibited the most pronounced elevations in pro-inflammatory cytokines, specifically IL-6 and TNF-α (p <0.001). Fibrosis markers, including FIB-4, APRI, and CK-18, were significantly raised (p <0.001), reflecting a greater risk of fibrotic progression.

The OB-MAFLD group had the highest BMI and higher TG levels than the L-MAFLD group (p <0.001), though not statistically different from the T2D-MAFLD group (p = 0.60). Liver enzymes were elevated and comparable to the T2D-MAFLD group, however, significantly higher than in the L-MAFLD group (p <0.05). TNF-α, and IL-6 levels were also higher than in the L-MAFLD group (p <0.05), however, showed no significant difference from the T2D-MAFLD group. Fibrosis indices were elevated, though lower than in the diabetic phenotype.

Although L-MAFLD patients had a lower BMI, they still exhibited central obesity, with a significantly smaller WC compared to the other two groups (p <0.001). Insulin resistance was less severe than in T2D-MAFLD patients (p = 0.003). This group showed the highest HDL-c levels and comparatively lower LDL-c, TG, and liver enzyme levels (p <0.05). Inflammatory markers (IL-6, TNF-α, and hs-CRP) were modestly elevated (p <0.05), and fibrosis markers were the lowest among the phenotypes.

Univariate analysis (Table III) revealed distinct biomarker associations across all phenotypes. WC was a strong predictor in all the groups, with the highest in the T2D-MAFLD group (OR: 2.05, p <0.001). SBP was most strongly associated with the L-MAFLD group (OR: 1.61, p <0.001). Lipid profiles showed variation, with TC and LDL-c most elevated in the T2D-MAFLD group, while TG was the highest in the OB-MAFLD group (OR: 53.4, p <0.001). HDL-c was inversely associated with the T2D- and OB-MAFLD groups. Liver enzymes were significantly associated across all the groups. Glucose-related markers (FPG, FSI, and HOMA-IR) showed significant associations across all the phenotypes. Among inflammatory markers, hs-CRP had the strongest associations in all the phenotypes, particularly in the L-MAFLD group (OR: 8.2, p <0.001). Adiponectin was the lowest in the OB-MAFLD group (p <0.001), while leptin levels were lower in the L-MAFLD group (p = 0.04). FIB-4 and APRI were more significantly associated with the T2D- and OB-MAFLD group than the L-MAFLD group. AIP was elevated across all phenotypes, most notably in the OB-MAFLD group (OR: 21.6, p <0.001).

Multivariate logistic regression was performed across four models, each representing a biomarker group (Table IV). Model 1 (lipid parameters) revealed a significant association between LDL-c and the T2D-MAFLD phenotype (OR: 2.6, p = 0.014), while HDL-c was inversely associated with both the T2D- and OB-MAFLD phenotypes (OR: 0.001, p <0.001). TG showed the highest odds in the L-MAFLD phenotype (OR: 84.9, p <0.001).

In Model 2 (liver enzymes), ALT was significantly associated with the T2D-MAFLD phenotype (OR: 1.3, p = 0.008) and AST with the OB-MAFLD phenotype (OR: 1.06, p = 0.004). GGT was significantly elevated in all phenotypes (p <0.05). In Model 3, (inflammatory biomarkers) hs-CRP (OR: 51.7, p = 0.001) and TNF-α (OR: 5.8, p = 0.005) showed the strongest associations with the OB-MAFLD phenotype, whereas IL-6 levels were raised in both the diabetic and obese groups. Adiponectin was significantly reduced in both the OB- and L-MAFLD phenotypes; leptin was associated with the OB-MAFLD phenotype (p = 0.004). CK-18 levels were elevated in all phenotypes, with the highest level in the OB-MAFLD phenotype. In Model 4 (calculated indices), HOMA-IR was significantly associated with all phenotypes, with the highest in the T2D-MAFLD phenotype (OR: 79.3, p <0.001). AIP was significantly elevated in all phenotypes, most prominently in the OB-MAFLD phenotype (OR: 14.8, p <0.001).

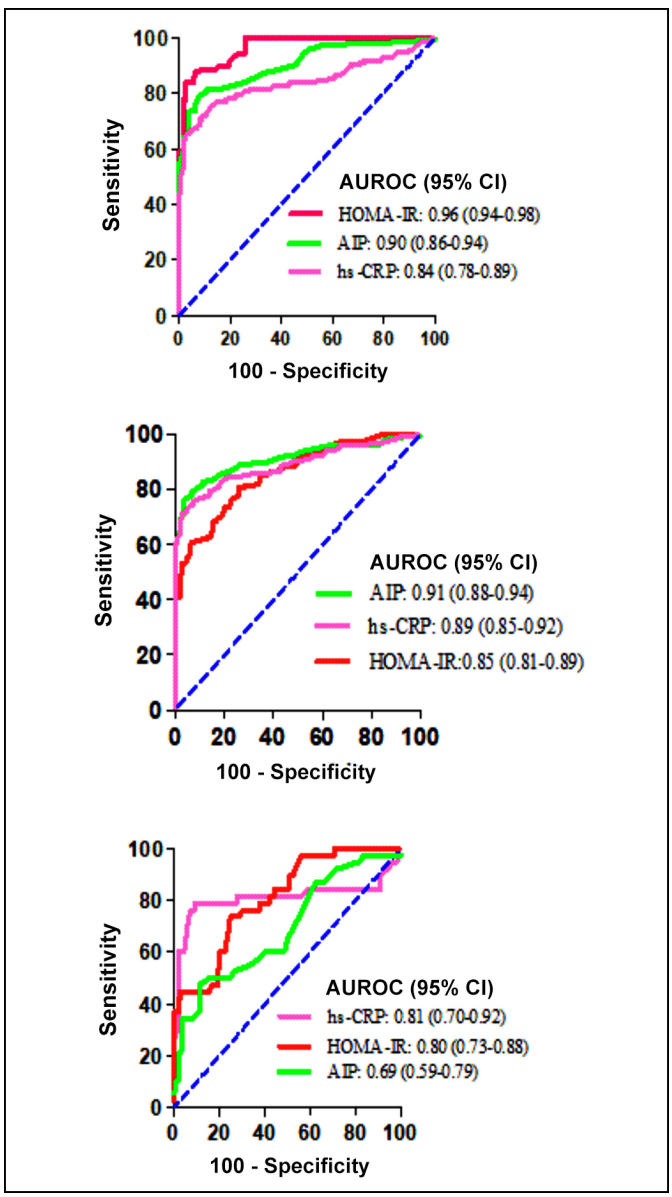

Figure 2: ROC curves of biomarkers that have high diagnostic accuracy in the T2D-MAFLD, OB-MAFLD, and L-MAFLD groups.

The ROC analysis showed the diagnostic performance of the biomarkers (Figure 2). HOMA-IR and AIP demonstrated the highest diagnostic accuracy for the T2D-MAFLD group (AUROC = 0.96, 0.90), whereas hs-CRP, TNF-α, and AIP were the most accurate in the OB-MAFLD group (AUROC = 0.89, 0.85, and 0.91 respectively). In the L-MAFLD group, hs-CRP, HOMA-IR, and AIP showed moderate accuracy (AUROC = 0.81, 0.79, and 0.69).

The diagnostic accuracy of biomarkers varied among the three MAFLD phenotypes at specific cut-off values (Table V). For the T2D-MAFLD group, HOMA-IR (83.6% sensitivity, 97.2% specificity; 2.58 cut-off), AIP (80.6%, 89%; 0.15), and hs-CRP (75.4%, 86.2%; 2.34) were the most accurate. In the OB-MAFLD group, AIP (81.4%, 89%), hs-CRP (78.3%, 86.7%), and HOMA-IR (53.4%, 97.2%) showed strong performance. In the L-MAFLD group, hs-CRP (78.9%, 86.2%) was the most reliable, with HOMA-IR (44.7.4%, 96.3%) and AIP (39.5%, 89%) showing moderate accuracy.

DISCUSSION

The study was conducted to explore phenotype-specific differences in metabolic and inflammatory profiles of individuals with MAFLD. The study demonstrated significant alterations in metabolic biomarkers, lipid parameters, and inflammatory cytokines in MAFLD patients compared to healthy controls. These changes reflected the underlying pathophysiology of MAFLD, characterised by insulin resistance, lipid dysregulation, and ongoing systemic inflammation, aligning with previous reports.[11,15]

Comparative analysis of the three MAFLD phenotypes revealed distinct clinical and biochemical characteristics that highlighted the heterogeneity of the disease. These findings aligned with previous research, emphasising the complex interplay between metabolic dysfunction, adiposity, and inflammation in the MAFLD progression.[1] The T2D-MAFLD phenotype exhibited the most pronounced metabolic abnormalities, consistent with earlier studies that linked diabetes with advanced liver pathology.[16] This group also showed severe insulin resistance, with the highest FPG and HOMA-IR levels, both of which are well-established indicators of metabolic derangement. Dyslipidaemia was also the most severe in this phenotype, contributing to an increased cardiovascular risk. Elevated IL-6 and TNF-α, with higher FIB-4, APRI, and CK-18 levels, suggested a greater predisposition to progressive hepatic fibrosis and a possible transition to cirrhosis in this group.

The OB-MAFLD phenotype showed the highest BMI and WC, highlighting the contribution of adiposity. The lipid profile was significantly elevated compared to the L-MAFLD phenotype but similar to the T2D-MAFLD phenotype, reinforcing the established link between obesity and dyslipidaemia in the MAFLD phenotype. Although inflammatory markers were

raised, they were slightly lower than in the T2D-MAFLD phenotype, suggesting that hyperglycaemia may exacerbate inflammation. These findings stressed the importance of lipid monitoring and lifestyle interventions to mitigate hepatic and cardiovascular risks.

The L-MAFLD group was characterised by a lower BMI but evident central obesity and metabolic disturbances, likely due to ectopic fat and insulin resistance. Although insulin resistance was less severe than in the T2D-MAFLD group, inflammatory markers remained moderately elevated. Fibrosis markers were the lowest in this group, consistent with other studies indicating a milder disease course.[17]

Regression analysis supported these findings. WC had the strongest association with the T2D-MAFLD phenotype, confirming the role of central obesity in the metabolic dysfunction. SBP was most significantly associated with the L-MAFLD phenotype, indicating metabolic dysregulation independent of obesity, consistent with the findings of Cheng *et al*.[18]

Differences in lipid markers were also evident, with TC and LDL-c strongly linked to the T2D-MAFLD group and the lowest in the L-MAFLD group, while TG was most elevated in the OB-MAFLD group. Elevated liver enzymes in the T2D-MAFLD and OB-MAFLD groups further supported metabolic stress-related liver injury.

Among the phenotypes, both the T2D-MAFLD and OB-MAFLD groups demonstrated the most significant elevations in hs-CRP, TNF-α, and IL-6 levels. Notably, hs-CRP also had a significant association with the L-MAFLD groups. AdpN was markedly lower in both the OB-MAFLD and L-MAFLD groups, while leptin was higher in the OB-MAFLD groups, consistent with known cytokine imbalances.[19] While FIB-4 was significant in univariate analysis, it did not remain significant in multivariate models. APRI and AIP showed strong associations with both T2D-MAFLD and OB-MAFLD groups, indicating elevated fibrosis risk. Although fibrosis risk was lower in the L-MAFLD group in the study cohort, other studies suggest lean individuals may still progress due to genetic factors.[20]

Several biomarkers demonstrated high diagnostic value for identifying the MAFLD phenotypes. HOMA-IR showed the highest accuracy for the T2D-MAFLD group, with a cut-off of 2.58, consistent with the previous studies reporting variable cut-offs depending on population and disease stage.[21,22] AIP showed the highest accuracy for the OB-MAFLD group (AUROC = 0.91); however, different studies had reported varying results. Duan *et al*. reported that the AUROC of AIP for MAFLD in non-obese subjects was significantly higher than that in obese subjects (0.78 *vs.* 0.57).[23] Another study further demonstrated that AIP was closely associated with hepatic steatosis severity regardless of BMI, highlighting its relevance to the cardiovascular risk.[24]

In the L-MAFLD group, hs-CRP had the highest predictive value (AUROC = 0.81). Elevated hs-CRP levels indicated systemic inflammation, which was a key feature of the L-MAFLD group. The hs-CRP was also associated with the adverse cardiovascular events, thereby linking the L-MAFLD phenotype with an increased cardiovascular risk.[25]

To the best of the authors' knowledge, this is the first study to provide a comprehensive comparison of metabolic and inflammatory biomarkers across all three MAFLD phenotypes. The variation in biomarker profiles reflects the heterogeneity of the disease and supports the concept of phenotype-specific pathways. The T2D-MAFLD group presented the most severe metabolic and inflammatory dysfunction, followed by the OB-MAFLD and L-MAFLD groups. These findings emphasise the need for personalised diagnostic tools and treatment approaches based on phenotype-specific risk.

The study has certain limitations. Liver biopsy was not performed, and hepatic steatosis was assessed using ultrasonography. Moreover, the inadequate sample size in the L-MAFLD group may have affected the power of the study to detect the differences between the groups. Additionally, the study was conducted in a specific geographical region, which may limit the generalisability of its findings.

CONCLUSION

The MAFLD phenotypes reflect the heterogeneous nature of this disease. The T2D-MAFLD group exhibited the most severe metabolic and inflammatory dysfunction, whereas the OB-MAFLD group showed moderate hepatic and lipid disturbances. The L-MAFLD group showed mild metabolic abnormalities. Phenotype-specific biomarker analysis holds promise for improving early diagnosis, risk assessment, and tailored treatment, thereby enhancing clinical outcomes.

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ETHICAL APPROVAL:
Ethical approval for this study was obtained from the Research Ethics and Academic Department (READ) of the Armed Forces Institute of Pathology, Rawalpindi, Pakistan (Approval No. READ-IRB/2021/024; Dated: 07.01.2021). Additional ethical approval was secured from the Institutional Review Board of Pakistan Aeronautical Complex Hospital Kamra, Punjab, Pakistan (Approval No. PACH-IRB/2021/06; Dated: 14.11.2021).

PATIENTS' CONSENT:
Written informed consent was obtained from all participants for the publication of their de-identified clinical information.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
SN: Drafted the manuscript.
DAK: Conceived and designed the study.
SN, MQAK, NC: Collected the data.
MQAK, SS: Performed data analysis.
DAK, MAP, NC: Reviewed and revised the manuscript.
All authors approved the final version of the manuscript to be published.

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