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Estimation of 8-iso-PGF2α as a marker of oxidative stress in T2DM patients with and without dyslipidemia

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ABSTRACT

Oxidative stress resulting from excessive production of reactive oxygen species (ROS) plays a pivotal role in the development of chronic diseases, including type 2 diabetes mellitus (T2DM) and its related metabolic disturbances. This study aimed to assess the biomarker 8-iso-prostaglandin-F2α (8-iso-PGF2α) as an indicator of oxidative stress in T2DM patients and to investigate its association with dyslipidemia, with the objective of determining its potential value in predicting early diabetesrelated complications. The study included 90 participants: 60 T2DM patients (30 with dyslipidemia and 30 without) and 30 age and sexmatched healthy controls. Fasting serum samples were analyzed for glucose and lipid profile using an autoanalyzer, while C-reactive protein (CRP) and 8-iso-PGF2α levels were measured using ELISA. Compared with healthy controls, patients with T2DM exhibited markedly elevated levels of oxidative stress and inflammatory markers, including significant increases in both 8-iso-PGF2α and CRP. Among individuals with T2DM, those with dyslipidemia showed substantially higher 8-iso-PGF2α concentrations and a slightly higher CRP levels compared with patients without dyslipidemia. Diagnostic assessment indicated that 8-iso-PGF2α showed moderate ability to distinguish T2DM patients with dyslipidemia from those without. These findings suggest that elevated levels of 8-iso-PGF2α, particularly in dyslipidemic T2DM patients, may serve as a promising early biomarker for assessing the risk of diabetes-related complications.

INTRODUCTION

Diabetes is a metabolic disease characterized by elevated blood glucose levels [1]. It is generally classified into two major types: type 1 diabetes mellitus (T1DM), which results from an absolute deficiency of insulin, and type 2 diabetes mellitus (T2DM), which arises from insulin resistance combined with partial insulin deficiency [2]. The prevalence of diabetes has increased dramatically in recent decades and is expected to reach epidemic proportions. According to World Health Organization projections, the global number of individuals with diabetes is projected to reach 366 million by the year 2030 [1,3-5]. One of the major risk factors contributing to cardiovascular disease among diabetic patients is dyslipidemia [6]. Individuals with T2DM and dyslipidemia commonly exhibit an atherogenic lipid profile characterized by elevated triglycerides and low-density lipoprotein (LDL) levels, accompanied by reduced highdensity lipoprotein (HDL) levels [7,8].

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Oxidative stress, defined as the excessive generation of reactive oxygen species (ROS), plays a significant role in the development and progression of chronic diseases beginning early in life [9]. ROS causes oxidative damage to proteins, lipids, and deoxyribonucleic acid (DNA). Recent research has demonstrated that ROS contributes to the pathogenesis of a wide range of disorders, including diabetes, atherosclerosis, metabolic syndrome, cancer, cardiovascular and neurodegenerative diseases, hepatic, gastrointestinal and renal disorders, infertility, and hypertension [10,11]. Oxidative stress can be monitored by assessing various biomarkers, including indicators of lipid and protein oxidation and changes in the antioxidant defense system [12]. Common lipid peroxidation markers include lipoperoxides, lipid hydroperoxides, and malondialdehyde (MDA) [13].

Another wellestablished lipid peroxidation biomarker is 8-iso-prostaglandin F2α (8-iso-PGF2α). It is considered one of the most reliable markers of lipid peroxidation because it is generated through non-enzymatic oxidation

of arachidonic acid within membrane phospholipids and is not significantly affected by dietary intake. This makes it more specific and sensitive than many other oxidative stress indicators, and its levels are modulated by endogenous antioxidant capacity [14]. Elevated concentrations of 8-iso-PGF2α have been implicated in the onset of diabetes and the development of diabetesrelated vascular complications. The measurement of 8-iso-PGF2α using modern analytical techniques provides valuable insights into the role of free radicals in diseases associated with oxidative stress and both acute and chronic inflammatory conditions [15]. Increased levels of this biomarker have been reported in patients with various chronic diseases, including diabetes, likely due to oxidative stress driven by factors such as insulin resistance, obesity, and impaired glucose tolerance [16]. Additionally, 8-iso-PGF2α contributes to mitogenesis, chronic platelet activation, and vasoconstriction, which may further accelerate the progression of diabetes and its complications [17]. Previous studies have demonstrated associations between 8-iso-PGF2α levels and several clinical parameters, including hemoglobin A1c (HbA1c), fasting glucose, and both acute and chronic glucose fluctuations [18,19].

To the best of our knowledge, 8-iso-PGF2α has not been previously investigated in Iraqi patients with T2DM. Therefore, the present study aims to evaluate 8-iso-PGF2α as a marker of oxidative stress and to examine its relationship with dyslipidemia in this patient population.

METHODS

This study included 90 participants: 60 patients diagnosed with type 2 diabetes mellitus (T2DM) (36 males and 24 females; mean age 45.73 ± 5.43 years) and 30 healthy, age- and sex-matched controls (18 males and 12 females; mean age 43.73 ± 5.89 years). Sample collection took place between November 2023 and February 2024 through collaboration between the Department of Chemistry, AlMustansiriyah University, and the Specialized Center for Diabetes and Endocrinology in Baghdad AlRusafa, Iraq. Ethical approval was obtained from the Ethics Review Board for Human Studies, Department of Chemistry, College of Science, AlMustansiriyah University (Approval No. BCSMU/0124/0007C).

T2DM diagnosis followed the 1999 World Health Organization criteria, defined as fasting plasma glucose ≥126 mg/dL or glycated hemoglobin (HbA1c) ≥6.5%, which reflects longterm glycemic control and was measured in all participants.

Based on their lipid profiles, patients were classified as having dyslipidemia if their triglyceride (TG) level exceeded 150 mg/dL, lowdensity lipoprotein cholesterol (LDL-C) exceeded 100 mg/dL, or highdensity lipoprotein cholesterol (HDL-C) was <50 mg/dL.

Individuals with the following conditions were excluded: heart failure, hepatic or renal impairment, myocardial infarction, angina pectoris, malignancy, or use of medications such as statins, steroids, nonsteroidal anti-inflammatory drugs, catechins, as well as smokers and pregnant women.

Blood Samples

Venous blood samples were collected in the morning after a 12hour overnight fast. Serum was separated immediately by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until subsequent analysis of lipid profile, glucose, C-reactive protein (CRP), and 8-iso-PGF2α.

Biochemical Parameter Determination

Fasting blood sugar (FBS), triglycerides (TG), total cholesterol (TC), and highdensity lipoprotein cholesterol (HDL-C) were measured using an automated analyzer (COBAS INTEGRA 400 PLUS, Roche, Germany). Lowdensity lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation [20]. The Castelli index (TC/HDL-C ratio) was calculated according to Castelli *et al.* [21]. HbA1c (%) was determined using the Diabetes Control and Complications Trial (DCCT) method [22].

Assay of CRP

We measured serum C-reactive protein (CRP) levels using a sandwich enzyme immunoassay kit (Cloud-Clone Corp., USA).

Assay of 8-iso-PGF2α

Serum 8-iso-PGF2α concentrations were determined using a competitive enzyme immunoassay kit (CloudClone Corp., USA), based on competitive inhibition principles.

Statistical Analysis

The data were analyzed using SPSS software, version 22 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean ± standard deviation (SD). Differences between groups were evaluated using Student's ttest followed by Levene's test for equality of variances. Pearson correlation analysis was used to assess the relationships among study variables. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal cutoff value, sensitivity, specificity, and area under the curve (AUC). Statistical significance was defined as $p < 0.05$ [23].

RESULTS

Table 1 summarizes the general characteristics and biochemical parameters of the study groups. No significant difference in age was observed between T2DM patients and healthy controls ($p = 0.26$). Body mass index (BMI) was significantly higher in T2DM patients compared with healthy subjects ($p < 0.05$). Additionally, significant differences ($p < 0.05$) were found in fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), LDL-C, HbA1c, 8-iso-PGF2α, and C-reactive protein (CRP), all of which were elevated in the patient group relative to controls.

Comparisons between T2DM patients with dyslipidemia (n = 30) and those without dyslipidemia (n = 30) are presented in Table 2. No significant differences were noted in age, disease duration, HDL-C, HbA1c, or CRP between the two groups. However, patients with dyslipidemia exhibited significantly higher BMI, FBG, TC, TG, LDL-C, and serum 8-iso-PGF2α levels compared with those without dyslipidemia.

Table 3, Figure 1, and Figure 2 demonstrate that no significant differences were observed in CRP ($p = 0.13$) or 8-iso-PGF2α ($p = 0.08$) between obese and non-obese diabetic patients.

Table 1. General features and biochemical parameters of patients and healthy subjects

Parameters	Control group (N=30)	Diabetic patients group (N=60)	p value
Age (y)	43.73 ± 5.89	45.73 ± 5.43	0.26
Disease duration (y)	–	6.47 ± 4.92	–
BMI (kg/m²)	23.13 ± 0.99	30.37 ± 4.40	0.00
FBG (mg/dl)	87.33 ± 5.60	216.26 ± 74.74	0.00
TC (mg/dl)	129.26 ± 20.54	208.26 ± 78.52	0.00
TG (mg/dl)	91.73 ± 22.38	181.26 ± 90.92	0.00
HDL-C (mg/dl)	49.53 ± 4.62	43.43 ± 14.62	0.04
LDL-C(mg/dl)	61.46 ± 20.10	127.08 ± 70.19	0.00
VLDL-C(mg/dl)	18.26 ± 4.47	36.25 ± 18.19	0.00
TC/HDL-C	2.61 ± 0.41	5.34 ± 3.06	0.00
HbA1C (%)	4.89 ± 0.24	8.88 ± 1.11	0.00
8-iso-PGF2α (pg/ml)	220.52 ± 14.82	794.77 ± 68.07	0.00
CRP (mg/dl)	2.75 ± 0.29	4.41 ± 0.90	0.00

The mean ± SD is employed to describe the data
To examine group differences, the student t-test and Levene's test were used. A p value of less than 0.05 was regarded statistically significant.
BMI – Body mass index; FBG – Fasting blood glucose; TC – Total cholesterol; TG – Triglycerides; HDL-C – High-density lipoprotein cholesterol; LDL-C – Low-density lipoprotein cholesterol; VLDL-C – Very low-density lipoprotein cholesterol; HbA1c – Hemoglobin A1c; 8-iso-PGF2α – 8-iso-Prostaglandin F2α; CRP – C-reactive protein

Table 2. General characteristics and biochemical parameters of diabetic patient's sub-groups depending on dyslipidemia

Parameters	Diabetic without dyslipidemia (N=30)	Diabetic with dyslipidemia (N=30)	p value
Age (y)	45.66 ± 5.87	42.80 ± 5.13	0.16
Disease duration (y)	7.46 ± 5.97	5.48 ± 3.51	0.27
BMI (kg/m²)	27.74 ± 3.10	33.00 ± 3.95	0.00
FBG (mg/dl)	179.13 ± 73.61	253.40 ± 56.55	0.00
TC (mg/dl)	146.06 ± 27.82	270.46 ± 60.90	0.00
TG (mg/dl)	106.33 ± 25.16	256.20 ± 66.79	0.00
HDL-C (mg/dl)	47.40 ± 17.65	39.46 ± 9.86	0.14
LDL-C (mg/dl)	74.40 ± 23.24	179.76 ± 60.99	0.00
VLDL-C (mg/dl)	21.26 ± 5.07	51.24 ± 13.35	0.00
TC/HDL-C (mg/dl)	3.28 ± 0.90	7.40 ± 3.08	0.00
HbA1C (%) (mg/dl)	8.78 ± 1.17	8.98 ± 1.08	0.63
8-iso-PGF2α (pg/ml)	764.97 ± 63.80	824.57 ± 60.20	0.01
CRP (mg/dl)	4.11 ± 0.34	4.72 ± 1.16	0.06

The mean ± SD is employed to describe the data. To examine group differences, the student t-test and Levene's test were used.
A p value of less than 0.05 was regarded statistically significant

Table 3. CRP and 8-iso-PGF2α levels in diabetes who are obese or not

Parameters	Diabetic without obesity (N=30)	Diabetic with obesity (N=30)	P (value)
8-iso-PGF2α (pg/ml)	761.16 ± 65.25	805.03 ± 62.16	0.08
CRP (mg/dl)	4.08 ± 0.37	4.49 ± 0.89	0.13

The mean ± SD is employed to describe the data. To examine group differences, the Student t-test and Levene's test were used.
A p-value of less than 0.05 was regarded statistically significant

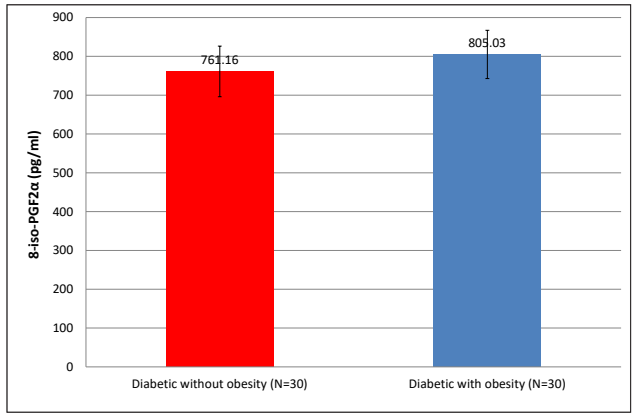

Figure 1. 8-iso-PGF2α levels in diabetic patients with and without dyslipidemia

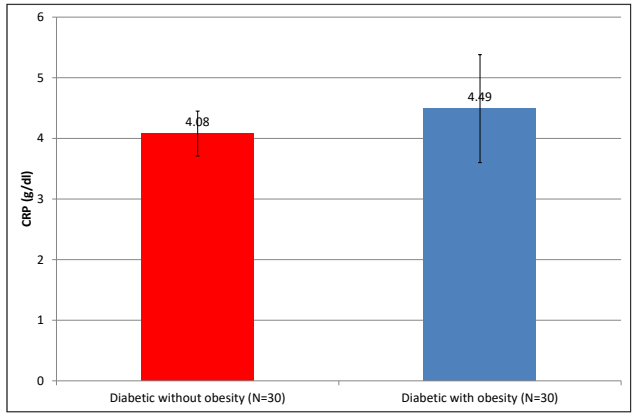

Figure 2. CRP levels in diabetic patients with and without dyslipidemia

Pearson correlation analysis revealed significant positive correlations between 8-iso-PGF2α and TC, LDL-C, and the TC/HDL-C ratio among diabetic patients (Table 4). Furthermore, among T2DM patients with dyslipidemia, 8-iso-PGF2α showed a significant correlation with CRP ($p = 0.04$).

Table 4. The Pearson correlation between 8-iso-PGF2α and various parameters in the study groups

Parameters	Diabetic patients group		Diabetic without dyslipidemia group		Diabetic with dyslipidemia group	
	r	P	r	P	r	P
Age (y)	-0.26	0.15	-0.07	0.78	0.10	0.71
Disease duration (y)	-0.03	0.84	0.16	0.55	-0.12	0.65
BMI (kg/m²)	0.19	0.29	-0.38	0.15	0.13	0.63
FBG (mg/dl)	0.30	0.10	0.23	0.39	-0.08	0.77
TC (mg/dl)	0.43*	0.01	-0.21	0.43	0.34	0.21
TG (mg/dl)	0.33	0.07	-0.16	0.56	-0.06	0.82
HDL-C (mg/dl)	-0.09	0.60	0.22	0.41	-0.33	0.21
LDL-C (mg/dl)	0.44*	0.01	-0.32	0.23	0.40	0.13
VLDL-C (mg/dl)	0.33	0.07	-0.12	0.65	-0.06	0.82
TC/HDL-C	0.42*	0.01	-0.46	0.08	0.43	0.10
HbA1C (%)	0.05	0.78	0.24	0.37	-0.26	0.34
8-iso-PGF2α (pg/ml)	1	–	1	–	1	–
CRP (mg/dl)	0.06	0.73	-0.52*	0.04	0.01	0.98

Data are presented as correlation coefficients (r) using Pearson's test to analysis the correlations. A p -value of less than 0.05 was considered statistically significant (* means p value of less than 0.05)

Figure 3 presents receiver operating characteristic (ROC) curve analysis, demonstrating that 8-iso-PGF2α achieved an area under the curve (AUC) of 0.73, with both sensitivity and specificity equal to 60% for distinguishing T2DM patients with dyslipidemia from those without.

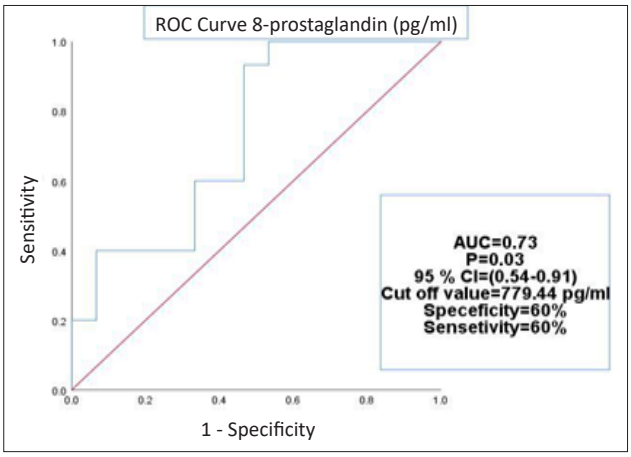

Figure 3. Receiver operating characteristic (ROC) curve for serum 8-iso-PGF2α levels between discriminating diabetic patients with and without dyslipidemia

DISCUSSION

Increased oxidative stress is a wellrecognized risk factor in the development of type 2 diabetes mellitus (T2DM), contributing to insulin resistance, dyslipidemia, βcell dysfunction, and impaired glucose tolerance [24]. Despite advances in managing hyperglycemia, diabetes remains associated with substantial morbidity and mortality, largely due to excessive free radical generation [25]. Accordingly, this study evaluated 8-iso-PGF2α as a marker of oxidative stress and investigated its association with dyslipidemia in T2DM patients.

The present findings demonstrate a significant elevation of 8-iso-PGF2α in patients with T2DM compared with healthy individuals. These results align with previous reports [1,26,27) indicating that elevated 8-iso-PGF2α levels may play a role in the early onset of T2DM, potentially through oxidative damage to pancreatic islet cells, leading to cellular dysfunction and disease progression [28,29]. Supporting these observations, Gopaul *et al.* [30) reported higher 8-iso-PGF2α levels in subjects with non-insulindependent diabetes mellitus (NIDDM) compared with controls, while Davi *et al.* [31) found increased urinary 8-iso-PGF2α and creatinine levels in NIDDM patients.

This study also identified elevated CRP levels in T2DM patients, reinforcing the link between oxidative stress and inflammation. These findings are consistent with previous work showing that inflammation, an important contributor to oxidative stress, promotes the release of proinflammatory mediators such as interleukins and adhesion molecules, which play crucial roles in the development of diabetic complications [32-34].

Marked lipid abnormalities were also detected among T2DM patients with dyslipidemia, including increased TC, TG, LDL-C, TC/HDL-C, and VLDL, accompanied by reduced HDL-C levels compared with T2DM patients without dyslipidemia. Although CRP levels tended to be higher in this group, the difference did not reach statistical significance ($p = 0.06$), potentially due to the limited sample size.

With regard to obesity, non-significant increases in CRP and 8-iso-PGF2α were observed in obese T2DM patients compared with non-obese patients ($p = 0.13$ and $p = 0.08$, respectively). These findings differ from those of Abulnaja *et al.* [35), who reported significant elevations, suggesting that obesity may contribute to oxidative stress and inflammation, though this effect was not statistically confirmed in our sample.

Importantly, this study demonstrated significant positive correlations between 8-iso-PGF2α and lipid parameters such as TC ($p = 0.01$, r = 0.43), LDL-C ($p = 0.01$, r = 0.44), and TC/HDL-C ($p = 0.01$, r = 0.42). These findings reinforce the association between dyslipidemia and oxidative stress, consistent with the observations of Mukhtar *et al.* [1].

To better identify earlystage risk indicators rather than outcomes, this study focused on detecting early biochemical alterations associated with dyslipidemia in T2DM patients. Although the crosssectional design limits causal inference and the ability to track longitudinal changes, it nonetheless provides valuable insight into the interaction between oxidative stress and lipid disturbances. Additionally, while factors such as diet and medication use were not fully controlled, this reflects realworld variation and therefore enhances the applicability of the findings to everyday clinical practice.

Receiver operating characteristic (ROC) analysis showed that 8-iso-PGF2α has moderate diagnostic value for differentiating T2DM patients with dyslipidemia from those without (AUC = 0.73; sensitivity = 60%; specificity = 60%; $p = 0.03$).

In summary, the observed increase in 8-iso-PGF2α among T2DM patients, particularly those with dyslipidemia, supports its potential usefulness as an early biomarker of oxidative stress. These findings further suggest that lipid abnormalities may exacerbate oxidative stress, contributing to a greater risk of future complications. Nevertheless, additional longitudinal studies with larger cohorts, particularly involving patients with established diabetic complications, are needed to confirm the prognostic significance of 8-iso-PGF2α.

CONCLUSIONS

The findings of this study demonstrate that patients with type 2 diabetes mellitus (T2DM) exhibit significantly elevated levels of 8-iso-PGF2α, with the highest concentrations observed among those with dyslipidemia. This pattern suggests a cumulative oxidative burden in these individuals, highlighting the interplay between lipid abnormalities and oxidative stress. Although further longterm studies are required to confirm the predictive and prognostic value of this biomarker, the present results indicate that 8-iso-PGF2α may serve as a promising early indicator for assessing the risk of diabetesrelated complications. Early identification of heightened oxidative stress in dyslipidemic T2DM patients may support more effective risk stratification and targeted therapeutic interventions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The study was approved by the Ethical Research Committee of the College of Science, Mustansiriyah University, Baghdad, Iraq (Ref.: BCSMU/0124/0007C, Jan 01, 2024).

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