Kufa Medical Journal | Vol. 21, No.2, 2025

Original article | DOI: https://doi.org/10.36330/kmj.v21i2.20678 | p-ISSN: 1993-517X e-ISSN: 2709-4464

Elevated Serum Cathepsin D Correlates with TNF-α, Insulin, and Myocardial Infarction in Type 2 Diabetes

Mohammed H. Hassan*[1] and Ferdous A. Jabir[2]

[1,2]*Department of Medical Chemistry, College of Medicine, University of AL-Qadisiyah, Al-Diywaniyah, Iraq.*

***Corresponding Authors Email:** * med.post24.61@qu.edu.iq, Ferdousabbas@gmail.com

ABSTRACT

Background: *Cathepsin D, a lysosomal enzyme, has a clear role in the metabolic and inflammatory processes associated with conditions such as type 2 diabetes mellitus and myocardial infarction (MI), particularly in the context diabetic complications.* ***Objective****: To evaluate serum cathepsin D levels in T2DM patients with MI and assess associations with TNF-α, insulin, and other metabolic/inflammatory parameters.* ***Methods****: Case-control study: This study included 120 participants divided into three equal groups (40 participants): 40 control groups, 40 individuals with type 2 diabetes and insulin resistance, and 40 individuals with diabetes who had suffered a MI.* ***Results****: Cathepsin D levels were significantly elevated in G3 (374 ± 118 ng/L) vs. G2 (291 ± 41.6 ng/L) and G1 (233.6 ± 55.8 ng/L; $p < 0.001$). Strong positive correlations existed between cathepsin D and TNF-α ($r = 0.514$, $p < 0.05$) and insulin ($r = 0.554$, $p < 0.05$). ROC analysis indicated high diagnostic accuracy for MI in diabetics: cathepsin D (AUC 0.94, sensitivity 88%, specificity 88%) and TNF-α (AUC 0.93, sensitivity 92%, specificity 80%). All metabolic (RBS, HbA1c%, insulin) and inflammatory markers (CRP, TNF-α) were significantly higher in G2 and G3 vs. G1 ($p < 0.001$).*

Conclusion: *Serum cathepsin D is significantly elevated in T2DM patients with MI and correlates strongly with TNF-α and insulin resistance. These findings support its potential as a biomarker for cardiovascular risk stratification in diabetic populations.*

Keywords: Cathepsin D, Type 2 Diabetes, Myocardial Infarction, Inflammation, TNF-α.

Article Information

Received: July 24, 2025; Revised: November 29, 2025; Online December, 2025

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by insulin resistance and secretion failure which leads to chronic hyperglycemia [1]. It had increased markedly on a worldwide scale, which is shown in IDF (International Diabetes Federation) estimated that the number of diabetic patients would rise to 783 million in **2045** (mainly in Corresponding author developing countries such as Iraq) [2]. At a local level, about one in every ten Iraqi adults is assumed to have diabetes [3, 4], with its increase being mainly associated with metabolic distortions, lack of physical activity, and non-nutritive high fat diet intake[5]. The risk of death from cardiovascular disease (CVD), especially myocardial infarction (MI), is highest in diabetic patients among the causes of death with a reported death rate of 83.2% as compared to non-diabetic (73.4%) [6]. This increased risk is due to the chronic inflammation and metabolic troubles with atherosclerosis coming faster and faster attacking the heart [7].

Pro-Inflammatory cytokines, particularly tumor necrosis factor alpha (TNF-a), play a central role in the development of insulin

resistance and endothelial dysfunction by reducing nitric oxide(NO) availability, thereby enhancing the progression of atherosclerosis and increasing MI risk in T2DM patients [8, 9]. Another emerging molecule is cathepsin D, a lysosome protease involved in proteins cascading apoptosis that has been progressively related to the inflammatory and metabolic imbalance seen both in diabetes and cardiovascular disease[10]. Myocardial cathepsin D is important in the integrated pathophysiology of diabetics with MI as a focal point crosstsalk between inflammation and alteration in metabolism. This endopeptidase aspartic proteinase, predominantly active in the lysosomes, is of wide interest for both its clinical relevance as a diagnostic marker and mechanistic mediator role under cardiovascular complications associated to diabetics.[11, 12].

A number of reports have documented a raised level of serum TNF-a and Cathepsin D in diabetics, more particularly who are with heart disease including those with myocardial infarction (MI)[8, 13] However, there remains a scarcity of studies evaluating this parameter in the local diabetes population, highlighting a gap in understanding their diagnostic and prognostic potential in this context. Therefore, this study aims to evaluate the serum levels of cathepsin D, TNF-a, and selected metabolic and inflammatory parameters among patients with type 2 diabetes and those with MI secondary to diabetes, in order to explore their association with MI risk.

METHODS

Study Design and Setting

This was a case control study that was carried out in the department of clinical biochemistry, College of Medicine, University of Al-Qadisiya during the period from December 2023 to March 2024.120 patients were enrolled consecutively on presentation in Al-Zahraa Teaching Hospital, Kut city, Wasit Governorate. The study population was divided into 3 age-stratified categories: Group-1 (clinically healthy controls) [n=40], Group-2: Patients with Type 2 diabetes Mellitus (T2DM) [n=40]; and, Group-3 were the T2DM patients with history of Myocardial Infarction (MI).

Participants

The study participants, comprising three groups (G1, G2, G3) initially matched for age, gender, and body mass index (BMI), demonstrated the following demographic characteristics (reported as mean ± standard deviation unless noted): age was 49.0 ± 5.8 years for G1, 52.3 ± 8.2 years for G2, and 57.4 ± 6.9 years for G3; male representation was 37% in G1, 40% in G2, and 55% in G3; BMI was 25.3 ± 3.1 kg/m² for G1, 28.4 ± 0.84 kg/m² for G2, and 28.9 ± 1.54 kg/m² for G3. Eligibility followed stringent inclusion criteria: individuals aged 40-70 years with a confirmed diagnosis of T2DM, either with or without a history of MI. Key exclusion criteria encompassed type 1 diabetes mellitus, current insulin therapy, the presence of other significant cardiac diseases, and chronic liver disease [14].

Sample Collection and Handling

Separation of serum. Serum was extracted from gel tubes by centrifuging them for ten minutes at 3000 rpm. Each participant had five milliliters of venous blood drawn. 2 ml were placed in an edta tube for glycated hemoglobin (hba1c) measurement, and 3 ml were collected in a gel tube for stored at −20°c until analysis.

Biochemical Analysis

The levels of insulin, cathepsin D and TNF-α in the sera were determine using enzyme-linked immunosorbent assay (ELISA) kits, according to provided instruction (Clinical Biochemistry Laboratory of Al-Zahraa Teaching Hospital). The ELISA kits in our experiments were: Insulin: Catalog No. [E0010Hu], Lot No.[202410010].. TNF-α:

Cat.No.[E0082Hu], Lot No.[It was repeated on]. Cathepsin D: Catalog No [E4668Hu], Lot No.[202410010] BT-Lab®, China. Random blood sugar (RBS) and c-reactive protein (CRP) were determined on an autoanalyzer (Mindray BS-240 using kits purchased from Human, Germany for CRP and Standard diagnostic Korea for RBS via standard enzymatic and turbidemetric methods respectively. HbA1c was determined as a part of glycated hemoglobin measurement using an Abbott analyzer system according to the manufacturer's instruction..

All samples were analyzed in duplicate, with proper calibration before each assay to ensure accuracy.

Statistical Analysis

Analysis Data were analyzed using GraphPad Prism (version 9.3.1). Continuous variables were described in terms of mean ± standard deviation (SD). We used the Shapiro-Wilk test to evaluate for normality. The comparisons among more than two groups were done by one-way ANOVA with Tukey's post hoc test. Chi-square test was used for the quantification of categorical variables. Pearson correlation coefficient was used to analyze the associations between continuous variables. The diagnostic value of parameters was evaluated using the receiver operating characteristic (ROC) curve analysis. The results were considered as significantly different at $p < 0.05$.

RESULTS

The table 1 summarizes the baseline demographic and clinical characteristics of the study cohort. Significant intergroup differences (one-way ANOVA, p < 0001) were observed inflammatory parameters (CRP, TNF-α and Cathepsin D) and metabolic parameters (RBS, HbA1C, Insulin)

Table 1: Demographic, Inflammatory and Metabolic Parameter profiles of Study participants

Parameter	Control (G1)	Diabetes (G2)	Diabetes + MI (G3)	P-value
Age (years)	49 ± 5.8	52.3 ± 8.2	57.4 ± 6.9	0.16 O ^{NS}
BMI (kg/m²)	25.3 ± 3.1	28.4 ± 0.84	28.9 ± 1.54	0.16 O ^{NS}
(RBS) mg/dL	110.9± 14.5	214 ± 86	245 ± 75	< 0.001 ***
HbA1c %	5.1 ± 0.4	8.2 ± 2.0	9.1 ± 2.3	< 0.001 ***
Insulin µIU/mL	11.2 ± 2.2	13.4 ± 3.9	18.3 ± 8.2	< 0.001 ***
CRP mg/L	1.9 ± 1.3	3.9 ± 2.3	23.5 ± 13.8	< 0.001 ***
TNF-α ng/L	98.8 ± 31.4	126 ± 62	160 ± 52.8	< 0.001 ***
CathepsinD ng/L	233.6± 55.8	291 ± 41.6	374 ± 118	< 0.001 ***

C: Chi-square test, O: one-way ANOVA; ***: significant at P <0.001. Statistical analysis was performed using one-way ANOVA variance with post-hoc Tukey's test for pairwise comparisons

ROC Curve Analysis for Diagnostic Biomarkers in Myocardial Infarction. Cathepsin D: Cut-off: >258.6 ng/L. Performance: Sensitivity = 88%, specificity = 88%, PPV = 88%, NPV = 88%, accuracy = 75%. **AUC: 0.94** (p < 0.0001), indicating excellent discriminatory power. TNF-α: Cut-off: >122.3 ng/L. Performance: Sensitivity = 92%, specificity = 80%, PPV = 82%, NPV = 91%, accuracy = 72%. **AUC: 0.93** (p < 0.0001), confirming strong diagnostic accuracy. Both biomarkers exhibit high sensitivity (>88%) and specificity (>80%) for MI detection in diabetes. Cathepsin D (**AUC 0.94)** marginally outperforms TNF-α (**AUC 0.93),**

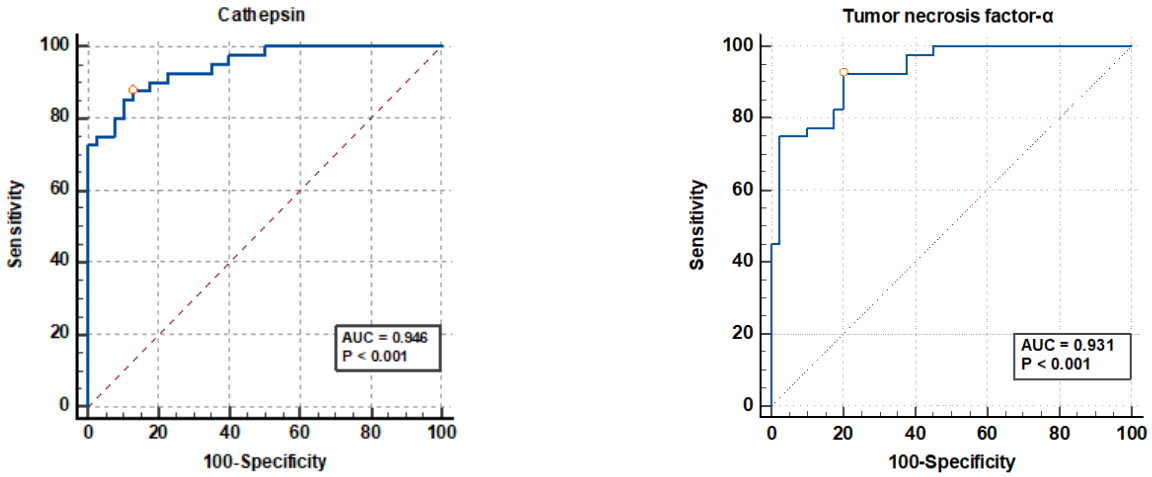

Figure 1: The ROC curve for Cathepsin and TNF-α

Correlations between metabolic and inflammatory markers were highly significant (all $p < 0.05$). We found an association between cathepsin and TNF-alpha ($r = 0.514$), which suggests common inflammatory pathways. Insulin was associated with inflammatory markers ($r = 0.392$ with TNF-alpha). The cathepsin was also related to insulin ($r=0.554$).

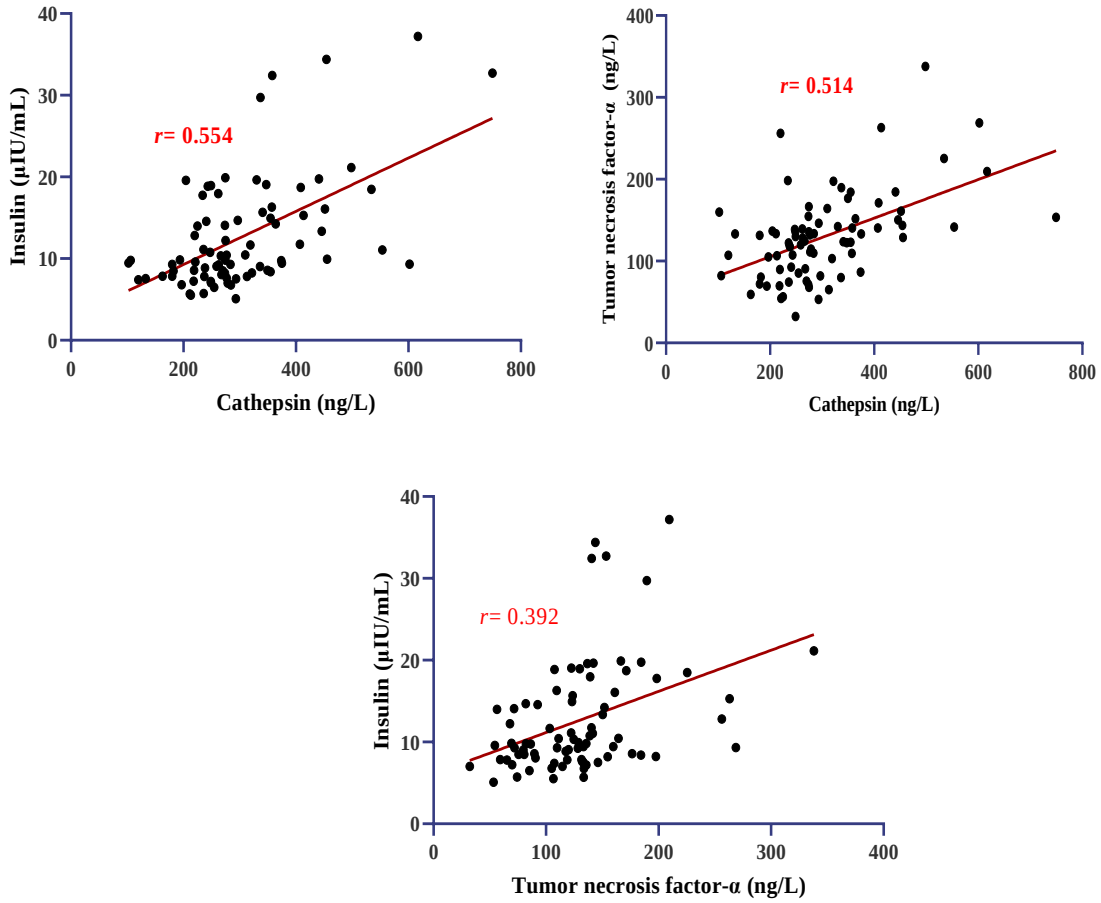

Figure 2: The different charts for correlation between variable.

DISCUSSION

In this study, the demographic variables, namely age and BMI, did not show statistically significant associations with the biochemical markers (p = 0.16 for both). This may be attributed to factors such as sample size, disease duration, and the degree of diabetes control among patients, which could attenuate the expected effects of these variables. Although previous studies, such as that by Ellulu et al. (2022), have demonstrated potential associations between age, BMI, and inflammatory status, our findings are consistent with the view that these relationships may be less evident in well-monitored diabetic populations under treatment [15].

For metabolic indices, three parameters including random blood sugar, glycated hemoglobin and insulin were measured. The study showed a substantial increase in RBS and HbA1c levels, suggesting uncontrolled glycemia, and long-standing exposure to hyperglycemia. Such chronic exposure is instrumental in the formation of advanced glycation end products (AGEs), which activate oxidative and inflammatory cascades that may contribute to pre-existing cardiovascular damage. These findings are consistent with those of Bansal et al. (2023) who associated higher metabolic markers with greater oxidative and inflammatory activity in diabetes subjects [16].

Insulin resistance that is frequently observed in T2DM. Another interesting finding is the fact that insulin levels correlated positively with TNF antagonism thing that demonstrates once more, that insulin resistance is not only a problem of metabolism but it's intertwined with inflammation at systemic level. This finding is consistent with Al-Zamli et al. (2020) which showed similar relationship between TNF-α and insulin resistance in diabetics [8]. Furthermore, a significant positive correlation was observed between cathepsin D and fasting insulin concentration, suggesting that the enzyme activity might be up-regulated through inflammatory pathways by insulin resistance [17].

Consistent with these findings, a similar immune response was also observed in the same study and marked by increased TNF-α compared to control (as previously described),30 which suggests that TNF-α plays an essential role in mediating low-grade chronic inflammation during diabetes and MI interventions. This was associated with increased systemic inflammation as measured by C-reactive protein (CRP). They as well modulate vascular function and in general NO bioavailability, thus leading to endothelial dysfunction. These sov behaviors are in agreement with the observations of Zhenfeng et al. (2023) and Zeng et al. (2024) the TNF-α and CRP high levels association with MI was verified and validated according to the MI pathogenesis and prognosis [18, 19]. Additionally, Guo et al. (2020) reported that NO deficiency in diabetics contributes to endothelial damage and vascular inflammation [20].

In the same group of diabetic patients, the levels of cathepsin D increased to a statistically significant degree, probably in response to both metabolic and inflammatory stimuli. Although cathepsin D is generally said to act in the digestion of damaged proteins and cell components, its overexpression may even worsen tissue damage, especially in the heart. Liu et al. (2017) has shown that cathepsin D is associated with insulin resistance, inflammation, and cardiovascular diseases including MI [17]. Similarly, Molvin et al. (2020) demonstrated that higher plasma cathepsin D was associated with worse cardiac events including myocardial infarction (MI) and heart failure, mainly in diabetic patients [21]. The cathepsin D interaction with pro-inflammatory cytokines is even more obvious in the group of diabetic patients with foot ulcers,

among whom, the level of cathepsin D is positively correlated with the amount of TNF-α and interleukin-6 (IL-6). In particular, patients with diabetic foot ulcers have significantly higher median levels of cathepsin D in plasma than do diabetic subjects without ulcers [22].

Correlation analysis was performed to provide an insight into the pathophysiological relationships among the measured parameters. The greatest positive relationship between insulin and TNF-α (1) of the ones tested above suggests some connection of insulin resistance with inflammation. TNF-α was evidenced to inhibit insulin signal transduction, and to lead to decreased insulin-stimulated glucose transport and impaired glycaemic control. This is concordant to that of Alzamil. et al. (2020) who reported similar correlation TNF-α,-insulin-resistance and the metabolic parameters such as HbA1c [8].

Figure 2 Furthermore, Figure We also observe in the histogram a positive relationship between insulin and cathepsin D levels indicating that the abnormal metabolism and increased activity of an enzyme will follow by mutual negative feedback. Ding et al. (2021) verified this correlation and indicated the mechanism that insulin resistance upregulates cathepsin D expression through inflammation signals in T2DM patients [23].

STUDY LIMITATIONS

Limitations and Interpretation This study has several limitations that need to be considered when interpreting our results. Small sample size may limit the generalization of the findings, especially since only one center was involved, in which the study was performed in Iraq. Furthermore, the study measured biochemical markers at a single time point only, which might not represent long-term status of inflammation or metabolism. Finally, we have no longitudinal follow-up of the study population and could not evaluate whether cathepsin D is sufficiently predictive of future cardiovascular events in this population.

CONCLUSION

In this study the serum levels of cathepsin D, TNF-α as well as other inflammatory and metabolic markers including XO were found to be significantly increased in patients with type 2 DM especially those with MI. These results indicate a potential use for the assessment of cardiovascular risk in patients with diabetes mellitus. Further studies are recommended to validate its diagnostic and prognostic value in larger cohorts.

ACKNOWLEDGMENT

The authors would like to thank the Clinical Biochemistry Department, College of Medicine, Al-Qadisiyah University, and the staff of the Clinical Biochemistry Laboratory at Al-Zahraa Teaching Hospital for their technical support and assistance during sample analysis.

Ethical approval

The present study which is conducted by authors ((Mohammed H. Hassan, Ferdous A Jabir) was approved by the local Department of Medical Chemistry committee.

Competing interests

The authors have nothing to disclose.

Author contribution

The authors contributed equally to the conceptualization of the research, collected data, and participated in data analysis and write-up, editing, and review.

Funding: Non.

REFERENCES

1. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. Introduction and methodology: standards of care in diabetes—2023 [Internet]. Vol. 46, Diabetes care. American Diabetes Association; 2023. p.

S1–4. Available from: https://doi.org/10.2337/dc23-Sint

2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119.
3. Pengpid S, Peltzer K. Prevalence and factors associated with undiagnosed type 2 diabetes among adults in Iraq: analysis of cross-sectional data from the 2015 STEPS survey. BMJ Open. 2022;12(11):e064293.
4. Saad AL-Harris S, Mohammed Hashim J, Dhafer M. Sultan, Diah Zain Al Deen MJ, Roaa Ali Musa, Talib Alharmosh MJ. Post COVID-19 Pediatric Acute Myocardial Infarction In a Known Case of Acute Lymphoblastic Leukemia (A case Report). Kufa Med J [Internet]. 19(1 SE-Original Articles):1–6. Available from: https://doi.org/10.36330/kmj.v19i1.10651
5. Zamil AH, Amin SS. The prevalence of metabolic syndrome among university students in Wasit, Iraq. Saudi Med J. 2022;43(11):1240.
6. Kerola AM, Juonala M, Kytö V. Short- and long-term mortality in patients with type 2 diabetes after myocardial infarction–a nationwide registry study. Cardiovasc Diabetol. 2024;23(1):390.
7. Zhang Q, Wu C, Liu Y, Tan X, Li C, Li L, et al. Chronic inflammation plays a role of a bridge between cardiovascular disease and hyperglycemia. Metab Syndr Relat Disord. 2023;21(8):468–74.
8. Alzamil H. Elevated serum TNF-α is related to obesity in type 2 diabetes mellitus and is associated with glycemic control and insulin resistance. J Obes. 2020;2020(1):5076858.
9. Liu Z, Lu J, Sha W, Lei T. Comprehensive treatment of diabetic endothelial dysfunction based on pathophysiological mechanism. Front Med. 2025;12:1509884.
10. Kobayashi S, Hahn Y, Silverstein B, Singh M, Fleitz A, Van J, et al. Lysosomal dysfunction in diabetic cardiomyopathy. Front Aging. 2023;4:1113200.
11. Liu CL, Guo J, Zhang X, Sukhova GK, Libby P, Shi GP. Cysteine protease cathepsins in cardiovascular disease: from basic research to clinical trials. Nat Rev Cardiol. 2018;15(6):351–70.
12. Mijanovic O, Petushkova AI, Brankovic A, Turk B, Solovieva AB, Nikitkina AI, et al. Cathepsin D-Managing the Delicate Balance. Pharmaceutics. 2021 Jun;13(6).
13. Velders MA, Calais F, Dahle N, Fall T, Hagström E, Leppert J, et al. Cathepsin D improves the prediction of undetected diabetes in patients with myocardial infarction. Ups J Med Sci. 2019;124(3):187–92.
14. Mohammed HR, Othman R Ben, Alghurabi H, Hussein RM, Al-Obaidi Z, Abdesselem H. Comparison of clinical characteristics, microvascular complications and inflammatory markers in type 2 diabetic patients under insulin versus metformin treatment: A cross-sectional study at Karbala Diabetic Center, Iraq. Medicine (Baltimore). 2024;103(44):e40330.
15. Ellulu MS, Samouda H. Clinical and biological risk factors associated with inflammation in patients with type 2 diabetes mellitus. BMC Endocr Disord.

2022;22(1):16.

16. Bansal S, Burman A, Tripathi AK. Advanced glycation end products: Key mediator and therapeutic target of cardiovascular complications in diabetes. World J Diabetes. 2023;14(8):1146.

17. Liu L, Chen B, Zhang X, Tan L, Wang DW. Increased cathepsin D correlates with clinical parameters in newly diagnosed type 2 diabetes. Dis Markers. 2017;2017(1):5286408.

18. Guo J, Hu Z, Ren L, Zhao W, Zuo R, Guo S, et al. Circulating tumor necrosis factor-α, interleukin-1β, and interleukin-17A estimates increased major adverse cardiac event risk in acute myocardial infarction patients. J Clin Lab Anal. 2023 Mar;37(5):e24853.

19. Zeng Q, Xu T, Luo Z, Zhou H, Duan Z, Xiong X, et al. Effect of inflammatory factors on myocardial infarction. BMC Cardiovasc Disord. 2024;24(1):538.

20. Guo H, Zhang Q, Yuan H, Zhou L, Li F fang, Wang SM, et al. Nitric oxide mediates inflammation in type II diabetes mellitus through the PPARγ/eNOS signaling pathway. PPAR Res. 2020;2020(1):8889612.

21. Molvin J, Jujić A, Melander O, Pareek M, Råstam L, Lindblad U, et al. Proteomic exploration of common pathophysiological pathways in diabetes and cardiovascular disease. ESC Hear Fail. 2020;7(6):4151–8.

22. Ahmad J, Zubair M, Malik A, Siddiqui MA, Wangnoo SK. Cathepsin-D, adiponectin, TNF-α, IL-6 and hsCRP plasma levels in subjects with diabetic foot and possible correlation with clinical variables: a multicentric study. Foot. 2012;22(3):194–9.

23. Ding L, De Munck TJI, Oligschlaeger Y, Verbeek J, Koek GH, Houben T, et al. Insulin resistance is positively associated with plasma cathepsin D activity in NAFLD patients. Biomol Concepts. 2021;12(1):110–5.