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Association of Inflammatory and Oxidative Stress Markers of Type 2 Diabetes Mellitus with Glycemic Control and Microvascular Complications

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ABSTRACT
Background: Type 2 diabetes mellitus (T2DM) is defined by persistent hyperglycemia which leads to oxidative stress and low-grade inflammation which causes microvascular complications. The interconnection between inflammatory signs, oxidative stress, and glycemic control is imperative to understanding of patients at risk. Purpose: The purpose of the study was to compare inflammatory (IL-6, TNF-a, hs-CRP) and oxidative stress (MDA, TAC, SOD) parameters between T2DM patients and healthy controls and determine the relationship between them and microvascular complications and glycemic control. Methods: 150 T2DM patients (75 with complications, 75 without complications) and 50 healthy controls were involved in this case-control study. Blood samples were fasted and compared in terms of HbA1c, fasting glucose, lipids profile, inflammatory (IL-6, TNF-a, hs-CRP), and oxidative stress (MDA, TAC, SOD) levels. The analysis of the predictive performance to complications was done using receiver operating characteristic (ROC) analysis. Findings: IL-6 (18.4+-4.2 vs 4.2+-1.1 pg/mL, P<0.001), TNF-a (28.6+-6.4 vs 8.4+-2.1 pg/mL, P<0.001), hs-CRP (6.8+-1.8 vs 1.2+-0.4 mg/L, P<0.001) were found to be significantly higher in T2DM patients than controls. The level of oxidative stress was high: the MDA level doubled, and the TAC fell by 48%. Further increases in IL-6 (24.6+-5.8 vs 12.4+-3.2 pg/mL, P<0.001) and MDA (8.4+-2.1 vs 4.8+-1.2 nmoI/mL, P<0.001) were found in patients with complications. The highest predictive accuracy of microvascular complications was IL-6/TAC ratio (AUC=0.92). Conclusion: There is a great increase of inflammatory and oxidative stress markers in T2DM patients, and additional increases in microvascular complication patients. The IL-6/TAC ratio can be used as an effective biomarker to detect patients with a risk of complications.

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1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a significant health issue of the world as about 537 million adults are currently diagnosed with this condition, and the number is expected to grow to 783 million by 2045. It is a disease with insulin resistance and progressive b-cell impairment that generates chronic hyperglycemia and is the basis of the development of both microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (cardiovascular disease, stroke) complications [1].

The long-term effects of hyperglycemia cause protein kinase C activation, hexosamine and advanced glycation end-products (AGE) formation, activation of polyol pathway, and flux of the

hexosamine pathway. All of these processes come together to cause a rise in the production of reactive oxygen species(ROS) creating an oxidative stressful state by which cellular components such as lipids, proteins and DNA are destroyed [2].

In T2DM, oxidative stress and inflammation are closely associated. Overproduction of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-a) by activating nuclear factor kappa B (NF-kB) and other inflammatory transcription factors is precipitated by excess ROS. These cytokines also worsen insulin signaling, and it forms a vicious cycle of metabolic dysfunction [3].

Although inflation and oxidative stress profiles in patients with T2DM in Western populations have been widely studied, little information is available in the literature on the Middle East population, with complex genetic and lifestyle factors. Moreover, the usefulness of combined inflammatory-oxidative markers as predictors of microvascular complications needs to be investigated more.

Consequently, the aim of the study was to: (1) compare inflammatory (IL-6, TNF-a, hs-CRP) and oxidative stress (MDA, TAC, SOD) markers to assess the differences between T2DM patients and healthy controls; (2) compare the markers between patients with and without microvascular complications; (3) determine the correlation between the markers and glycemic control (HbA1c); and (4) evaluate predictive capabilities of these markers of microvascular complications using ROC analysis

2-MATERIALS AND METHODS

2.1. Design and participants of the study.

This case-control study will be carried out in the Diabetes Center, Baghdad Teaching Hospital in the period between January 2024 and August 2024. The Institutional Ethics Committee gave the approval to the study (Protocol No. EC-2024-012), and informed consent was signed by all participants.

The participants were enrolled (200 of them, 150 T2DM patients and 50 healthy controls). T2DM patients further were stratified into two groups depending on the presence of microvascular complications, Group A (T2DM without complications, n=75), Group B (T2DM with complications, n=75). The list of complications included: diabetic retinopathy (confirmed by fundus), diabetic nephropathy (creatinine albuminuria >30mg/g, eGFR <60mL/min/1.73m2), or diabetic neuropathy (confirmed by nerve conduction studies).

Inclusion criteria: Age 35-65 years, diagnosed with T2DM [?]5 years (patients), HbA1c less than 5.7% and fasting glucose less than 100mg/dl (controls). Exclusion criteria Type 1 diabetes, recent infection (less than 2 weeks), autoimmune diseases, malignancy, pregnancy, use of antioxidant supplements, corticosteroid treatment.

2.2. Biochemical analysis and collection of samples.

Blood samples (10 mL) were taken after an overnight fasting (10-12 hours). Sampling was done within a 2-hour period with serum stored at -80 deg C pending analysis. The HbA1c was examined through HPLC (Bio-Rad D-10). Fasting glucose, lipid profile (TC, TG, HDL-C, LDL-C), urea and creatinine were done by automated analyzer (Roche Cobas c501).

2.3. Inflammatory markers

Enzyme-linked immunosorbent assay (ELISA) kits of serum IL-6 and TNF-a were used to measure 0.7 pg/mL and 0.5 pg/mL sensitivities, respectively (R&D Systems, USA). The determination of high-sensitivity C-reactive protein (hs-CRP) was done by immunoturbidimetric method (Roche Diagnostics). All inflammatory markers had intra-assay and inter-assay CVs of less than 8% and 10% respectively.

2.4. Oxidative stress markers

As an indicator of lipid peroxidation, Malondialdehyde (MDA) was determined using thiobarbituric acid reactive substances (TBARS) method. The ferric reducing antioxidant power (FRAP) was used to determine the total antioxidant capacity (TAC). A commercial kit (Randox Laboratories, UK) was used to measure the activity of superoxide dismutase (SOD). The MDA/TAC ratio was obtained as a compound marker of oxidative balance.

2.5. Statistical analysis

The SPSS version 26.0 and R software were used to analyze the data. Kolmogorov-Smirnov test was used to test normalcy. One way ANOVA was applied to compare the continuous variables with either post-hoc test of Tukey or the Kruskal-Wallis test. Chi-square test was employed in the comparison of categorical variables. Markers and glycemic parameters were analyzed by Pearson correlation coefficients.

The predictive accuracy of inflammatory and oxidative stress markers to microvascular complications was estimated using the receiver operating characteristic (ROC) curve analysis. Area under the curve (AUC), optimal cut-off values

(Youden index), sensitivity, specificity and accuracy were computed. Multiple logistic regression was used to determine independent predictors of complications. Statistical significance was set at P<|human|>The level of significance was considered to be P<|human|>The level of significance was taken as P.

3-RESULTS

3.1. Demographic and clinical statistics.

Table 1 shows demographic characteristics. There was no significant difference in terms of age, sex ratio and BMI between the three groups (P>0.05). T2DM patients had very high values in HbA1c, fasting glucose, and dyslipidemia than the controls. The longer the duration of diabetes (12.4+-3.8 vs 7.2+-2.6 years, P<0.001) and higher the HbA1c (9.2+-1.6 vs 7.4+-1.2% P<0.001) was observed in patients with complications versus non-complicated patients.

3.2. Inflammatory markers

T2DM patients exhibited a large inflammatory marker presence relative to healthy controls (Table 2). The overall T2DM patients had higher levels of IL-6 (18.4+-4.2 vs 4.2+-1.1 pg/mL, P<0.001). There was additional increase in patients with complications: IL-6 (24.6+-5.8 vs 12.4+-3.2 pg/mL, P<0.001), TNF-a (36.8+-8.2 vs 20.4+-4.8 pg/mL, P<0.001), and hs-CRP (9.4+-2.4 vs 4.2+-1.2 mg/L, P<0.001) than in those without complications

Table 1. Clinical and demographic data of study subjects.

Parameter	Controls (n=50)	T2DM-A (n=75)	T2DM-B (n=75)	P value
Age (years)	52.4±8.6	54.2±9.4	55.8±8.2	>0.05
Sex (M/F)	24/26	38/37	40/35	>0.05
BMI (kg/m²)	26.4±3.2	28.6±4.2	29.4±4.8	>0.05
Diabetes duration (years)	—	7.2±2.6	12.4±3.8*	<0.001
HbA1c (%)	5.2±0.4	7.4±1.2†	9.2±1.6*†	<0.001
FBG (mg/dL)	92.4±8.6	148.6±32.4†	186.4±42.8*†	<0.001

*T2DM-A: Without complications; T2DM-B: With complications. *P<0.05 vs T2DM-A; †P<0.05 vs Controls. FBG: Fasting blood glucose.*

Table 2. Inflammatory markers of study groups.

Parameter	Controls	T2DM-A	T2DM-B	P value
IL-6 (pg/mL)	4.2±1.1	12.4±3.2†	24.6±5.8*†	<0.001
TNF-α (pg/mL)	8.4±2.1	20.4±4.8†	36.8±8.2*†	<0.001
hs-CRP (mg/L)	1.2±0.4	4.2±1.2†	9.4±2.4*†	<0.001
IL-6/hs-CRP ratio	3.5±0.8	2.9±0.6†	2.6±0.5*†	<0.05

**P<0.05 vs T2DM-A; †P<0.05 vs Controls. IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-alpha; hs-CRP: High-sensitivity C-reactive protein.*

3.3. Oxidative stress markers

The oxidative stress markers exhibited high changes among T2DM patients (Table 3). There was an increase of MDA of 2.4+-0.6 nmol/ mL (Control) to 4.8+-1.2 nmol/mL (T2DM-A), and 8.4+-2.1 nmol/mL (T2DM-B), which was 3.5- folds higher in patients with complications. TAC in T2DM-A and T2DM-B reduced to 1.24+-0.28 mmol/mL (33 and 48% respectively) and 0.96+-0.22 mmol/mL respectively. The greatest difference was observed in the MDA/TAC ratio whose value rose 7-fold (9.24+-2.48) (T2DM-B) compared to the controls (1.32+-0.34).

3.4. Correlations with glycemic control

There were significant correlations between inflammatory/oxidative markers and HbA1c (Table 4). There was a significant positive relationship between IL-6 and HbA1c (r=0.72, P<0.001), between MDA (r=0.68, P<0.001) and TNF-a (r=0.64, P<0.001). HbA1c had negative correlations with TAC and SOD (r= 0.58 and r= 0.52, respectively). The MDA/TAC ratio showed the best relationship with HbA1c (r=0.76, P<0.001).

3.5. ROC analysis as a predictive of microvascular complications.

The IL-6/TAC ratio was found to be the most potent predictor of microvascular complications on ROC analysis followed by MDA/TAC ratio, IL-6 alone, and TNF-a (AUC=0.92, 0.88, 0.96, respectively). The best cut-off point was above 15.0 and it had 90.4% sensitivity, 86.8% specificity, and 88.4% accuracy

Table 3. Markers of oxidative stress on study groups.

Parameter	Controls	T2DM-A	T2DM-B	P value
MDA (nmol/mL)	2.4±0.6	4.8±1.2†	8.4±2.1*†	<0.001
TAC (µmol/mL)	1.86±0.32	1.24±0.28†	0.96±0.22*†	<0.001
SOD (U/mL)	124.6±18.4	86.4±14.8†	62.4±12.6*†	<0.001
MDA/TAC ratio	1.32±0.34	4.12±1.08†	9.24±2.48*†	<0.001

**P<0.05 vs T2DM-A; †P<0.05 vs Controls. MDA: Malondialdehyde; TAC: Total antioxidant capacity; SOD: Superoxide dismutase.*

Table 4. Prediction of microvascular complications by ROC analysis.

Biomarker	AUC	Cut-off	Se (%)	Sp (%)
IL-6/TAC ratio	0.92	>15.0	90.4	86.8
MDA/TAC ratio	0.90	>6.5	88.6	84.4
IL-6 (pg/mL)	0.88	>18.0	86.4	82.6
TNF-α (pg/mL)	0.86	>28.0	84.2	80.4

AUC: Area under curve; Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; Acc: Accuracy.

4-DISCUSSION

This paper has shown very high levels of inflammatory and oxidative stress indicators among Iraqi T2DM patients and further higher levels among those who have microvascular complications. The results of the study that the IL-6/TAC ratio has the best predictive capacity (AUC=0.92) of complications indicate the significance of combined inflammatory-oxidative evaluation instead of a single marker in isolation.

The increased IL-6 level in our T2DM patients was 4.4 times, which correlates with the results reported by other researchers who reported chronic low-grade inflammation in diabetes. SOFS3 activation and hepatic gluconeogenesis caused by IL-6 leads to hyperglycemia and metabolic dysregulation. The additional 2-fold rise in the number of cases with complications indicate that IL-6 can be a marker and mediator of microvascular destruction.

Substantial oxidative stress in our patients as depicted by 3.5-fold higher MDA and 48 percent lower TAC results demonstrate surpassing antioxidant defenses with the production of ROS by chronic hyperglycemia. The positive correlation between the MDA/TAC ratio and HbA1c (r=0.76) highlights the relationship between the glycemic and oxidative balance.

The clinical implications of our findings are: (1) the IL-6/TAC and MDA/TAC ratios could be helpful as biomarkers of identifying the patients with type 2 diabetes who are at risk of complications; (2) the interventions designed to target the mitigation of the inflammation and oxidative stress may be of an added value to the glycemic control; and (3) these markers can be used to track the response to therapy.

STUDY LIMITATIONS

(1) Cross-sectional design does not allow one to determine a cause. The study can be limited due to (2) Single-center study. (3) Individual forms of microvascular complications were not individually analyzed by the reason of sample size. (4) Inflammatory markers (statins, metformin) were not controlled completely under the effects of medication. (5) The intake of dietary antioxidants was not measured.

CONCLUSION

This paper establishes that the inflammatory markers (IL-6 4.4-fold, TNF- a 3.4-fold, hs-CRP 5.7-fold) and oxidative stress markers (MDA 3.5-fold increase, TAC 48% decrease) are highly elevated in T2DM patients, which increases more in patients with microvascular complications. The IL-6/TAC ratio was the best predictor of complications (AUC=0.92) and can be useful in a biomarker that can be used to predict high-risk patients. These results offer evidence in the incorporation of inflammatory and oxidative stress measurement into the daily practice of diabetes

management, especially in populations where there is a high rate of complications.

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CONFLICT OF INTEREST

The authors strictly state that they have no conflicts of interest.

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