Evaluation of Oxidative Stress in Iraqi Male Patients with Myocardial Infarction and Type 2 Diabetes Mellitus

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Abstract
One of the most prevalent illnesses in developing countries is myocardial infarction (MI), which develops when the heart's blood supply is suddenly interrupted and causes tissue damage. It is connected to several metabolic risk factors, including diabetes, hypertension, and obesity. The objective of this study was to assess how oxidative stress (OS) contributed to the pathophysiology of MI and T2DM. The current study examined 152 male samples, including 52 MI patients without T2DM (G1), 50 MI patients with T2DM (G2), and 50 seemingly healthy men as controls (C). The levels of fasting blood sugar (FBS), C-reactive protein (CRP), total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), malondialdehyde (MDA), and total peroxidase activity were assessed. Based on the results, CRP levels increased significantly in both the G1 and G2 groups compared to the C group, with G2 reporting greater significant increases than G1. Both the G1 and G2 groups considerably exceeded the C group in terms of TOS, OSI, MDA, and total peroxidase activity. In contrast to the C group, the TAS was found to be much lower in the G1 and G2 groups. Additionally, there were no noticeable variations in the TOS, TAS, OSI, MDA, or total peroxidase activity between the G1 and G2 groups. According to our findings, individuals with MI and T2DM had an increased OS and a diminished antioxidant system. Consequently, OS may be crucial to the pathophysiology and prognosis of MI and T2DM.

Keywords: Myocardial infarction, Oxidative stress, Type 2 diabetes mellitus, C-Reactive Protein, Malondialdehyde

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بِاحتشاء عضلة القلب و داء السكري من النوع الثاني، تم فحص 152 عينة من الذكور في الدراسة الحالية وشملت 52 مريضاً بِاحتشاء عضلة القلب دون داء السكري من النوع الثاني (G1) و 50 رجلاً بدون انهم يتمتعون بصحة جيدة كمجموعة سيطرة (C). تم تقييم مستويات السكر في الدم أثناء الصيام (FBS)، والبروتين التفاعلي C (CRP)، وحالة الكلية (TAS)، وحالة مضادات الأكسدة الكلية (TOS)، والأكسدة الكلية (TOS)، والقدرة على مكافحة أكسدة النيتروجين (MDA) وظائف البيروكسيداز الكلية. بناءً على النتائج، زادت مستويات CRP بشكل ملحوظ في كلا المجموعتين G1 و G2 من خلال مقارنة مع مجموعة C. وجد أن G2 عانت بشكل ملحوظ من الالتهابات الكلية في كلا المجموعتين G1 و G2، وظائف البيروكسيداز الكلية في كلا المجموعتين G1 و G2 قليلة بشكل ملحوظ. G2 G1 من خلال مقارنة مجموعات TAS وTAS وTAS، وظائف البيروكسيداز الكلية، والقدرة على مكافحة أكسدة النيتروجين. إلى ذلك، لم تكن هناك فروقات ذات دلالة إحصائية في TAS وTAS وTAS، وظائف البيروكسيداز الكلية، والقدرة على مكافحة أكسدة النيتروجين، وتلك التي يكون في النهاية نتيجة زيادة في الالتهابات الكلية لدى المرضى الذين يعانون من احتشاء عضلة القلب و داء السكري من النوع الثاني مع انخفاض في نمط مضادات الأكسدة. وبالتالي، قد يلعب الالتهابات الكلية دورًا مهمًا في التنبؤ بالإصابة بمرض احتشاء عضلة القلب و داء السكري من النوع الثاني والتكهن به.

1. Introduction:
A myocardial infarction (MI), commonly known as a heart attack, happens when the blood supply to a portion of the heart is stopped or reduced, damaging the heart muscle [1]. The most typical symptom is chest pain or discomfort, which can also occur in the shoulder, arm, back, neck, or jaw [2]. It usually lasts for several minutes and affects the left or center of the chest. It’s possible that the discomfort will occasionally feel like heartburn. Other symptoms and indicators include fatigue, shortness of breath, nausea, dizziness, and faintness [3]. Coronary artery disease is the main cause of MIs (CAD). High blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor nutrition, and excessive alcohol use are only a few of the risk factors. The primary cause of a MI is typically a rupture of an atherosclerotic plaque, which results in a complete blockage of a coronary artery [4,5]. The most frequent cause of MI is atherosclerotic disease of an epicardial coronary artery (or arteries) severe enough to result in insufficient perfusion of the myocardium supplied by the affected coronary artery and a local decrease in myocardial blood flow [6].

Blood glucose testing can be used to identify the illness known as diabetes mellitus (DM), which is characterized by inefficient carbohydrate metabolism. Type 1 diabetes (T1DM), type 2 diabetes (T2DM), gestational diabetes, and monogenic diabetes are the many kinds of DM [7]. The blood vessels, kidneys, eyes, nerves, heart, and nerves are the key organs that chronic hyperglycemia is associated with that result in long-term dysfunction and organ failure [8]. Untreated T2DM patients had much higher rates of peripheral vascular disease (PVD), stroke, and coronary artery disease (CAD) than the general population [9].

Along with DM, oxidative stress (OS) is a key factor in the development of MI. The two main types of reactive species are reactive oxygen species (ROS) and reactive nitrogen species (RNS) [10]. All aerobic cells have the capacity to generate RONS (reactive oxygen and nitrogen species) [11]. Numerous macromolecules, including lipids, proteins, and nucleic acids, are damaged as a result of RONS overproduction and/or a decreased capacity to repair or neutralize them. Thus, an imbalance between the generation and elimination of RONS leads to OS [10]. The most precise way for estimating the severity of OS is the oxidative stress index (OSI). The ratio between the total oxidant status (TOS) and total antioxidant status (TAS) values in blood samples was used to calculate it [12]. Malondialdehyde (MDA), a byproduct of long-chain fatty.
Acid peroxidation, is a significant OS biomarker [13]. MDA, which is produced by the peroxidation of polyunsaturated fatty acids, is the biomarker of lipid peroxidation and OS that has been the subject of most research [14].

One of the most often employed indicators for inflammation and cardiovascular (CV) risk is C-reactive protein (CRP), which has been linked to endothelial dysfunction, prothrombotic states, atherosclerotic plaque remodeling, and destabilization [15,16]. Furthermore, in individuals with CAD, elevated CRP levels are linked to atherosclerotic load and significant adverse cardiovascular events [17].

Therefore, the objectives of the current study are to assess OS's contribution to the pathophysiology of MI and T2DM along with the potential for using it as a biochemical marker for these conditions and their development.

2. Samples and Methods
2.1 Patients and Control Subjects
The 102 male patients with recently diagnosed MI participated in the research. According to the presence or absence of T2DM, the MI patients were divided into two groups: G1 (MI patients without T2DM, n=52 aged between 43 and 63 years), and G2 (MI patients with T2DM, n=50 aged between 48 and 65 years). The Ibn Al-Bitar Hospital and Ibn Al-Nafees Hospital for Cardiac Surgery in Baghdad City were used to select all patients. Additionally, 50 healthy participants with ages ranging from 40 to 61 were included in this study as the control (C group) for comparison.

2.2 Exclusion Criteria
Alcohol consumption, smoking, use of antioxidant supplements, and other disorders such as kidney and liver disease, active inflammatory conditions, and malignancies that may interfere with this study were the exclusion criteria for patients and controls.

2.3 Blood Samples
Each participant fasted for 8-12 hours before having 5 ml of venous blood taken from them. The blood samples were centrifuged at 3000 rpm for 10 minutes after being allowed to clot at room temperature, and they were then kept at -20 °C until they were utilized for analysis.

2.4 Methods
Fasting blood sugar (FBS) was measured by a glucose oxidase kit (Linear Chemicals, Spain). The CRP Titer was measured by the automated biochemistry analyzer Gesan Chem 200 (Italy). Both TOS and TAS were assessed using the Erel's methods [18,19] respectively, in serum specimens from patients and control. The findings were expressed for TOS as (micromolar hydrogen peroxide equivalent per liter; μmol H₂O₂ Eq./L) and for TAS as (millimolar glutathione per liter; mmol glutathione Eq./L). The OSI, a measure of OS severity, was computed using the following formula [20]:

\[ \text{OSI} = \frac{\text{TOS (mmol H}_2\text{O}_2 \text{ Eq.}/\text{L})}{\text{TAS (mmol glutathione Eq.}/\text{L})} \]

Thiobarbituric acid (TBA) was used as the active component in a precipitation process to measure the amount of MDA in the serum [21]. The MDA result was given in (nmol/ml), and the MDA molar extinction coefficient was 1.52×10⁵ M⁻¹cm⁻¹.

The activity of peroxidases was measured using phenol, 4-aminoantipyrine (4-AA), and hydrogen peroxide as dye-generating chemicals [22]. The dissociation of H₂O₂ per time was used to evaluate the activity of peroxidases.

2.5 Statistical Analysis
The data were displayed in mean± standard deviation (±SD), and analyzed by SPSS (Version 22). In order to compare the parameters among the groups, a one-way analysis of variance (ANOVA) was used. The difference between groups was defined to be statistically highly significant if the $p<0.01$, significant if the $p<0.05$ and non-significant if the $p>0.05$. Pearson correlation coefficient was used to determine the correlations between variables. The receiver operating characteristic curve (ROC) was used to determine a diagnostic test's optimal specificity and sensitivity.

3. Results

Figure 1 shows the FBS levels for patients and controls. The findings revealed no significant differences between the G1 and C groups, however the amount of FBS was significantly higher in the G2 group when compared to the C and G1 groups.

The significant difference when *$p < 0.05$, **$p < 0.01$. The small letters a: significant difference between C with G1 and G2, b: significant difference between G1 and G2.

CRP levels increased significantly for both patient groups (G1 and G2) as compared to the control, as indicated in Table 1, and significantly varied across the G1 and G2 groups. Table 1 displays the levels of TOS and OSI in G1, G2 and C groups. The TOS and OSI levels in G1 and G2 groups were significantly higher when compared to the C group. In contrast to their level in the C group, there was a highly significant decline in the serum TAS levels in the G1 and G2 groups. Additionally, no discernible variations in TOS, TAS, and OSI levels between G1 and G2 were seen.
Table 1: The comparison of biochemical parameters in patients and control groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (n=50)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.451 ± 0.23</td>
</tr>
<tr>
<td>TOS (μmol/L)</td>
<td>30.14 ± 3.25</td>
</tr>
<tr>
<td>TAS (mmol/L)</td>
<td>2.154 ± 0.098</td>
</tr>
<tr>
<td>OSI</td>
<td>0.014 ± 0.001</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>6.783 ± 1.106</td>
</tr>
<tr>
<td>Peroxidase activity (U/L)</td>
<td>7.292 ± 4.41</td>
</tr>
</tbody>
</table>

The significant difference when *p < 0.05, **p < 0.01. The small letters a: significant difference between C with G1, b: significant difference between C and G2, c: significant difference between G1 and G2.

The results in Table 1 showed a significantly considerable increase in serum MDA levels for both patient groups (G1 and G2) as compared to the C group. Furthermore, the MDA levels across the G1 and G2 groups did not show any obvious differences. Additionally, the levels of peroxidase activity in the G1 and G2 groups were much greater than those in the C group. The G1 and G2 groups were not really all that different from one another. Tables 2 and 3, for the G1 and G2 groups, respectively, show the results of the correlation analysis between all examined parameters. Table 2 results show that there is a highly substantial positive association between TOS and OSI, TOS and peroxidase activity, and (OSI and peroxidase activity). Furthermore, there is a very strong association between (TOS and MDA). In the G1 group, the results showed a very significant negative correlation between (TAS and OSI) and a strong negative correlation between (FBS and MDA).

Table 2: Pearson correlation coefficients between the biochemical parameters in G1 group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CRP</th>
<th>TOS</th>
<th>TAS</th>
<th>OSI</th>
<th>MDA</th>
<th>Peroxidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS</td>
<td>-0.050</td>
<td>-0.044</td>
<td>-0.177</td>
<td>0.016</td>
<td>-0.277*</td>
<td>0.139</td>
</tr>
<tr>
<td>CRP</td>
<td>0.171</td>
<td>-0.020</td>
<td>0.178</td>
<td>0.125</td>
<td>0.180</td>
<td></td>
</tr>
<tr>
<td>TOS</td>
<td>-0.111</td>
<td>0.789**</td>
<td>0.321*</td>
<td>0.471**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAS</td>
<td>-0.650**</td>
<td>0.172</td>
<td></td>
<td>-0.185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OSI</td>
<td></td>
<td>0.180</td>
<td></td>
<td>0.502**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td></td>
<td></td>
<td></td>
<td>0.056</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*.Correlation is significant at the 0.05 level
**.Correlation is significant at the 0.01 level

In G2 group, a highly significant positive correlation between (TOS and OSI), (TOS and MDA), and (OSI and MDA) were found. Moreover, TAS and OSI showed a highly significant negative correlation, as shown in Table 3.
Table 3: Pearson correlation coefficients between the biochemical parameters in G2 group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CRP</th>
<th>TOS</th>
<th>TAS</th>
<th>OSI</th>
<th>MDA</th>
<th>Peroxidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS</td>
<td>0.078</td>
<td>0.084</td>
<td>-0.126</td>
<td>0.111</td>
<td>-0.145</td>
<td>-0.007</td>
</tr>
<tr>
<td>CRP</td>
<td>0.167</td>
<td>0.081</td>
<td>0.050</td>
<td>0.070</td>
<td>0.098</td>
<td></td>
</tr>
<tr>
<td>TOS</td>
<td>-0.197</td>
<td>0.889**</td>
<td>0.538**</td>
<td>0.045</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAS</td>
<td>-0.562**</td>
<td>-0.262</td>
<td>0.111</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OSI</td>
<td>0.555**</td>
<td>0.028</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.147</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level  
**Correlation is significant at the 0.01 level

The ROC curves analysis for CRP, TOS, OSI, MDA and peroxidase activity to (control and G1 groups) and (control and G2 groups), are presented in Figure 2 and Table 4.

Figure 2: The ROC curves between (A) control and G1 groups, and (B) control and G2 groups.
Table 4: The ROC curve analysis for (A) control and G1 groups, and (B) control and G2 groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Test Result Variable(s)</th>
<th>Area under the curve (AUC)</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Cut off value</th>
<th>Area under the curve (AUC)</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Cut off value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CRP (mg/L)</td>
<td>1.000</td>
<td>100</td>
<td>100</td>
<td>8.07</td>
<td>1.000</td>
<td>100</td>
<td>100</td>
<td>8.44</td>
</tr>
<tr>
<td>B</td>
<td>CRP (mg/L)</td>
<td>0.970</td>
<td>94</td>
<td>92</td>
<td>36.19</td>
<td>0.993</td>
<td>96</td>
<td>98</td>
<td>36.67</td>
</tr>
<tr>
<td></td>
<td>TOS (μmol/L)</td>
<td>1.000</td>
<td>100</td>
<td>100</td>
<td>0.022</td>
<td>1.000</td>
<td>100</td>
<td>100</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>OSI</td>
<td>0.963</td>
<td>98</td>
<td>94</td>
<td>8.78</td>
<td>0.966</td>
<td>100</td>
<td>96</td>
<td>9.06</td>
</tr>
<tr>
<td></td>
<td>MDA (nmol/ml)</td>
<td>0.763</td>
<td>89</td>
<td>64</td>
<td>7.07</td>
<td>0.716</td>
<td>82</td>
<td>60</td>
<td>6.83</td>
</tr>
<tr>
<td></td>
<td>Peroxidase activity (U/L)</td>
<td>0.763</td>
<td>89</td>
<td>64</td>
<td>7.07</td>
<td>0.716</td>
<td>82</td>
<td>60</td>
<td>6.83</td>
</tr>
</tbody>
</table>

4. Discussion

As the global population ages and obesity rates rise, diabetes mellitus (DM), a prevalent public health problem, will become more common [23]. Diabetes patients have a higher risk of developing CV issues, including MI, which is the primary cause of death in those with T2DM [24]. The main causes of CV issues in diabetics are the formation of atheroma plaque and ventricular dysfunction [25]. According to the findings in Figure (1), group G2 had a considerably greater FBG than groups G1 and C. These results support earlier research by Peng et al. [26]. Al-Musawi et al. observed that individuals with DM had poor glycemic control and that variable FBG levels may contribute considerably to OS, maybe even more so than chronic hyperglycemia [27].

Atherosclerosis, endothelial cell dysfunction, glycosylation of extracellular matrix proteins, and vascular denervation are further conditions that are accelerated by high blood glucose levels and are all caused by vascular injury, mostly to the vulnerable coronary arteries. Therefore, ongoing vascular damage results in inflammation, stiffness, stenosis, thrombosis, or even rupture of the coronary arteries, which results in MI [28].

A blood test called CRP is used to determine if the body is infected or inflamed, and the fact that it is a strong indicator of CV events supports the argument that it is a more accurate predictor of the risk of CV events than LDL cholesterol [29]. In addition, in the short time following a MI, inflammation has been shown to significantly speed up the formation of atherosclerotic plaque [30]. It has been previously mentioned [31] that fatal coronary artery lesions exhibit a link between serum CRP and histology CRP staining. The results of this study are consistent with prior studies [32,33] in that they demonstrate a highly significant rise in CRP levels for both patient groups (G1 and G2) when compared to the C group. Determining the level of blood antioxidants and OS markers as a diagnostic and prognostic tool for the medical management of MI is essential given the imbalanced oxidative state and antioxidant activity in MI patients [34]. Biomarkers for OS and antioxidants may be useful predictors or indicators of the risk of MI. OS markers have an impact on the aetiology of MI, and too much ROS make antioxidants unstable [35]. The current research supported Bhat and Gandhi’s findings, who investigated serum TOS in MI patients and discovered that TOS was substantially larger in the MI patient groups than in the control group [36]. Patients with MI had greater TOS levels than the control group, according to Aksoy et al. [37]. Free radical generation is normally regulated by antioxidants. Antioxidants have the ability to stabilize or inactivate free radicals before they cause harm to the various cell components [38]. The findings
in Table 1 above were in line with other studies, including those by Gökdemir et al. who examined TAS in MI patients and found a significant decrease in the patient group compared to that of healthy participants [39] and Shahzad et al. who also found a decrease in TAS in MI patients [40].

It's interesting that the OSI is the best tool for determining the extent of OS [41]. The current findings are consistent with studies by Gökdemir et al. that showed an increase in OSI in MI patients' serum [39] and Shahzad et al. that indicated that CAD patients had higher OSI values than healthy individuals [40]. Serum TAS, TOS, and OSI levels are indicators of the redox balance between oxidant and antioxidant state [42]. In the present investigation, researchers investigated these OS markers in MI patients and discovered a significant association between higher OSI levels, decreased TAS, and MI progression. The OSI and TAS increased across the board for all of the patient groups that were evaluated. According to a report by Shahzad et al., the etiology of MI was associated with increases in OSI and a decrease in TAS, which is consistent with our findings [40]. As a result, OS plays a significant role in the genesis of several chronic diseases, including diabetes and cardiovascular disease [43].

MDA, however, is a useful marker of OS and lipid peroxidation. Monitoring MDA levels in various biological systems can therefore be used to detect lipid peroxidation both in vitro and in vivo for a variety of chronic human diseases [14]. The results of this study are in line with a number of previous investigations that revealed higher blood MDA levels in MI [44] and T2DM [45]. All main groups of biomolecules are damaged by the chemically reactive free radicals, with lipids most likely being the most at risk. Cell membranes contain a lot of polyunsaturated fatty acids, which are easily harmed by oxidizing radicals [46]. The oxidative damage inflicted by free radicals to polyunsaturated fatty acids is referred to as lipid peroxidation and OS. Rising OS levels are crucial to the etiology and development of MI as well diabetes [43].

It should be noted that the study's findings indicated that MI patients with T2DM had a higher level of MDA than MI patients without T2DM. Increased levels of free radicals in cells caused by hyperglycemia can result in OS and the production of ROS or RNS. Due to an increase in free radical levels that leads to an increase in the synthesis of MDA, one of the byproducts of peroxidation, the generated OS accelerates up the development and progression of diabetes as well as the peroxidation of cell membrane lipids [47]. Therefore, the higher MDA levels may be primarily caused by an increase in free radicals and OS in DM. Patients with T2DM are typically twice as likely to get MI as people without DM [48]. A study by Kitano et al. found that DM had more oxygen free radicals than non-DM [49]. Because ROS attack cell membranes and boost MDA levels, growing OS also entails rising ROS levels [13]. When compared to healthy individuals, MDA concentrations were significantly greater in the group of MI patients who had T2DM. Our results were consistent with research conducted by Saad et al., who noted that MDA increased in MI patients with a notable elevation in the diabetic group when compared to the non-diabetic and healthy control groups [50]. Another study by Mahreen et al. [51] also found an elevated MDA levels in diabetics with MI than healthy control groups. The possible reason for significantly high MDA levels in diabetic with MI cases is due to tissue damage caused by MI resulting in increased rate of free radicals production as assessed by lipid peroxidation. According to previous study who claimed that measuring MDA is a one of the suitable marker of radical stress of the acute myocardial infarction (AMI) [52].

A wide class of enzymes known as peroxidases is crucial to many biological processes. It contributes to the pathophysiology of various illnesses, innate immunity, hormone production, and the oxidation of ROS. According to several suggested pathways, various prevalent illnesses
including cancer, cardiovascular disease, and diabetes are directly or indirectly linked to variable expressions of peroxidases. As a result, the status of peroxidases can be employed as a diagnostic for many disorders [53]. In our investigation, researchers observed higher peroxidase activity in both the G1 and G2 groups when compared to the control, which may be related to increased ROS production, as demonstrated by the rise in TOS, MDA, and OSI.

The ROC curve is a graph that shows, as the discrimination threshold of a double classifier system is changed, how diagnostically capable it is. ROC test for CRP and OSI markers showed an excellent AUC=1.00 for both groups with 100% sensitivity and 100% specificity indicating that CRP and OSI considered as a perfect diagnostic marker. While, the ROC test for TOS and MDA markers showed a good AUC (0.970, 0.993, 0.963 and 0.966) with sensitivity (94%, 96%, 98% and 100%) and specificity (92%, 98%, 94% and 96%), respectively; indicating that TOS and MDA considered as a good diagnostic marker. Meanwhile, ROC test for peroxidase activity marker showed fair AUC (0.763 and 0.716) for both groups (MI without T2DM) and (MI with T2DM) with sensitivity (89% and 82%) and specificity (64% and 60%) respectively; indicating that peroxidase activity considered as a fair diagnostic marker.

Conclusions
The recent findings clearly showed an increase in OS in patients with MI and T2DM along with a decrease in antioxidant levels in these patients, demonstrating that OS is crucial to the pathophysiology and development of MI in T2DM patients. The ROC curve results indicate that CRP, TOS, OSI, and MDA may aid in the diagnosis of MI patients with and without T2DM. Our results suggest that the modulation of such indicators may be therapeutic in upcoming MI and DM preventative strategies.

Ethical Clearance
This research was ethically approved by the Research Ethical Committees of the College of Sciences/University of Baghdad, Iraq.

Conflict of Interest
The authors declare that they have no conflict of interest.

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