Int. J. Pharm. Investigation, 2026; 16(2):708-714.
https://www.jpionline.org

Original Article

Cardiovascular Disease Risk Factors in Diabetics with and without Metabolic Syndrome in NAFLD Patients

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ABSTRACT

Background: Globally, Non-Alcoholic Fatty Liver Diseases (NAFLD) is considered as the frequent chronic liver diseases strongly linked with metabolic dysfunction and cardiovascular risk specifically in Type 2 Diabetes Mellitus (T2DM) cases. Metabolic Syndrome (Mets) is generally characterized by various cardiometabolic risk factors that frequently coexist with NAFLD, which elevates the chances of cardiovascular diseases (CVDs). This research aims to evaluate and compare CVD predictive factors in diabetic NAFLD individuals with or without Mets and to assess how clinical and biochemical profiles differ across different groups and fatty liver disease grades. **Materials and Methods:** Observational cross-sectional single-centric research was performed over 12 months in the Department of Medicine. A total of 120 diabetic patients with NAFLD were enrolled and stratified into two different groups based on the presence (*n*=61) and absence (*n*=59) of metabolic syndrome. Demographic data, anthropometric measurements, and clinical and biochemical parameters of all enrolled participants were evaluated and comparatively analyzed. Statistical comparisons were carried out using independent sample t-tests and chi-square tests. **Results:** Insignificant variations were observed in the age, gender, and BMI distribution of patients in any group. However, HDL cholesterol showed a significant reduction (*p=0.025*) and serum ALT revealed a significant increase in Mets patients than in patients not having Mets (*p=0.020*). Other clinical and biochemical parameters demonstrated insignificant distribution between diabetic NAFLD individuals having Mets or those without Mets. Furthermore, the severity of FLD does not have a notable influence on most cardiovascular risk variables. **Conclusion:** Diabetic NAFLD patients with metabolic syndrome demonstrated unfavorable lipid profiles and higher levels of liver enzymes, which emphasize increased cardiovascular risk. Presence of Mets in diabetic NAFLD individuals underlines the urgency of early detection and targeted intervention to alleviate the risk of cardiovascular morbidity.

Keywords: Cardiovascular Disease Risk, Metabolic Syndrome, Non-alcoholic Fatty Liver Disease, Type 2 Diabetes Mellitus.

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Received: 05-09-2025;
Revised: 24-10-2025;
Accepted: 16-12-2025.

INTRODUCTION

Metabolic Syndrome (Mets) is a complex disease that is generally characterized by cardio metabolic risk factors, insulin resistance, Hypertension (HTN), and dyslipidaemia (Ahima, 2024). Globally, the incidence of Mets is rising slowly because of a sedentary lifestyle and obesity, which make it a prime medical issue (Saklayen, 2018; Grundy *et al*., 2005). Individuals with Mets are at increased risk of progression of co-morbidities, including Type 2 Diabetes Mellitus (T2DM), cardiovascular diseases (CVDs), and Non-Alcoholic Fatty Liver Diseases (NAFLD) (Dhondge *et al*., 2024).

T2DM is a metabolic disorder recognized by impaired secretion of insulin and insulin resistance, which is often considered a consequence of Mets (Abdul-Ghani *et al*., 2024). Existence of T2DM along with Mets increases the risk of vascular complications and accelerates damage to the target organ, specifically the liver (Voulgari *et al*., 2011). Amidst hepatic complications, fatty liver has surfaced as one of the leading pancreatic manifestations in patients of T2DM with Mets (Choudhary, 2019).

Globally, NAFLD accounts for the most frequent chronic liver disease, which is prevalent in nearly 25 to 30% of the general population. NAFLD is recognized as a multifunctional disease interlinked with metabolic dysfunction and increased risk of

DOI: 10.5530/ijpi.20260150

cardiovascular diseases (Younossi *et al.*, 2016). NAFLD also has a broad histopathological spectrum, which ranges from steatosis to non-alcoholic steatohepatitis that may later lead to cirrhosis, fibrosis, and liver cancer. Patients with NAFLD are highly prone to comorbidities such as T2DM and Mets; these conditions are pathophysiological associated with insulin resistance, oxidative stress, dyslipidaemia, inflammation, and endothelial dysfunction (Mantovani *et al.*, 2024). On the other hand, metabolic syndrome is interrelated to risk factors such as obesity, hypertension, and hyperglycaemia, which elevate the risk of CVD and macrovascular complications. The occurrence of T2DM and Mets in patients with NAFLD elevates the progression of hepatic diseases and cardiovascular morbidity and mortality. Various studies have reported that diabetes patients with NAFLD possess a higher probability of suffering from CVDs compared to NAFLD patients without diabetes (Chalasani *et al.*, 2018). Fatty liver is histologically categorized as mild (Grade I), moderate (Grade II), and severe (Grade III) as per the extent of hepatic fat accumulation. In patients with T2DM, the presence of hepatic steatosis reflects metabolic dysregulation along with oxidative stress, mitochondrial dysfunction, fatty acid reflux, and pro-inflammatory cytokine release (Lonardo *et al.*, 2015; Brunt, 2016).

Despite this well-known interrelationship, clinical studies evaluating the distribution of Mets across different grades of fatty liver diseases are limited, especially in patients with T2DM. A better understanding of clinical and biochemical differences in T2DM patients with or without Mets, stratified by fatty liver grades, can provide beneficial and significant insight of disease progression, risk stratification, and therapeutic interventions (Cornier *et al.*, 2008; Issa *et al.*, 2017; Targher *et al.*, 2010).

The presented study aims to assess the association of metabolic symptoms with demography, anthropometry, clinical, and biochemical factors in T2DM patients, and explore how these parameters alter across different grades of fatty liver disease. Characterization of these interrelationships will help to identify early metabolic dysfunction and potential markers predictive of hepatic involvement in T2DM.

MATERIALS AND METHODS

Study design and setting

The presented research was an observational, cross-sectional, single-centric study, which was performed for 12 months in the MLT-Biochemistry laboratory of the College of Applied Medical Sciences, Jazan University, in Jazan, Saudi Arabia.

Study population

A total of 120 patients aged in the range of 30-70 years diagnosed with T2DM and NAFLD and who were willing to give their consent for research, were enrolled in the study. The NAFLD status of patients was confirmed by abdominal ultrasonography. However, exclusive cases were alcoholic patients, individuals with chronic kidney disorder, congestive heart failure, pregnant and lactating females, and patients with chronic liver disease due to other etiologies like hepatitis B and C, as well as autoimmune disorders.

Sample size

Patients enrolled were stratified into two categories as per the presence or absence of Mets.

Group A - Diabetic cases with NAFLD and Mets.

Group B - Diabetic individuals with NAFLD and without Mets.

In addition, fatty liver grading was also performed, and patients were stratified as; Grade I (Mild), Grade II (Moderate), and Grade III (Severe).

Statistical analysis

Statistical evaluation was performed via descriptive and inferential statistics using the chi-square test, Student's *t*-test, and one-way ANOVA. Continuous data were represented as average±Standard Deviation (SD), and categorical data were shown as frequency and percentage. The software SPSS 24.0 version and GraphPad Prism version 7.0 were utilized for the analysis, and $p<0.005$ was set to show a significant value.

RESULTS

In this study, a total of 120 participants with T2DM and NAFLD were enrolled, of whom 61 (50.83%) patients have Metabolic Syndrome (Mets), and the remaining 59 (49.16%) patients are without metabolic syndrome. Table 1 reveals the demographic and clinical profile of the candidates, along with their distribution between the two groups. The data revealed statistically insignificant distribution in age among the participating patients with and without metabolic syndrome ($p<0.79$). The number of individuals with Mets was found to be high within 51 to 60 years (26.22%), followed by 31-40 years (24.59%) and 41-50 years (22.95%). On the other hand, in the patient group without Mets, the maximum patients belonged to 61-70 years and 31-40 years (27.11% each). In the younger age group (≤30), there were only 3 (4.91%) cases in the Mets category and 1 (1.69%) patient in the non-Mets category.

Gender distribution was insignificantly linked to the presence of Mets ($p=0.86$). Of the total 61 male participants, 49.18% patients ($n=30$) had Mets, whereas 50.82% patients ($n=31$) are non-Mets. Similarly, among the total 59 females, 50.84% ($n=30$) had Mets and 49.16% ($n=29$) were non-Mets, indicating a nearly equal distribution. However, the overall association was found statistically significant, which suggests potential interaction effects.

An elevated trend in the incidence of Mets was noted along with an increase in BMI (Body Mass Index). In the Mets group, 46.6% patients have a BMI of 18.5 - 24.9 kg/m^2, and 52% patients are with 25 - 29.9 and 30 - 34.9 kg/m^2. On the other hand, in the non-Mets group, most patients (53%) are in BMI 18.5 - 24.9 kg/m^2, and 47% patients each are with 25 - 29.9 and 30-34.9 kg/m^2 (*p=0.87*).

The clinical and biochemical profile of patients with or without Mets is shown in Figure 1 and Table 2. Mean duration of T2DM was found comparable between Mets (9.21±6.07) and non-Mets (9.93±6.24) group (*p=0.206*). Fasting blood glucose (174.98±42.26 vs 178.66±39.39; *p=0.349*) and HbA_{1C} levels (8.08±1.30 vs 8.20±1.28; *p*=0.321) were not found to be significantly different in both the Mets and non-Mets groups. Similar findings were also observed in SBP (145.02±21.10 vs 147.32±21.14; *p=0.242*) and DBP (88.49±12.03 vs 88.61±11.18; *p=0.915*), both these parameters were not found significantly different in Mets and non-Mets groups. Waist circumference was found to be similar in both groups (101.14±12.39 vs 101.66±11.35; *p=0.648*); a statistically insignificant association was noted. Lipid profile of patients evaluated revealed no significant differences in the levels of triglyceride levels (245.74±91.05 vs 243.93±90.58; *p=0.831*), LDL (131.73±33.79 vs 133.16±33.13; *p=0.648*), and total cholesterol (212.64±49.1 vs 207.94±54.16; *p=0.307*). However, HDL was found notably higher in individuals with Mets than non-Mets cases (40.4±8.80 vs 38.57±8.89; *p=0.025*). Liver enzyme Alanine Aminotransferase (ALT) was found substantially high in Mets cases than in non-Mets cases (47.30±18.29 vs 43.27±18.49; *p=0.020*), which suggested potential hepatic involvement. However, Aspartate Aminotransferase (AST) levels showed no significant difference and were found to be similar in both groups (44.46±18.30 vs 44.74±17.37; *p=0.870*).

Table 3 represents comparison of clinical and biochemical characteristics in individuals with FLD grades. All the participants were stratified as per FLD as Grade I (*n*=41, 34.16%), Grade II (*n*=44, 36.66%) and Grade III (*n*=35, 29.16%).

No significant difference was found among all three FLD grades in the duration of T2DM (Grade I: 9.07±6.11; Grade II: 9.26±6.08; Grade III: 9.21±6.10; *p=0.53*), Glycaemic parameters such as fasting blood glucose (Grade I: 74.04±41.57; Grade II: 174.04±41.57; Grade III: 174.31±42.16; *p=0.964*) and HbA_{1C} (Grade I: 7.99±1.29; Grade II: 8.08±1.31; Grade III: 8.03±1.29; *p=0.539*) were also found comparable across all three FLD grades. Anthropometric measurements such as mean waist circumference revealed no significant difference among FLD grades (Grade I: 101.10±12.38; Grade II: 101.22±12.40; Grade III: 101.19±12.44; *p=0.71*). However, systolic blood pressure revealed statistically significant difference (Grade I: 144.31±21.50; Grade II: 144.94±21.17; Grade III: 144.30±21.06; *p=0.002*) among all FLD groups.

Whereas diastolic blood pressure (Grade I: 88.68±11.83; Grade II: 88.51±12.08; Grade III: 88.58±11.97; *p=0.261*) revealed non-significant difference among all FLD groups. Lipid profile parameters were comparable across FLD grades. Triglyceride levels (Grade I: 245.36±90.15 mg/dL; Grade II: 245.21±91.25 mg/dL; Grade III: 245.77±90.63 mg/dL; *p*=0.616), HDL cholesterol (Grade I: 40.22±8.77 mg/dL; Grade II: 40.31±8.79 mg/dL; Grade III: 40.21±8.70 mg/dL; *p=0.602*), LDL cholesterol (Grade I: 130.83±34.64 mg/dL; Grade II: 131.41±33.75 mg/dL; Grade III: 131.16±33.91 mg/dL; *p*=0.267), and total cholesterol (*p*=0.093) did not exhibit statistically significant differences. Liver enzyme parameter ALT and AST levels were also not found significantly different among the three FLD grades. ALT values were 46.86±19.43 U/L, 47.36±18.99 U/L, and 47.46±19.07 U/L in Grades I, II, and III respectively (*p*=0.849). Whereas AST levels remained consistent among all grades (Grade I: 43.44±18.13 U/L; Grade II: 44.36±18.34 U/L; Grade III: 44.25±18.18 U/L; *p*=0.50).

DISCUSSION

Cardiovascular Diseases (CVDs) remain the primary cause of morbidity and mortality among patients with underlying diabetes mellitus. Additionally, the presence of Mets is considered as a key contributor to elevating the overall risk, which is identified by the concurrent presence of multiple metabolic abnormalities, such as insulin resistance, elevated BP, hypertriglyceridemia, and reduced HDL cholesterol. Mets are closely associated with a range of pathophysiological processes, which include low-grade chronic inflammation, oxidative stress, and endothelial dysfunction. Notably, Mets are commonly observed in T2DM cases, which, in combination, amplify the progression of CVDs. The main scope of the investigation presented was to explore and determine the risk of CVDs among diabetic NAFLD patients with or without Mets to provide better clinical risk prediction with overall management strategies.

We observed that diabetic NAFLD patients with Mets were significantly more prevalent in older age group. Similar results were reported by (Wang *et al.*, 2015), where the incidence of NAFLD with Mets was elevated in older individuals. In another study revealed that NAFLD cases in diabetes were higher in older patients (Song *et al.*,2022). The gender distribution of patients with or without Mets was equally distributed; the number of male patients were found similar to the number of female patients in both with or without Mets is found in sync with the Jiang *et al.*'s observation (Jiang *et al.*, 2018), where the prevalence of Mets was similar in male and female patients. We have observed a positive correlation between the BMI of patients and the prevalence of Mets (*p=0.87*), which aligns with the observations (Slagther *et al.*, 2017), who published that the occurrence of Mets increases with BMI and age. In various literature reports a strong association of obesity with both NAFLD and Mets is reported which revealed that obese patients or patients with higher BMI are predisposed

to NAFLD and Mets which include underlying mechanisms such as insulin resistance and inflammation (Frankowski *et al.*, 2023; Dharmalingam *et al.*, 2018; Latif *et al.*, 2024).

In this current study, insignificant variation was noted in fasting glucose and HbA_{1C} between the Mets and non-Mets groups. This suggests that Mets in our study is not driven by extreme glycaemia, but perhaps by mild to moderate insulin resistance and lipid abnormalities, which is in line with the results (Latif *et al.*, 2024), who reported that Mets components cluster even with modest individual change in glycemic parameters.

Interestingly, HDL levels were significantly higher (42.4 vs 38.6 mg/dL; *p=0.025*), and LDL levels are lower in Mets cases in comparison to non-Mets (131.73 vs 133.16;*p=0.648*). This aligns with the classical definitions of Mets, which state a typical association of Mets with lower levels of HDL (Karadag *et al.*, 2009; Fernandez *et al.*, 2010).

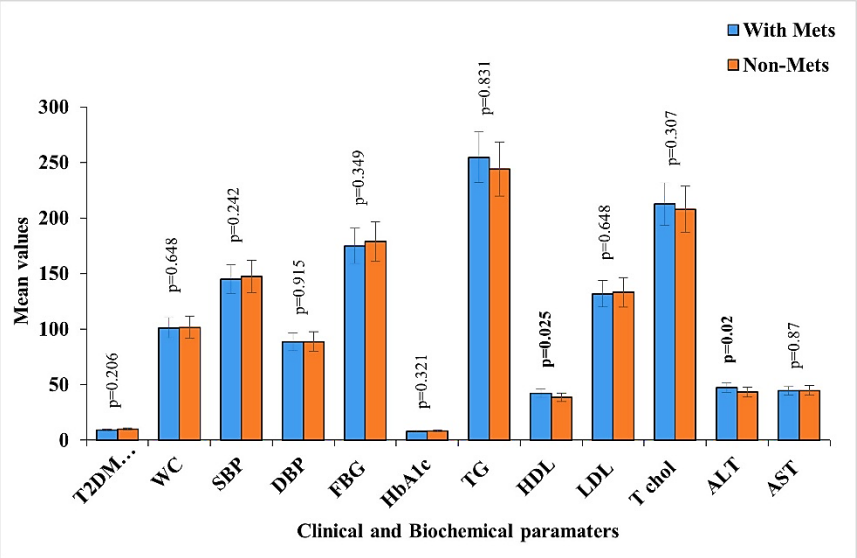

Figure 1: Mean values of clinical and biochemical parameters with or without Mets in diabetic NAFLD patients. Data are shown as Mean±SD; *p*-values computed using independent t-test; ALT: Alanine transferase, AST: Aspartate aminotransferase, DBP: Diastolic blood pressure, FBG: Fasting Blood Glucose, HDL: High Density Lipoprotein cholesterol, LDL: Low Density Lipoprotein cholesterol, SBP: Systolic blood pressure, T Chol: Total cholesterol, T2DM: Type 2 diabetes mellitus, TG: Triglycerides, WC: Waist Circumference.

Table 1: Demographics and anthropometric Characteristics of enrolled patients.

Variables	Overall (*n*=120)	With Mets (*n*=61)	Non-Mets (*n*=59)	*p*-Value
Age (Year)				
≤ 30	4	3 (4.91)	1 (1.69)	0.79
31 - 40	31	15 (24.59)	16 (27.11)	
41 - 50	25	14 (22.95)	11 (18.64)	
51 - 60	31	16 (26.22)	15 (25.42)	
61 - 70	29	13 (21.31)	16 (27.11)	
Sex				
Male	61	30 (49.18)	31 (50.82)	0.86
Female	59	30 (50.84)	29 (49.16)	
BMI (Kg/m²)				
18.5 - 24.9	30	14 (46.66)	16 (53.34)	0.87
25 - 29.9	42	22 (52.38)	20 (47.62)	
30 - 34.9	48	25 (52.08)	23 (47.92)	

Data are shown as numbers and percentage; *p*-values computed using chi-square test; BMI, Body mass index.

Table 3: Comparative analysis of anthropometric and cardiometabolic profiles as per the FLD grade.

Variables	FLD Grade			*p*-Value
	I (*n*=41)	II (*n*=44)	III (*n*=35)	
T2DM duration	9.07±6.11	9.26±6.08	9.21±6.10	0.53
WC (cm)	101.10±12.38	101.22±12.40	101.19±12.44	0.71
SBP (mmHg)	144.31±21.50	144.94±21.17	144.30±21.06	0.002
DBP (mmHg)	88.68±11.83	88.51±12.08	88.58±11.97	0.261
FBG (mmHg)	174.04±41.57	175.47±42.09	174.31±42.16	0.964
HbA_{1C} (%)	7.99±1.29	8.08±1.31	8.03±1.29	0.539
TG (mg/dL)	245.36±90.15	245.21±91.25	245.77±90.63	0.616
HDL (mg/dL)	40.22±8.77	40.31±8.79	40.21±8.70	0.602
LDL (mg/dL)	130.83±34.64	131.41±33.75	131.16±33.91	0.267
T chol (mg/dL)	213.99±49.20	212.98±49.16	213.17±49.49	0.093
ALT (U/L)	46.86±19.43	47.36±18.99	47.46±19.07	0.849
AST (U/L)	43.44±18.13	44.36±18.34	44.25±181.18	0.50

Data are shown as Mean±SD; *p*-values computed using One-way ANOVA; ALT: Alanine transferase, AST: Aspartate aminotransferase, DBP: Diastolic blood pressure, FBG: Fasting Blood Glucose, HDL: High Density Lipoprotein cholesterol, LDL: Low Density Lipoprotein cholesterol, SBP: Systolic blood pressure, T Chol: Total cholesterol, T2DM: Type 2 diabetes mellitus, TG: Triglycerides, WC: Waist Circumference.

Table 2: Clinical and biochemical characteristics of Mets and non-Mets individuals

Variables	With Mets (*n*=61)	Non-Mets (*n*=59)	*p*-value
T2DM duration (Years)	9.21±6.07	9.93±6.24	0.206
WC (cm)	101.14±12.39	101.66±11.35	0.648
SBP (mmHg)	145.02±21.10	147.32±21.14	0.242
DBP (mmHg)	88.49±12.03	88.61±11.18	0.915
FBG (mg/dL)	174.98±42.26	178.66±39.39	0.349
HbA_{1C} (%)	8.08±1.30	8.20±1.28	0.321
TG (mg/dL)	254.74±91.05	243.93±90.58	0.831
HDL (mg/dL)	42.4±8.80	38.57±8.89	0.025
LDL (mg/dL)	131.73±33.79	133.16±33.13	0.648
T chol (mg/dL)	212.64±49.1	207.94±54.16	0.307
ALT (U/L)	47.30±18.29	43.27±18.49	0.020
AST (U/L)	44.46±18.30	44.74±17.37	0.870

Data are shown as Mean±SD; *p*-values computed using independent *t*-test; ALT: Alanine transferase, AST: Aspartate aminotransferase, DBP: Diastolic blood pressure, FBG: Fasting Blood Glucose, HDL: High Density Lipoprotein cholesterol, LDL: Low Density Lipoprotein cholesterol, SBP: Systolic blood pressure, T Chol: Total cholesterol, T2DM: Type 2 diabetes mellitus, TG: Triglycerides, WC: Waist Circumference.

Overall lipid levels such as triglyceride and total cholesterol were found elevated in Mets group. Our results align with the findings (Krishan *et al.*, 2016) who have shown that association of dyslipidaemia with NAFLD as an integral part in patients with T2DM. Further, comparable consequences were also noted (Thomas *et al.*, 2020).

ALT was substantially increased in cases with Mets compared to non-Mets (47.3 vs. 43.3 U/L; *p=0.020*), while AST remained unchanged. Chen reported that elevated ALT is a hallmark of hepatic involvement and correlates with the association with Mets (Chen *et al.*, 2008). Further, Hassan has also reported the association of liver enzymes with Mets in patients with T2DM (Hassan *et al.*, 2021). In another study, similar findings were reported, which showed that liver enzymes are markedly elevated in T2DM patients with Mets (Music *et al.*, 2015), and it was also reported that T2DM in association with Mets plays a significant role in the elevation of risk of liver inflammation and injury (Shahwan *et al.*, 2019).

Further stratification of diabetic patients across FLD grades I, II, and III, our findings for glycemic control, BMI, waist

circumference, and liver enzyme parameters remained similar across groups. However, an increase in lipid profile across all grades aligns with findings, who has reported obesity being a major NAFLD predictive factor (Younossi *et al*., 2016).

A significant difference in SBP (*p=0.002*) was observed, which indicates a possible role of hemodynamic stress in advancing FLD grade in diabetic patients with Mets (Singh *et al*., 2025).

In various studies higher incidence of NAFLD was reported in individuals with Mets. It is evident that there is a noteworthy relation between NAFLD and Mets; NAFLD should be considered as an additional hepatic manifestation of Mets. This highlights the urgency to explore more evidence-based clinical studies to have a better understanding of NAFLD in diabetic patients with Mets (Thomas *et al*., 2020; Acharya *et al*., 2019; Radu *et al*., 2023).

CONCLUSION

Accordingly, we have found that diabetic NAFLD patients with Mets have a close relationship with age, BMI, liver enzymes, and haemodynamic parameters such as SBP and DBP. This emphasizes the present underlying mechanism of insulin resistance, inflammation, and dyslipidaemia. Further evaluation revealed presence of different grades of NAFLD in diabetic patients with Mets increased the risk of hepatic inflammation, hepatic injury, and CVDs.

ACKNOWLEDGEMENT

The authors thank the hospital staff and patients for their support of this study.

ABBREVIATIONS

NAFLD: Non-Alcoholic Fatty Liver Diseases; **T2DM:** Type 2 Diabetes Mellitus; **CVDS:** Cardiovascular diseases; **METS:** Metabolic Syndrome; **HTN:** Hypertension; **SD:** Standard Deviation; **BMI:** Body Mass Index.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Cite this article: Alam MS, Mjery Y, Ahmad K, Kaabi YA, Akhter MS, Jabeen F, *et al*. Cardiovascular Disease Risk Factors in Diabetics with and without Metabolic Syndrome in NAFLD Patients. Int. J. Pharm. Investigation. 2026;16(2):708-14.