

Evaluation of Serum Myeloperoxidase and Malondialdehyde Levels as Biomarkers for Complications in Iraqi Patients with Type 2 Diabetes Mellitus

Hasan Hussein Idan¹, Halla Ghazi Mohamoud¹

¹ Dept. of Biochemistry, College of Medicine, University of Baghdad, Iraq

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is characterised by chronic oxidative stress and inflammation, which contribute to disease progression and the development of complications. Myeloperoxidase (MPO) and malondialdehyde (MDA) are biomarkers associated with oxidative stress and inflammatory processes, making them potential indicators for assessing complications related to type 2 diabetes mellitus (T2DM). **Objective:** This study aimed to evaluate the clinical significance of serum MPO and MDA levels as biomarkers for complications in Iraqi patients with type 2 diabetes mellitus (T2DM). **Methods:** A total of 130 participants were enrolled, comprising 70 patients with type 2 diabetes mellitus (T2DM) and 60 healthy controls. Serum MPO and MDA levels were measured using enzyme-linked immunosorbent assay (ELISA), while glycated haemoglobin (HbA1c) was analysed using an automated biochemical analyser. Statistical analysis was performed using Pearson's correlation and Student's t-test. The diagnostic accuracy of MPO and MDA was assessed through receiver operating characteristic (ROC) curve analysis. **Results:** Serum levels of MPO and MDA were significantly elevated in T2DM patients compared to healthy controls ($p < 0.01$). Pearson correlation analysis demonstrated a strong positive correlation between MPO, MDA, and HbA1c levels, indicating an association with glycemic control and disease severity. ROC curve analysis revealed high area under the curve (AUC) values for both MPO (0.98) and MDA (0.96), suggesting their potential utility as predictive biomarkers for T2DM complications. **Conclusion:** The findings suggest that elevated MPO and MDA levels are strongly associated with oxidative stress and metabolic dysfunction in patients with type 2 diabetes mellitus (T2DM). Both biomarkers show promise as potential indicators for identifying individuals at high risk of developing diabetes-related complications. Further studies are recommended to explore their clinical applicability in disease monitoring and management.

Keywords: Myeloperoxidase, Malondialdehyde, Type 2 Diabetes Mellitus, Diabetes Mellitus, Complications of Diabetes

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders that are related to a complicated interaction of genetic and environmental triggering factors. It is characterised by increased levels of glucose in the blood resulting from deficiencies in insulin production, insulin action, or both [1, 2]. In Iraq, the prevalence of diabetes has increased dramatically, rising from 19.58 per 1,000 in 2000 to 42.27 per 1,000 in 2015, a remarkable 115% increase [3, 4]. Type 2 diabetes mellitus (T2DM) prevalence in Iraq is estimated between 8.5% and 13.9% [5]. Patients with T2DM often must follow complex treatment strategies involving medications, dieting, and regular monitoring for any complications, all of which require lifestyle modifications [6]. Type 2 diabetes mellitus (T2DM), as well as its complications, is one of the leading healthcare concerns worldwide [7]. Myeloperoxidase (MPO), secreted by neutrophils and monocytes, is responsible for the normal processes of immune system function and becomes highly active during an infection. Chronic inflammation occurs in the early stages of T2DM, characterised by elevated levels of circulating monocytes, which become incredibly active in the course of the disease. Chronic inflammation occurs in the early stages of T2DM, characterised by elevated levels of circulating monocytes and neutrophils. This chronic activation process renders MPO more susceptible to activating chemically reactive substances, which may lead to tissue damage [8-10]. Oxidative stress occurs when cells fail to maintain the right balance between free radical production and antioxidant levels inside cells. In T2DM, continuously higher blood glucose levels lead to an imbalance, which is followed by

an excessive production of free radicals. Free radicals damage tissues and cells in the body, leading to the development of complications such as neuropathy, nephropathy, retinopathy, and cardiovascular disease associated with T2DM [11-13]. Malondialdehyde (MDA), a byproduct of the cellular oxidation of polyunsaturated fatty acids, is widely used as an indicator of oxidative stress. In T2DM, there has been a significant increase in MDA [14, 15].

Methods

Study Design and Participants

This case-control study was conducted between October 2023 and January 2024 at Al-Karkh General Hospital in Baghdad City, Iraq. After obtaining written informed consent, 130 Iraqi individuals were divided into two groups: Group 1 (G1) consisted of 70 individuals with type 2 diabetes mellitus (T2DM), and Group 2 (G2) included 60 healthy individuals serving as controls. The study received approval from the College of Medicine at the University of Baghdad.

Exclusion Criteria

Participants with the following conditions were excluded: Type 1 diabetes, alcoholism, smoking, pregnancy, current use of antioxidant supplements, cardiovascular disease, renal disease, liver disease, or other chronic medical conditions that might affect the results. Additionally, individuals who had undergone recent surgery were also excluded.

Outcome Measurements

Eight millilitres of venous blood were collected from each participant. Blood was separated into two portions: 3 ml in an EDTA tube for immediate HbA1c measurement using a fully automated analyser (Cobas C111, Germany) with an immunochemical assay method. The remaining 5 ml were used for serum collection. After a 10-minute incubation in a water bath, the serum was centrifuged at 3,500 rpm for 5 minutes and stored at -20°C for subsequent measurement of MPO and MDA levels using a sandwich enzyme-linked immunosorbent assay (sandwich ELISA) developed by Human, USA.

Data Analysis by Statistics

The statistical analysis was performed using SPSS® version 26.0 software on a Windows computer. A student's t-test (two-tailed) was applied for investigating continuous data (HbA1c, MPO, MDA) in the two groups. Pearson's correlation coefficient (r) was adopted to assess the strength of association between HbA1c and MPO/MDA levels. Visualisations were enhanced by plot graphs, which showed the relationships between the data. For statistical purposes, a p-value of less than 0.05 was regarded as significant.

Result

The mean age of participants in G1 was 57.91 ± 5.85 years, while G2 had a mean age of 53.30 ± 6.16 years. G1 comprised 40 females and 30 males, while G2 consisted of 32 females and 28 males. The distribution of age or gender between the groups shows no statistically significant differences ($p > 0.05$). G1 had significantly higher levels of MPO and MDA compared to G2 ($p\text{-value} < 0.05$). Table 1 lists the results of each biochemistry test, including the mean, standard deviation (mean $\pm$ SD), and p-value. A correlation analysis between MPO and MDA levels and HbA1c revealed a robust positive correlation. This correlation is statistically significant, as illustrated in Figures 1 and 2, respectively. The diagnostic effectiveness of MDA and MPO was evaluated using receiver operating characteristic (ROC) curve analysis. The Area Under the Curve (AUC) values of 0.98 ($p < 0.01$) for MPO and 0.96 ($p < 0.01$) for MDA are presented in the results, as shown in Table 2 and Figure 3. A sensitivity of 98% and a specificity of 100% characterised the MPO cutoff point at 230 ng/ml. The sensitivity and specificity for the MDA cutoff point were 88% and 91%, respectively, at a concentration of 16 ng/mL.

Table 1: Biochemical Parameters in T2DM Patients and Healthy Controls.

Parameter	Study Group		P- Value
	G1 N=70	G2 N=60	
Gender (M, F)	30, 40	28, 32	0.728
Age (years) (Mean $\pm$ SD)	57.91 ± 5.85	53.30 ± 6.16	0.781
HbA1c (%) (Mean $\pm$ SD)	8.93 ± 1.55	5.27 ± 0.22	$< 0.001^*$
MPO (ng/ml) (Mean $\pm$ SD)	387 ± 49	161 ± 28	$< 0.001^*$
MDA (ng/ml) (Mean $\pm$ SD)	39.98 ± 9.22	11.21 ± 3.36	$< 0.001^*$

* Difference between independent means is statistically significant at 0.05 level. N: The number of participants in each group.

M: Male. F: Female

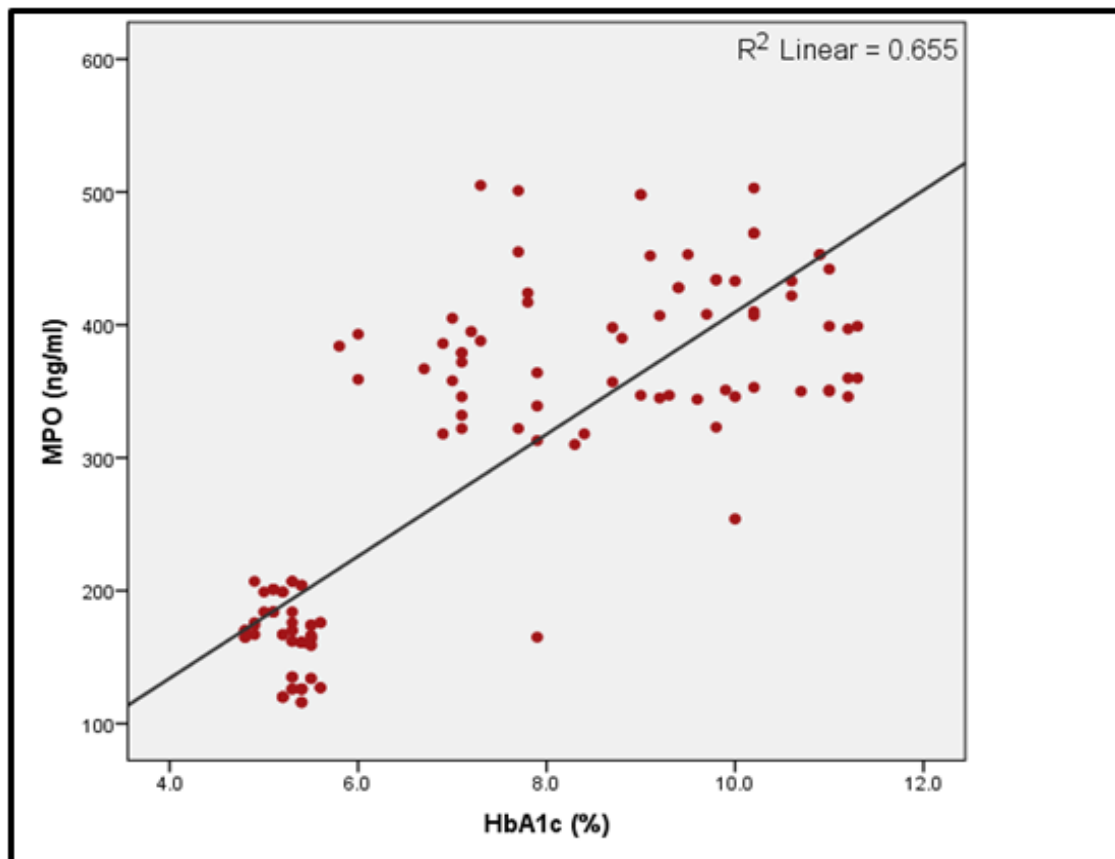


Figure 1: The Pearson Correlation Analysis Demonstrated a Highly Significant Positive Correlation ($P < 0.01$, $r = +0.81$) Between MPO and HbA1c

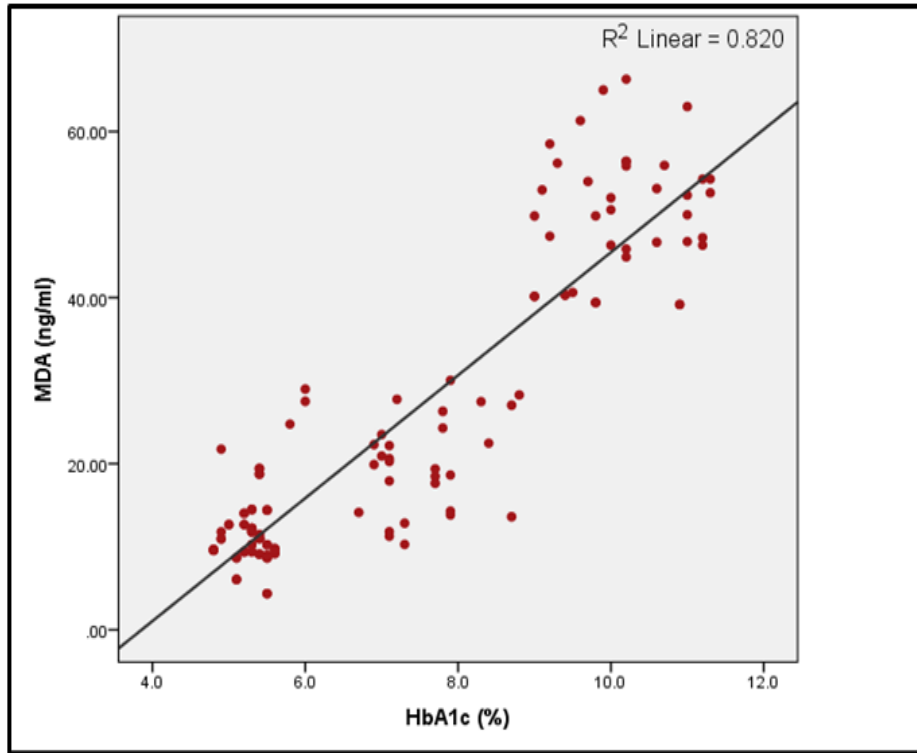


FIGURE 2: The Pearson Correlation Analysis Demonstrated a Highly Significant Positive Correlation ($P < 0.01$, $r = +0.91$) Between MDA and HbA1c

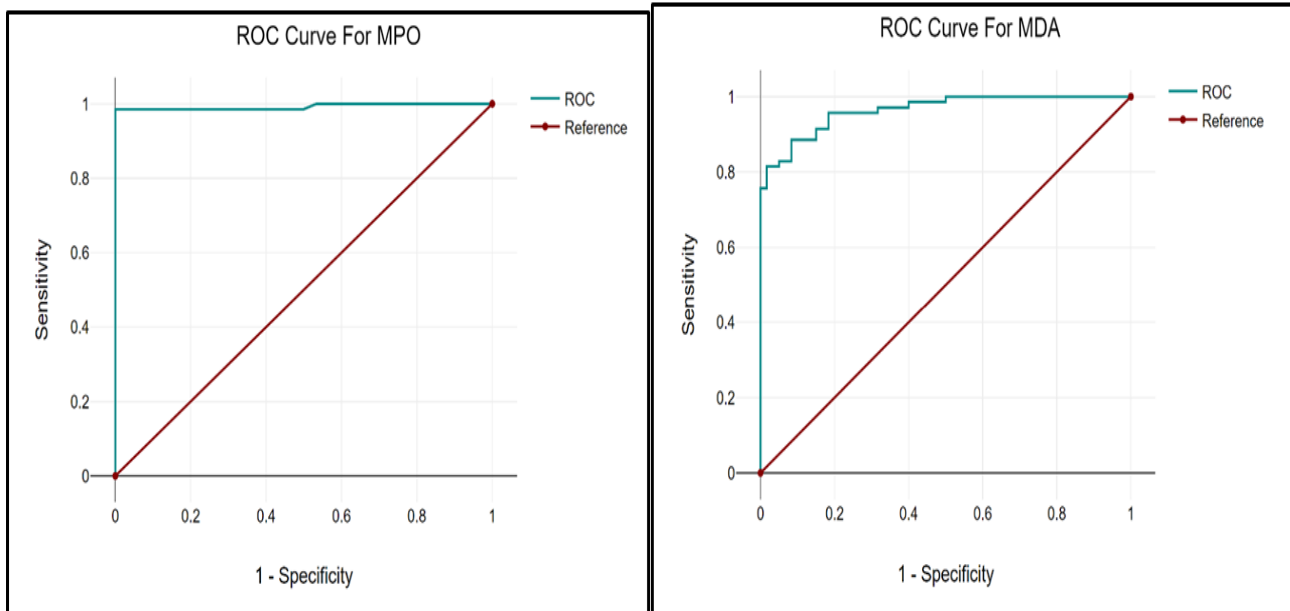


FIGURE 3: The ROC Curve for MPO and MDA

TABLE 2: ROC Curve Analysis Details for MPO and MDA

Parameter	Cut-off	AUC	SN	SP	P-value
MPO (ng/ml)	230	0.98	98%	100%	< 0.001
MDA (ng/ml)	16	0.96	88%	91%	< 0.001

SN: Sensitivity, SP: Specificity

Discussion

This finding demonstrates that patients with T2DM exhibit significantly higher levels of MPO compared to healthy controls, suggesting that inflammation is a common feature of this population. MPO is an enzyme primarily found in neutrophils and other immune

cells, and its release during inflammation can lead to tissue damage and is associated with various diseases [9]. This result aligns with previous research demonstrating elevated serum MPO levels in T2DM patients compared to healthy controls [16-18]. However, some studies have presented contrasting results, indicating a reduction in MPO concentrations among those with T2DM [19, 20]. Possible reasons

vary depending on different study populations, illness severity, or variations in methodology. Consequently, the current study's findings are in harmony with most existing literature, which hypothesises an increase in MPO in T2DM. In addition, during the examination of the healthy group compared to the T2DM group, it was found that the MDA levels in the T2DM group were significantly higher. This outcome, which aligns with previous studies, confirms the elevated levels of MDA in T2DM patients [21-23]. Excess levels of MDA can be toxic to cells, resulting in structural disruption of cell membranes, the release of inflammatory mediators, and have the potential to serve as indicators for assessing oxidative stress [24, 25]. Prolonged oxidative stress and inflammation, as indicated by high MPO and MDA levels, play a significant role in the development and progression of complications associated with T2DM, including nerve damage, kidney disease, retinopathy, and heart disease [26, 27].

The diagnostic potential of MPO and MDA was also evaluated by using ROC curve analysis. The results demonstrate excellent

performance for MPO and MDA, as indicated by AUC measurements ($AUC = 0.98$ and $AUC = 0.96$, respectively). The fact that AUC values are excellent for both biomarkers suggest a powerful discriminant capacity of these biomarkers and their possible use in risk evaluation of T2DM, associated with the development of complications. Moreover, the statistical analysis demonstrated a statistically significant positive correlation between the values of MPO, MDA, and HbA1c. Higher HbA1c levels, a well-established indicator of long-term blood glucose control, are correlated with elevated levels of MPO and MDA, markers of oxidative stress and inflammation. This is additionally possible and will render MPO and MDA suitable as markers to detect individuals at risk for secondary complications resulting from T2DM.

Conclusion

The present study suggests that elevated MPO and MDA may serve as potential biomarkers for identifying patients with T2DM who are at an increased risk of developing complications.

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