ORIGINAL ARTICLE

CONTENTS

Decline zyxin and CALLY index as predictors of cardiovascular diseases in patients with T2DM

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ABSTRACT

Aim: To find out if a CALLY index and zyxin can be used as new, easy-to-find, and low-cost biomarkers for finding and classifying T2DM people who are more likely to have heart problems like an AMI or stroke early on.

Materials and Methods: A total of 60 T2DM patients (30 male and 30 female), along with 30 healthy controls matched by age and BMI between patients and controls. Various biochemical and inflammatory markers were assessed using ELISA and colorimetric methods. Lipid profiles and atherogenic indices such as CALLY index, metabolic inflammatory index (MII), AIP, CRI-I, and CRI-II index were also calculated.

Results: Results revealed that T2DM patients had significantly higher glucose levels, dyslipidemia, atherogenic indices while decreased zyxin. There was a significant increase in CRP and MII but lymphocyte counts and CALLY index were reduced. Receiver operation characterization (ROC) curve analysis showed that zyxin and CALLY index were the most effective markers for diagnosing cardiovascular risk in T2DM.

Conclusions: T2DM is associated with significant cardiometabolic disturbances characterized by dyslipidemia, chronic inflammation, immune imbalance, and structural protein alterations that increase cardiovascular risk. The significant elevation of MII in T2DM patients ($p < 0.001$), together with reduced zyxin and CALLY index levels, highlights enhanced metabolic and inflammatory stress contributing to endothelial dysfunction and cardiovascular complications. These findings support the potential use of MII, zyxin, and the CALLY index as simple and cost-effective biomarkers for early cardiovascular risk assessment in patients with T2DM.

KEY WORDS: inflammatory, type 2 diabetes, cardiovascular diseases, zyxin and CALLY index

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by high blood sugar levels, which can lead to major problems because it is long-lasting and hard to spot [1]. The major reasons for T2DM are that the body doesn't make enough insulin or can't use the insulin it does make properly .The onset of T2DM is often silent and many years may pass before diagnosis [2]. Many causes of T2DM are a combination of lifestyle factors, genetics, and age [3]. Key risk factors include obesity and physical inactivity, which contribute to the body becoming less responsive to insulin. Genetics and family history also play a significant role, as does increasing age, with the risk rising significantly for people aged 45 and older [4]. About 537 million people between the ages of 20 and 79 had T2DM in 2021. This condition caused 1.6 million deaths, and 47% of all diabetes deaths were before the age of 70 [5]. In Iraq, around 1.4 million of people have diabetes. Reported T2DM prevalence in Iraq ranges from 8.5% (IDF—age adjusted) to 13.9% [6]. Hyperglycemia, dyslipidemia, oxidative stress, and inflammation, common features of T2DM, are recognized cardiovascular risk factors [7]. Cardiovascular diseases (CVDs) are the main cause of death among patients with T2DM [8]. In T2DM, persistent low-grade inflammation is a vital connection between metabolic inefficiency and the onset of CVD. Elevated blood glucose levels induce inflammation, resulting in vascular damage, facilitating atherosclerosis, and heightening the risk of myocardial infarctions, cerebrovascular accidents, and cardiac insufficiency. This inflammatory condition is frequently induced by factors such as obesity and insulin

resistance, resulting in the secretion of cytokines and other pro-inflammatory substances. CVD frequently co-exists with DM, potentially due to the shared risk factors, including atypical inflammatory responses or aberrant lipid metabolism [9]. Recent findings indicate that atherosclerosis is not merely a consequence of T2DM within the context of metabolic syndrome, but also exhibits a comparable pattern of inflammation generated by metabolic stress [10]. Inflammation is regarded as a critical risk indicator for systemic atherosclerosis and is a notable characteristic of vascular problems resulting from diabetes [11]. High blood sugar levels may cause changes in the microvasculature and higher levels of inflammatory substances, such as CRP [12]. The CALLY index measures inflammation, nutrition, and immune system status by looking at serum CRP concentration, serum Albumin concentration, and peripheral lymphocyte count [13]. Zyxin, a part of focal adhesions, may be vital for keeping cardiomyocytes alive. Zyxin has a similar effect on cardiomyocytes, cardiac fibroblasts, and microvascular endothelial cells, which may protect the heart [14]. Zyxin functions as a mechanotransducer in vascular cells, perhaps enhancing cardiomyocyte survival [15].

AIM

Aim of this study is to find out if a CALLY index and zyxin can be used as new, easy-to-find, and low-cost biomarkers for finding and classifying T2DM people who are more likely to have heart problems like an AMI or stroke early on.

MATERIALS AND METHODS

SUBJECT

The present study comprised 60 Arab Iraqi patients with T2DM, consisting of 30 males and 30 females. The average age was 56.8 ± 3.73 years, and the body mass index (BMI) was 26.61 ± 2.51 (kg/m^2). The fasting blood glucose (FBG) was 169.93 mg/dl, and the HbA1c level was 8.4%. The "Al-Najaf Hospital" in Najaf, Iraq, recorded these patients from January to April 2025. Clinical manifestations, symptoms, and biochemical assays were employed for each patient to ascertain their diagnosis of diabetes mellitus. The present investigation excluded participants with any diseases, inflammation, or heart conditions.

CONTROLS

The selection process consisted of thirty people, 15 men and 15 women, all of whom appeared to be in good health. The average BMI of these individuals was 26.07 ± 3.35 and similar age to the patients 55.34±3.11. The workings of the FBG. FBG and HbA1c were measured to have mean values of 92.47±5.6 mg/dl and 5.55±0.45%, respectively. Individuals who were suffering from chronic, systemic disorders and anemia were not allowed to participate in the study.

BIOCHEMICAL RESEARCH

The diagnosis of diabetes was made in accordance with the criteria established by the American Diabetes Association (ADA). This was done based on the patient's medical history, the medication they were currently taking, or both. A HbA1c of 6.5% or a fasting blood glucose level of more than 126 mg/dl (or 7.1 mmol/L) was used to define type 2 diabetes. In accordance with the WHO guidelines, the measurements of height and weight were obtained, and the BMI was determined by applying the formula (weight/(height)^2) (kg/m^2). Consent was given by each patient to the control person. Using two gel tubes and two anticoagulant tubes, the blood was split into two separate tubes. In order to separate the serum from the blood in the gel tube, the blood was centrifuged for five minutes at a speed of four thousand revolutions per minute. After fifteen minutes at room temperature, the serum was transferred into brand-new tubes that were disposable.

INSTRUMENTS FOR EXPERIMENTATION

All analytical procedures were performed under strictly controlled laboratory conditions to minimize environmental interference. The instrumentation listed in Table 1 underwent daily calibration and standardized quality control checks to ensure linear measurement accuracy and high-resolution detection. To maintain the biochemical integrity of the specimens, automated processing was prioritized to reduce human-induced variability and manual handling errors. Furthermore, the integration of high-performance centrifugation and precision thermal incubation protocols ensured that all derived data points and calculated indices were based on stable, non-degraded samples. This rigorous methodological framework supports the analytical validity of the diagnostic thresholds and the statistical precision reported throughout this study.

CHEMICALS

As shown in Table 2, commercially available spectrophotometer and ELISA kits from certified manufacturers

Table 1. Instruments and apparatus for laboratories

Instruments	Company	Origin	Description
Glass gel tube	Q.L.lab	China	Used for sample collection and handling
Micropipette	Dragon	China	Used for accurate measurement and transfer of small liquid volumes
Water bath	Hettich	Germany	Maintains constant temperature for sample incubation
Centrifuge	Hettich	Germany	Separates components based on density by spinning
Refrigerator	Hitachi	Japan	Used for short-term storage of samples at 2–8°C
Deep freezer	Hitachi	Japan	Used for long-term storage at −20°C or −80°C
Spectrophotometer	Spctra721	Taiwan	Measures absorbance to determine concentration of analytes
ELIA Microplate reader	BioTek	USA	Measures optical density in ELISA assays
UniCel DxH 800 analyzer	Beckman	Japan	Automated hematology analyzer for blood cell analysis

Note: The table includes a brief description of each instrument and kit, including their function and principle of measurement
Source: Compiled by the authors of this study

Table 2. Chemical compounds and study kits

Type of Kits	Company/Country	Description
Blood Glucose	Biolabo/ France	Enzymatic colorimetric assay for glucose determination
Total cholesterol	Biolabo/ France	Enzymatic method for cholesterol measurement
Triglyceride	Biolabo/ France	Enzymatic colorimetric method
HDL-c	Biolabo/ France	Direct enzymatic assay for HDL cholesterol
Albumin	Biolabo/ France	Colorimetric assay for serum albumin
HbA1c	BT LAB/ China	ELISA-based assay for glycated hemoglobin
CRP	LTD/Britain	Immunoassay for C-reactive protein detection
Zyxin	BT LAB/ China	ELISA kit for protein quantification

Notes: The calculation for the CALLY index was: albumin concentration (g/dL) × lymphocyte count (10^9/L) ÷ [CRP (mg/dL) × 10] [16].
Lipids and atherogenic indices such as AIP, CRI-I, CRI-II and AC were also calculated.
LDL-C = TC- (TG/5+HDL-C)
VLDL-C = TG/5
CRI-I= TC/HDL-c
CRI-II= LDL-C / HDL-c
AIP= Log (TG/HDL-c)
AC= (TC - HDL-C/HDL-c)
Metabolic inflammatory index (MII)=Platelet*lymphocyte *HbA1c/ albumin
Source: Compiled by the authors of this study

were used for the quantitative determination of the studied biomarkers. All procedures were carried out according to the manufacturers' protocols to ensure the reliability and validity of the obtained results.

STATISTICAL ANALYSIS

The Kolmogorov-Smirnov test was used to examine the distribution types of the results group. The results were expressed for the variable normally distributed, like (mean ± standard deviation). The control and patient groups were compared by using a pooled t-test on the measured parameters. The distinction among groups is considered like different of statistically significant when $p < 0.05$. SPSS Statistics version 26 and IBM-USA performed all statistical analyses. While the numbers were structured using Excel, Microsoft Office 2016.

ETHICAL APPROVAL & INTERNATIONAL COMPLIANCE

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The research protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine of Kufa University prior to the commencement of recruitment. All participants provided written informed consent after being fully briefed on the study's objectives, methodology, and potential risks. To maintain participant privacy, all data were anonymized in strict compliance with local confidentiality regulations and international standards for human subject research.

Table 3. Lipid profile and atherogenic index between patients and controls

Parameters	Patients Mean± SD	Control Mean± SD	p-value
TC [mg/dl]	205.82±33.49	172.67±21.52	<0.001
TG [mg/dl]	230.87±92.58	127.3±44.52	<0.0001
HDL-c [mg/dl]	42.22±5.23	41.83±3.09	0.712
VLDL-c [mg/dl]	46.17±18.51	25.46±8.9	<0.0001
LDL-c [mg/dl]	117.42±32.49	103.37±15.69	0.058
TG/HDL-c	5.53±2.24	3.04±1.02	<0.0001
AIP	0.708±0.103	0.422±0.091	<0.001
CRI-I	5.01±1.03	4.13±0.51	<0.001
CRI-II	2.85±0.92	2.53±0.4	0.017
AC	3.97±1.03	3.13±0.51	0.001

Note: TC: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, VLDL: very low density lipoprotein, and LDL: low density lipoprotein. AIP: atherogenic plasma index AC: atherogenic coefficient, CRI-I: Castelli's Risk Index I, and CRI-II: Castelli's Risk Index II
Source: Compiled by the authors of this study

Table 4. Inflammatory markers between patients and controls

Parameters	Patients Mean± SD	Control Mean± SD	p-value
S.Albumin [g/dl]	4.51±0.36	4.61±0.33	0.225
CRP [mg/dl]	7.17±3.42	2.92±1.85	0.007
Lymph [* 10^9/L]	2.32±1.03	3.70±1.73	0.034
CALLY index	363.27±147.21	629.09± 290.31	<0.001
MII	132.18±62.79	61.85±25.61	<0.001

Note: MII; metabolic inflammatory index.
Source: Compiled by the authors of this study

Table 5. Zyxin parameter between patients and controls

Parameters	Patients Mean± SD	Control Mean± SD	p-value
Zyxin [ng/l]	1579.21±647.48	2081.65±444.99	0.0064

Source: Compiled by the authors of this study

Table 6. ROC curve to zyxin and CALLY index

Test result variable(s)	Cut-off concentration	Sensitivity [%]	Specificity [%]	Area	95% CI of AUC	P-value
Zyxin ng/l	1738.94	77	70	0.785	0.691-0.879	0.000
CALLY index	4273.58	76	69	0.755	0.653-0.858	0.000
MII	74.95	76	70	0.808	0.721-0.896	0.000

Source: Compiled by the authors of this study

RESULTS

All measurements were performed using the laboratory devices shown in Table 1. Some parameters were determined using a spectrophotometer, while other parameters were measured using ELISA kits listed in Table 2.

There is a significant increase in TC, TG, VLDL-c, AIP, CRI-I, CRI-II and AC in Patients than controls. While there is no significant difference in HDL-c, and LDL-c between patients and controls. Table 3 illustrated these lipids and atherogenic index.

In table 4, there is a significant increase in CRP, MII in patients than healthy group. While a significant decrease in lymphocytes and CALLY index in patients compared with controls.

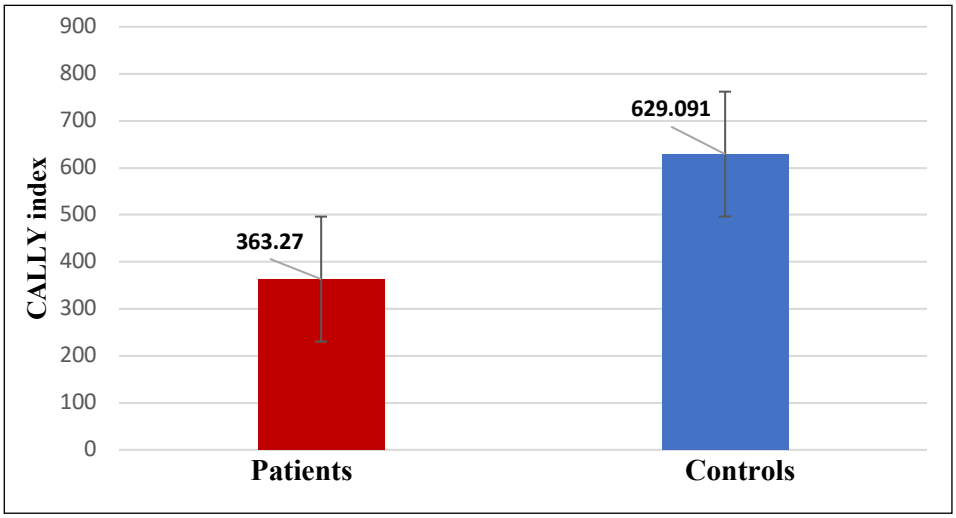

Fig. 1. Explain for comparison between patients and control groups in the CALLY index
Source: Own materials

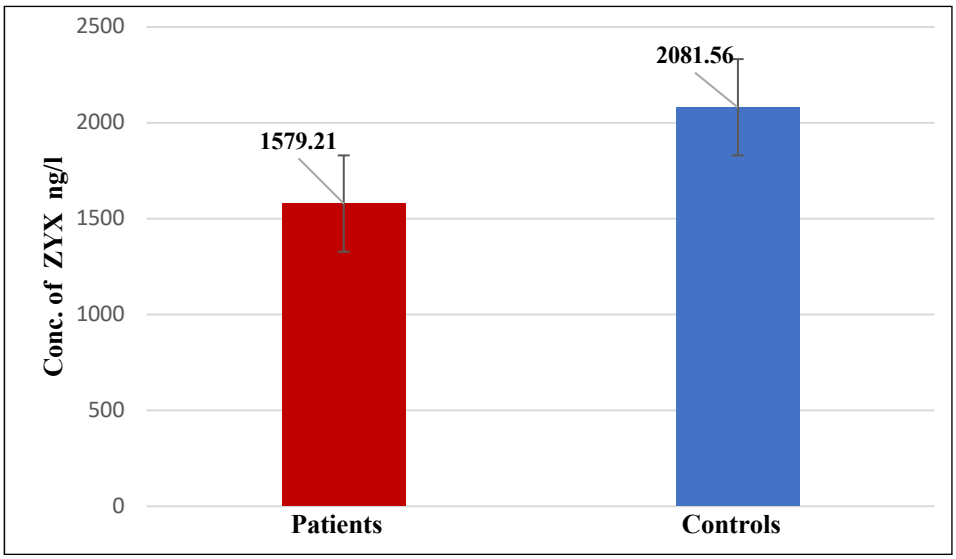

Fig. 2. Explain for comparison between patients and control groups in zyxin
Source: Own materials

The distribution of the CALLY index was compared between the patient group and the control group, as illustrated in Figure 1. The analysis revealed a significantly lower CALLY index in patients compared to healthy controls ($P < 0.001$), suggesting a distinct immunological status between the two cohorts.

There is a significant decrease in zyxin in patients as compared with controls showed table 5.

To investigate the potential of zyxin as a biological marker, its expression levels were compared between the patient group and the healthy control group. As illustrated in Figure 2, a significant decline in zyxin levels was observed in the patient cohort ($P < 0.05$), suggesting its involvement in the pathophysiological mechanism for patients with T2DM.

To determine the diagnostic efficacy and predictive power of both zyxin levels and the CALLY index in identifying patients with T2DM, a combined ROC curve analysis was performed. As illustrated in Figure 3, the AUC for each marker was calculated to compare their respective sensitivity and specificity. This analysis allows for a direct comparison of the clinical utility of these two parameters as potential biomarkers for the disease.

the diagnostic performance of the Metabolic Inflammatory Index (MII) was evaluated independently to determine its efficacy in identifying T2DM cases. As illustrated in Figure 4, a ROC curve was generated, showing the trade-off between sensitivity and specificity. The AUC was calculated to assess the overall accuracy of MII as a standalone metabolic-inflammatory marker

Table 6 showed the ROC curve analysis was performed to evaluated the diagnostic performed of the studies biomarkers. The area under the curve (AUC), sensitivity, and specificity were calculated to determine their ability to discriminate between patients and controls.

DISCUSSION

The number one cause of mortality worldwide is still CVDs. Individuals who suffer from T2DM are at a higher risk of developing cardiovascular diseases such as strokes, heart attacks, and heart failure, as well as peripheral artery dis-

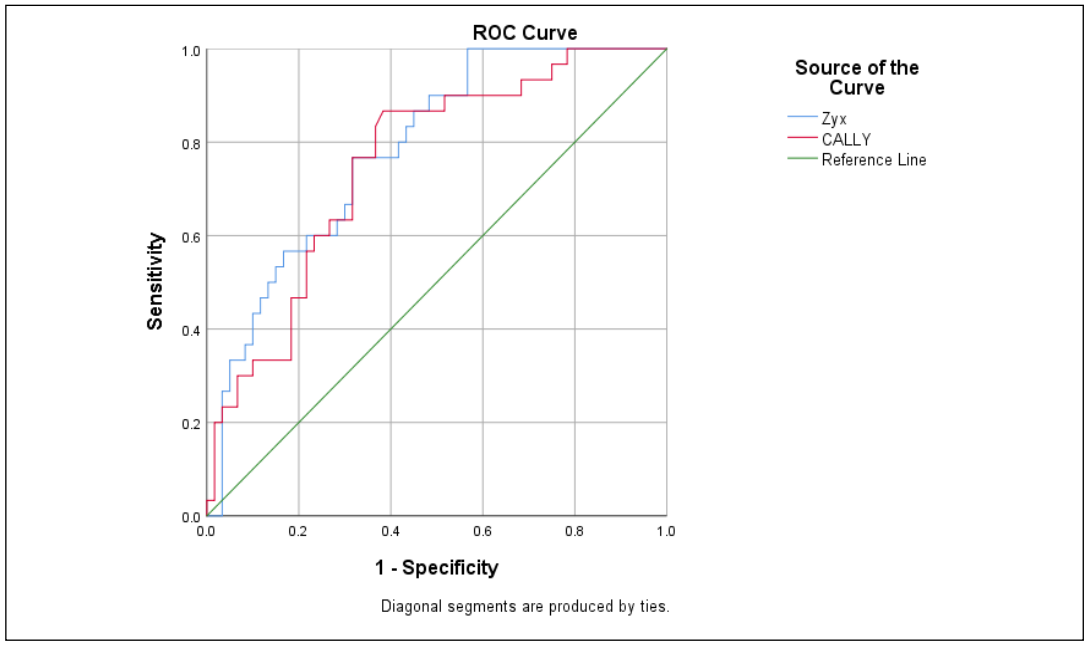

Fig. 3. Explain ROC curve to zyxin and CALLY index
Source: Own materials

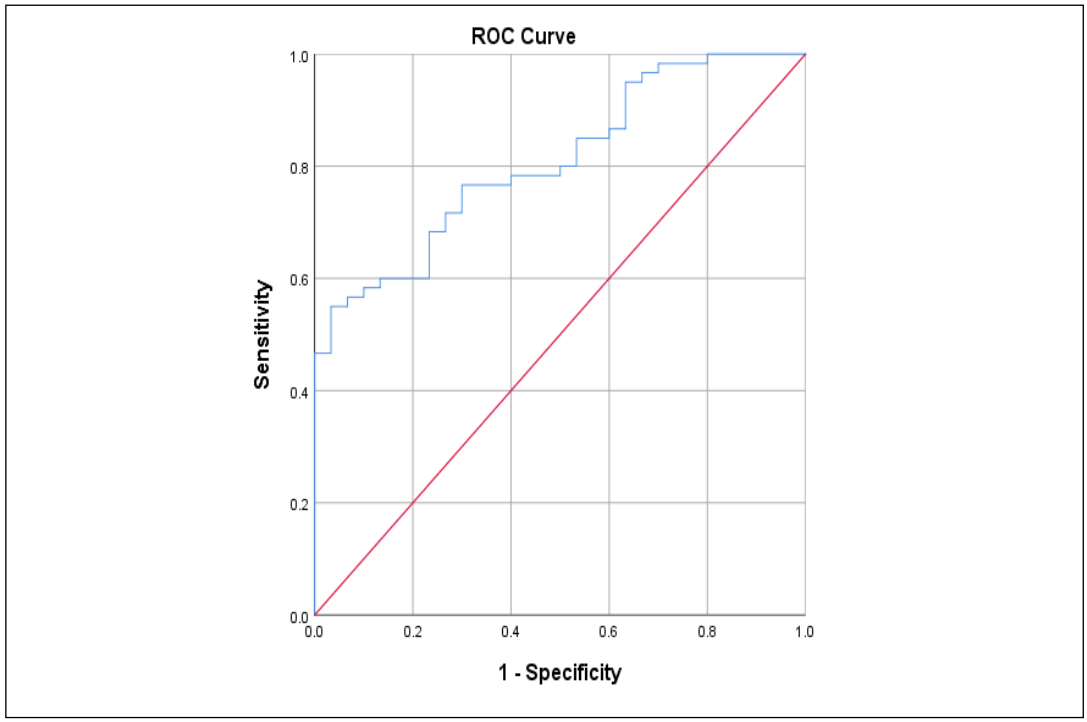

Fig. 4. Explain ROC curve to metabolic inflammatory index
Source: Own materials

ease, compared to individuals who do not have diabetes. This increased risk can be attributed to a number of factors, including insulin resistance, high blood sugar, and abnormal lipid metabolism. These variables are intricately intertwined and have a significant impact on one another. Low-grade chronic inflammation, in particular, is associated with a variety of pathways that contribute to the development and progression of CVD in individuals who have diabetes. In patients with T2DM, the CALLY index and zyxin were evaluated with the purpose of identifying CVD at an early stage. Diabetes and lipid profile are important predictors of metabolic abnormalities, such as dyslipidemia, hypertension, and cardiovascular diseases. The present study found that individuals with diabetes had significantly higher mean serum concentrations of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c) compared to normative values showed in Table 3. This increase is either an insufficient supply of insulin or resistance to insulin, carbohydrates are diverted from muscle glycogen stores and instead used for de novo lipogenesis in the liver, which ultimately leads to an increase in the level of triglycerides in the plasma. When it comes to people who have diabetes, hypertriglyceridemia is the most common lipid condition that is noticed. In addition to this, the oxidation of LDL-c particles results in their increased incorporation into the wall of the artery by way of a receptor-independent mechanism.

This contributes to a rise in the number of people who have diabetes mellitus and also suffer from heart and brain illnesses. The measurement of cholesterol levels in plasma is a crucial factor in determining the probability of cardiovascular events occurring in individuals with diabetes [17]. People with T2DM frequently experience consequences from CVD, and this is particularly noticeable in those who also have other risk factors including obesity and dyslipidemia [18]. In individuals afflicted with T2DM, the occurrence of insulin resistance results in an increase in free fatty acids due to the lipolytic process, which in turn leads to an enhanced release of VLDL-c, including triglyceride [19]. In patients with T2DM, the TG/HDL-c ratio is a significant predictor of CVD risk and can also be used to identify patients at risk of developing T2DM. Elevated TG/HDL-C ratios are associated with increased CVD risk and poorer glycemic control in T2DM [20]. AIP outperforms standard lipid indicators in predicting CVD risk in T2DM patients, and elevated AIP is associated with an increased risk of major adverse cardiovascular events. AIP can be used as a risk stratification and prognosis tool, and its integration into clinical assessments could guide more targeted interventions [21]. Atherogenic Coefficient (AC), Castelli's Risk Index I (CRI-I), and Castelli's Risk Index II (CRI-II) are lipid ratios used to evaluate the risk of cardiovascular disease (CVD), especially in people with T2DM. These indices can be useful in identifying those who are at higher risk for CVD, and elevated levels of them are linked to increased risk. Since diabetes predicts a very high risk of coronary heart disease, it is not surprising that our T2DM patients have raised AIP and CRI I, CRI II, and AC cardiovascular markers. AIP and CRI I, CRI II, and AC have been found to significantly correlate in one study. These indicators show that the coronary arteries have a significant density of plaque [22]. Consequently, their increased prevalence among research participants suggests a significant risk of CAD that requires immediate attention. Dyslipidemia, recognized as a metabolic disorder, is often correlated with diabetes mellitus. Disturbances in lipid metabolism have been documented in individuals diagnosed with diabetes mellitus, which is concomitant with an elevated risk of cardiovascular atherosclerosis [23]. As established risk factors for CVD in this population.

CRP, lymphocyte, CALLY index and MII significantly differ between T2DM patients and controls Table 4. Chronic inflammation is a major factor in the onset and advancement of CVD in people with type 2 diabetes. In those with type 2 diabetes, elevated levels of inflammatory markers such as CRP are linked to an increased risk of CVD. This inflammation damages blood vessels and encourages the accumulation of plaque, which leads to atherosclerosis, a leading cause of CVD. The liver produces the protein known as CRP in reaction to internal inflammation. Active inflammation is indicated by a higher level of this protein in the circulation [22]. It is strongly linked with heart disorders since it serves as an indirect indicator of chronic inflammation in blood vessels, a contributing factor to atherosclerosis and coronary heart disease [24]. The correlation between CRP and cardiac health CVD, particularly atherosclerosis, involve not only the accumulation of lipids in the arteries but also represent chronic inflammatory disorders impacting the vascular walls [25]. According to this study, compared to non-diabetic individuals, diabetes patients had a higher maximal CRP, a lower minimal lymphocyte count, and a declining CALLY index. For the diagnosis and treatment of cardiovascular diseases, lymphocytes are essential. Their altered levels indicate inflammatory or immunological abnormalities that could promote the progression of the disease [26]. Lymphocytes are a category of leukocytes that facilitate adaptive immunity. They are categorized as T cells, which are responsible for cellular immunity. B cells generate antibodies, while Natural Killer (NK) cells directly target infected or tumor-like cells. Lymphocytes comprise around 20–40% of the overall leukocyte population [27]. Lymphocytes are a specialized category of white blood cells that are essential to the body's immunological response, particularly in recognizing and eliminating pathogenic organisms such as viruses and bacteria. Any change in their quantity may signify inflammation or immunological malfunction [28]. In T2DM, a decrease in lymphocyte count with increased risk of CVD. According to a study in the National Institutes of Health (NIH) on patients with T2DM both very low lymphocyte has been linked to higher cardiovascular mortality risk. The medical perspective on these disorders has progressed to recognize the immune system's function, especially chronic inflammation, as a crucial aspect in their etiology [29]. Lymphocytes are a prominent component of the immune system, actively participating in inflammatory and immunological responses inside the cardiovascular system [30]. Atherosclerosis is a persistent inflammatory condition in which lymphocytes are pivotal. T cells secrete cytokines that exacerbate the destabilization of atherosclerotic plaques, heightening the risk of problems.

CRP, albumin levels, and lymphocyte count are all combined to create the CALLY index, a composite biomarker. A greater CALLY index that shown in Figure 1 is strongly linked to a lower risk of cardiovascular and all-cause mortality in people with CVD, according to research. For cardiovascular risk stratification, the CALLY index could be a helpful tool. In this study decrease CALLY index in T2DM patients that indicator to more exposure patients to CVD than healthy people this result explained in figure 1. On the other hand, other study used CALLY index to deliver a thorough evaluation of a patient's with T2DM systemic inflammatory status,

nutritional state, and immunological competence [31]. A higher CALLY score indicates stronger overall health state of the patient [32]. Other study consider it may function as a protective factor or prognostic indicator in cardiovascular patients the CALLY index proves to be a straightforward and economical solution. A low CALLY index typically signifies elevated inflammation (high CRP), deficient nutritional status (low albumin), or immunological suppression (low lymphocytes), indicates an intensified inflammatory condition and compromised physiological resistance all of which are detrimental prognostic indicators in cardiovascular illnesses [33]. Chronic hyperglycemia in diabetes induces a persistent inflammatory and immune dysregulation state, characterized by elevated HbA1c, increased PLR, and reduced albumin levels. The integration of these parameters into MII suggesting enhanced metabolic and inflammatory imbalance associated with diabetic progression. This elevation may reflect increased cardiometabolic stress and supports the role of MII as a potential indicator of disease severity and cardiovascular risk in individuals with T2DM.

In Table 5 shown there is a significant decline in zyxin level ($p<0.05$) in T2DM patients than controls. This result explained Figure 2. Zyxin, a focal adhesion protein, plays a crucial role in various aspects of CVD and it is a mechanosensitive protein that facilitates cellular responses to mechanical stress, the regulation of the cytoskeleton and cellular motility playing an essential role in heart shape and communication pathways [34]. It appears to be involved in protecting against hypertension-induced cardiac dysfunction, potentially by promoting cardiomyocyte survival and inhibiting fibrosis. Additionally, zyxin is implicated in vascular repair and endothelial migration, suggesting a role in managing vascular injuries associated with CVD. Depending on these reasons, decrease zyxin in patients. Based on these scientific facts, patients in this study are more likely to develop cardiovascular diseases than healthy individuals, and these diseases can be predicted before symptoms appear, and their occurrence can be reduced. One possible reason for the decrease in Zyxin among patients with T2DM is the inefficiency of the heart muscle function due to diabetes. Consequently, the heart muscle cannot produce Zyxin protein, depending on the finding of the following research in circumstances such as heart failure or myocardial infarction, the heart may diminish its capacity to synthesis sufficient Zyxin, resulting in inadequate tissue regeneration and increased fibrosis [35]. As individuals age, Zyxin levels progressively diminish in small arteries, compromising their capacity to endure strain and heightening the likelihood of cardiovascular disease [15]. Therefore, the ages of patients and healthy individuals were taken to be identical or close to avoid such reasons, which is the effect of increasing age on the zyxin protein. Other study investigation has examined the function of zyxin in cardiac muscle activity. zyxin is essential for preserving optimal muscle architecture, cardiac growth, and functionality. Zyxin positively influences cardiomyocytes, and it lack may result in cardiomyocyte death and excessive fibrosis, thereby impairing cardiac function [36]. Recent research indicates that zyxin shortage in the heart results in detrimental effects, especially in hypertension situations. The deficiency of this protein enhances cardiomyocyte apoptosis, diminishing the heart's contractile capacity. Furthermore, research with genetically modified animals deficient in the zyxin gene has demonstrated that vascular smooth muscle cells maintain their contractile nature, hence inhibiting vascular remodeling [15, 37]. Zyxin modulates the expression of genes associated with fibrosis, and its deficiency may result in excessive collagen accumulation and cardiac fibrosis [34].

The diagnostic evaluation of Zyxin, the CALLY index, and MII demonstrates that all three parameters are highly significant biomarkers for the target condition ($p < 0.001$) illustrated in Table 6. Figure 3 shown that MII emerged as the most superior indicator, yielding an AUC of 0.808 (95% CI: 0.721–0.896), which signifies "excellent" discriminative ability according to standard diagnostic criteria. Figure 4 shown that zyxin and the CALLY index also provided robust results with AUC values of 0.785 and 0.755, respectively, indicating their reliability in clinical stratification. However, the relatively moderate specificity across all three markers (ranging from 69% to 70%) suggests that while these biomarkers effectively identify affected individuals, there is a consistent ~30% risk of false positives. Given that the 95% confidence intervals for all variables remain well above the 0.50 null-hypothesis threshold, these findings suggest that incorporating these biomarkers - particularly the MII - into a multi-parametric diagnostic model could significantly enhance overall clinical precision and predictive accuracy.

CONCLUSIONS

Type 2 diabetes mellitus is associated with a pronounced cardiometabolic derangement characterized by atherogenic dyslipidemia, systemic low-grade inflammation, immune dysregulation, and altered structural protein expression. Elevated TC, TG, LDL-c, and increased atherogenic indices (AIP, CRI-I, CRI-II, and AC) reflect enhanced hepatic *de novo* lipogenesis, increased VLDL secretion, impaired lipid clearance, and intensified LDL oxidation, collectively promoting endothelial dysfunction and atherogenesis. In addition, the significant increase in MII observed in T2DM patients further indicates aggravated metabolic and inflammatory imbalance contributing to cardiovascular risk progression.

Concomitantly, elevated CRP, reduced lymphocyte count, and a decreased CALLY index indicate persistent activation of inflammatory signaling pathways, impaired immunological homeostasis, and compromised protein synthetic ca-

pacity, all of which contribute to vascular injury and plaque instability. The significant reduction in zyxin levels further suggests disruption of focal adhesion signaling, cytoskeletal integrity, and mechanotransduction in cardiomyocytes and vascular smooth muscle cells, potentially facilitating adverse cardiac remodeling and fibrosis.

Together, these biochemical and molecular alterations underscore the multifactorial pathophysiology of cardiovascular risk in T2DM and support the combined use of lipid-derived indices, inflammatory biomarkers, MII, the CALLY index, and zyxin as integrated tools for early cardiovascular risk assessment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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