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Mustafa. s. shareef
Department of Biochemistry, College of Health and Medical Techniques, AL-Bayan University, Baghdad, Iraq, mustafa123saleem@gmail.com

Ali B. Roomi
Department of Chemistry, College of Science, University of Thi-Qar, Thi-Qar, 64001, Iraq, dr.ali_bader@utq.edu.iq

Bassem Charfeddine
Department of Biochemistry, Faculty of Medicine, University of Sousse, Tunisia, charfeddinebassem@yahoo.fr

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RESEARCH ARTICLE

Spexin is a Reliable Predictor of Diabetes Postmenopausal Iraqi Women: A Case-Control Study

Mustafa S. Shareef [1], **Ali B. Roomi** [2,*], **Bassem Charfeddine** [3]

[1] Department of Biochemistry, College of Health and Medical Techniques, AL-Bayan University, Baghdad, Iraq
[2] Department of Chemistry, College of Science, University of Thi-Qar, Thi-Qar, 64001, Iraq
[3] Department of Biochemistry, Faculty of Medicine, University of Sousse, Tunisia

ABSTRACT

A peptide called spexin (SPX) regulates blood glucose and lipid metabolism. The study primarily focused on the pathogenesis of type 2 diabetes mellitus (T2DM) in postmenopausal women, examining its relationship with serum SPX levels. A case-control study involved 180 postmenopausal women from Thi-Qar, Iraq were enrolled in the current study. Ninety women were recently diagnosed with diabetic postmenopausal women, while the other 90 were non-diabetic postmenopausal women. Blood samples were collected to test: Glycated hemoglobin (HbA1c), SPX, Malondialdehyde (MDA), Superoxide dismutase (SOD), C-reactive protein (CRP), fasting blood glucose (FSG), insulin, lipid profile and interleukein (IL-6). Results: FSG, HbA1c, insulin, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), values raised significantly ($P < 0.001$) in T2DM postmenopausal women. Also, levels of MDA, CRP, and IL-6 dramatically elevated, and the levels of SPX and SOD significantly decreased ($P < 0.001$). Significant lipid profile changes were observed in postmenopausal T2DM subjects. Additionally, the connection in T2DM postmenopausal women showed a negative relationship between SPX with waist-to-height ratio, SPX with FSG, SPX with HOMA-IR, MDA with SPX, as well as a positive relationship between SPX and MDA, SPX and age, CRP and IL-6, CRP and age, IL-6 and FSG, SOD and insulin. It was demonstrated that SPX is crucial to the pathogenesis of T2DM in postmenopausal women. In diabetic individuals, targeting SPX may offer a novel strategy for preserving adequate glucose control.

Keywords: CRP, Malondialdehyde, Spexin, Superoxide dismutase, T2DM

Introduction

Diabetes mellitus (DM) is a metabolic disease expressed as high blood sugar resulting from a dysfunction in insulin production, activity, or both. The eyes, kidneys, nerves, heart, and blood vessels are particularly vulnerable to long-term damage, dysfunction, and failure caused by chronic hyperglycemia, which is related to diabetes. [1]

The onset of diabetes is caused by multiple pathological mechanisms. These can vary from autoimmune damage to the pancreatic β-cells leading to insulin shortages to anomalies that cause insulin resistance. [2] DM is characterized by dysfunctional glucose, lipid, and protein metabolism, caused by insulin not acting on the tissues that need it. Inadequate secretion of insulin and/or reduced tissue responses to insulin at one or more points in the intricate hormone action pathways cause the insulin deficiency impact. [3]

Many patients have problems with both insulin secretion and action simultaneously, and it is frequently unclear which defect is the primary cause of hyperglycemia if one is alone. There are two types of diabetes symptoms: the first is marked, and the other is long-term. [4] Signs of severe hyperglycemia include the following: increased thirst, lack of appetite,

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* Corresponding Author.
E-mail addresses: mustafa123saleem@gmail.com (M. S. Shareef), dr.ali_bader@utq.edu.iq (Ali B. Roomi), charfeddinebassem@yahoo.fr (B. Charfeddine).

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hazy vision, and more frequent urine. Chronic hyperglycemia can lead to stunted growth and an increased risk of contracting certain illnesses.[5] Hyperglycemia with ketoacidosis, or ketoacidosis hyperosmolar syndrome, is one of the immediate and potentially fatal complications of uncontrolled diabetes.[6] Diabetes can cause retinopathy, which can lead to vision loss, and nephropathy, which can cause kidney failure.[7] Foot ulcers, amputations, and Charcot joints are all possible outcomes of peripheral neuropathy, while gastrointestinal, genitourinary, cardiovascular, and sexual dysfunction symptoms can be caused by autonomic neuropathy. Atherosclerosis of the coronary and peripheral arteries, as well as cerebrovascular disorders, are more common in diabetic patients. Diabetic individuals frequently exhibit hypertension and lipoprotein metabolism problems.[7]

Type 2 diabetes, termed noninsulin-dependent or adult-onset diabetes mellitus, is often associated with factors such as obesity, a sedentary lifestyle, a family history of diabetes, and increasing age. Management of Type 2 diabetes can often involve dietary interventions or the use of oral hypoglycemic agents. This form of diabetes accounts for a variable prevalence, encompassing 90–95% of all diabetes cases.[6] Research findings have indicated that reduced expression of the spexin gene and lower serum spexin concentrations are associated with obesity, T2DM, hypertension, and cardiovascular disease, particularly in the context of metabolic syndrome conditions.[7]

Materials and methods

Study population and samples collection

The study was approved by the Al-Hussein Teaching Hospital's Ethical Committee (IRB number: 01/2023).

The blood samples were collected from 180 postmenopausal women from Thi-Qar, Iraq; 90 of them visited the Thi-Qar Governorate's diabetes and endocrinology centers with T2DM disease, while the other 90 samples were collected from non-diabetic postmenopausal women who visited the governorate's Al-Hussein Teaching Hospital. The samples were collected between February 28 and April 17, 2023. The name, age, weight, height, waist circumference, and family history at the time of study of each woman were recorded.

Samples preparation

Two blood samples were taken; the first was taken in test tubes containing EDTA, and the second was taken in test tubes without EDTA and allowed to coagulate for at least fifteen minutes at room temperature. After that, the samples were centrifuged for ten minutes at 3600 rpm to separate the serum, which was then stored at −20 °C until it was needed for the biochemical analysis.

The whole blood was used to analyze glycated hemoglobin (HbA1c) (Catalogue number; 06378676190), while the serum was used for the analyses of SPX (Catalogue number; orb1140635), Malondialdehyde (MDA) (Catalogue number; EEA015) Superoxide dismutase (SOD) (Catalogue number; 15,665,069), C-reactive protein (CRP) (Catalogue number C0128), fasting blood glucose (FS (Catalogue number; 11351–05051), insulin (Catalogue number; 15140122), lipid profile, and interleukein 6 (IL-6) (Catalogue number; 341–36). The levels of FSG and insulin were used to evaluate the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) level.

Included criteria

Women with newly diagnosed diabetes were diagnosed by the doctors of the Thi-Qar Governorate's diabetes and endocrinology centers. This criterion was based on the causes of T2DM in the menopausal stage, which were frequent urination, obesity, autoimmunity, and insulin resistance.

Statistical analysis

The findings were expressed as mean ± SD by using BM's SPSS software version 24, and the difference between more than two groups was ascertained T-test. Bonferroni's analysis was employed. The parameters were correlated using the Pearson correlation (r) coefficient. SPX parameter's diagnostic viability was determined using ROC curve analysis or receiver operating characteristic curve analysis. Statistics were significant at $p < 0.001$.

Results and discussion

The anthropometric indications of healthy and postmenopausal women with T2DM are shown in Table 1. The mean age of the postmenopausal participants was in the mid-fifties, showing no significant difference ($P < 0.001$) between the control group (54.97 ± 4.31 years) and those with T2DM (56.21 ± 4.37 years). The waist circumferences of healthy postmenopausal women (78.98 ± 5.21 cm) and postmenopausal women with T2DM (80.70 ± 5.77 cm) were statistically significant ($P < 0.001$), and the

Table 1. The basic anthropometric criteria of the study subgroups expressed as means ± SD.

Parameter	Postmenopausal healthy women (N = 90)	Postmenopausal women with newly T2DM diagnosis (N = 90)	P-value
Age (year)	54.97 ± 4.31	56.21 ± 4.37	0.056
Waist circumference (cm)	78.98 ± 5.21	80.70 ± 5.77	0.037
BMI (kg/m^2)	25.70 ± 1.63	26.11 ± 1.64	0.096
WHtR	0.48 ± 0.03	0.49 ± 0.03	0.030

patients showed a higher waist circumference than the control. The differences in BMI were non-significant (P < 0.001) between postmenopausal healthy women (25.70 ± 1.63 kg/m^2) and postmenopausal women with T2DM (26.11 ± 1.64 kg/m^2). Postmenopausal women with T2DM (0.49 ± 0.03) showed significant (P < 0.001) differences in the waist-to-height ratio (WHtR) compared to postmenopausal healthy women (0.48 ± 0.03). Age is a crucial risk factor for many health disorders, including T2DM disease. Since this study was conducted on postmenopausal women, the age of the participants was above 45, and it was restricted upon the selection of the study participants to maintain the differences between control and patients in the non-significant region. So, the age was eliminated from the fluctuations of the studied parameters. Moreover, the BMI was treated similarly to avoid any influence of obesity or overweight on the levels of the studied parameters. Yet, there was a significant difference in the values of waist circumference and WHtR between the control and patients.[8,9]

The level of IL-6 was elevated significantly ($P < 0.01$) in postmenopausal women with newly diagnosed T2DM (13.45 ± 4.37 pg/mL) compared to postmenopausal healthy women (6.21 ± 2.13 pg/mL), as shown in Table 2. Stanimirovic et al. reported a significant increase in CRP levels in T2DM patients. They attributed this increase to the elevation of the overall inflammation occurring due to insulin resistance in T2DM patients.[9] Previous studies showed that even slight increases in the inflammatory marker CRP are indicative of a higher likelihood of experiencing cardiovascular events in both diabetic and non-diabetic individuals.[10] Furthermore, in individuals without prior health issues, T2DM risk raise in those with elevated C-reactive protein levels. Previous research suggested that CRP not only serves as a predictor for cardiovascular risk but may also play an active role in atherogenesis.[10,11] Sadeghabadi et al. observed a notable elevation in IL-6 levels in the serum of individuals with T2DM. They found a positive correlation between IL-6 levels and factors such as BMI, FSG, and HOMA-IR in these patients. This increase in IL-6 was attributed to the presence of insulin resistance among individuals with T2DM.[12,13]

The level of MDA elevated significantly (P < 0.01) in postmenopausal women with newly diagnosed T2DM (6.29 ± 2.02 μmol/L) compared to postmenopausal healthy women (1.72 ± 0.84 μmol/L) The level of SOD reduced significantly (P < 0.05) in postmenopausal women with newly diagnosed T2DM (4.08 ± 1.90 IU/mL) compared to postmenopausal healthy women (6.47 ± 1.96 IU/mL), as shown in Table 3.

The results of lipid profile parameters, including TG, TC, HDL, LDL, and VLDL, are shown in Table 4. The level of TG significantly increased (P < 0.001) in postmenopausal women with newly diagnosed T2DM (137.75 ± 27.63 mg/dL) compared to postmenopausal healthy women (120.15 ± 20.83 mg/dL). The level of TC non-significantly increased (P < 0.001) in postmenopausal women with newly diagnosed T2DM (167.17 ± 27.11 mg/dL) compared to postmenopausal healthy women

Table 2. The levels of inflammatory indicators in the study participants.

Parameter	Postmenopausal healthy women (N = 90)	Postmenopausal women with newly T2DM diagnosis (N = 90)	P-value
CRP (mg/L)	7.07 ± 2.30	14.12 ± 6.71	<0.001*
IL-6 (pg/dL)	6.21 ± 2.13	13.45 ± 4.37	<0.001*

Table 3. The levels of oxidative stress indicators in the study participants.

Parameter	Postmenopausal healthy women (N = 90)	Postmenopausal women with newly T2DM diagnosis (N = 90)	P-value
MDA (μmol/L)	1.72 ± 0.84	6.29 ± 2.02	<0.001*
SOD (IU/mL)	6.47 ± 1.96	4.08 ± 1.90	<0.001*

Table 4. The level of lipid profile parameters in the study participants.

Parameter	Postmenopausal healthy women (N = 90)	Postmenopausal women with newly T2DM diagnosis (N = 90)	P-value
TG (mg/dL)	120.15 ± 20.83	137.75 ± 27.63	<0.001*
TC (mg/dL)	159.82 ± 23.80	167.17 ± 27.11	0.055
HDL (mg/dL)	40.92 ± 5.66	39.00 ± 6.01	0.029
LDL (mg/dL)	94.87 ± 23.77	100.62 ± 25.90	0.123
VLDL (mg/dL)	24.03 ± 4.17	27.55 ± 5.53	<0.001*

Table 5. Correlation among parameters in T2DM postmenopausal women.

	SPX		CRP		IL-6	
Parameters	R	*P*	R	*P*	R	*p*
CRP	0.173	0.103	-	-	**0.330***	**0.001**
IL-6	−0.024	0.825	**0.330***	**0.001**	-	-
MDA	**0.214***	**0.042**	−0.054	0.613	−0.097	0.363
Age	**0.324***	**0.002**	**0.304***	**0.004**	0.075	0.485
FSG	**−0.250***	**0.018**	−0.074	0.488	**0.229***	**0.030**

(159.82 ± 23.80 mg/dL). The level of HDL reduced significantly (P < 0.001) in postmenopausal women with newly diagnosed T2DM (39.00 ± 6.01 mg/dL) compared to postmenopausal healthy women (40.92 ± 5.66 mg/dL). The level of LDL increased non-significantly (P < 0.001) in postmenopausal women with newly diagnosed T2DM (100.62 ± 25.90 mg/dL) compared to postmenopausal healthy women (94.87 ± 23.77 mg/dL). The level of VLDL significantly increased (P < 0.001) in posstmenopausal women with newly diagnosed T2DM (27.55 ± 5.53 mg/dL) compared with postmenopausal healthy women (24.03 ± 4.17 mg/dL). The outcomes of lipid profile parameters, including TG, TC, HDL, LDL, and VLDL, are shown in Table 4.

The level of TG significantly increased (P < 0.001) in postmenopausal women with newly diagnosed T2DM (137.75 ± 27.63 mg/dL) compared with postmenopausal healthy women (120.15 ± 20.83 mg/dL). Tejaswi et al. reported significant alteration in the levels of lipid profile parameters, where the levels of TG, TC, LDL, and VLDL elevated while the level of HDL decreased in T2DM patients. [14,15]

Postmenopausal women with newly diagnosed T2DM disease showed a significant positive correlation between CRP and IL-6 (r = 0.0.330, p = 0.001), CRP and age (r = 0.304, p = 0.004), IL-6 and FSG (r = 0.229, p = 0.030), as shown in Table 5. Postmenopausal women with newly diagnosed T2DM disease showed a significant positive correlation between SPX and MDA (r = 0.214, p = 0.042), SPX and age (r = 0.324, p = 0.002), SPX and FSG (r = −0.250, p = 0.018), as shown in Table 5. Tejaswi et al. reported a significant reduction of SPX levels in T2DM patients. They indicated that SPX levels correlate with T2DM in patients. [14]

The present study on postmenopausal women with Type 2 Diabetes Mellitus (T2DM) highlights the potential of interleukin-6 (IL-6) as a valuable biomarker for disease prognosis, with an excellent sensitivity of AUC = 0.941 and a cut-off value of 8.45 pg/dL, providing 91.1% sensitivity and 80.1% specificity. Additionally, malondialdehyde (MDA) and superoxide dismutase (SOD) were also evaluated as biomarkers for T2DM prognosis in postmenopausal women, showing promising results. MDA exhibited excellent sensitivity with an AUC of 0.990, a cut-off value of 2.90 μ mol/L, 97.8% sensitivity, and 90% specificity. On the other hand, SOD demonstrated good sensitivity with an AUC of 0.816, a cut-off value of 4.99 IU/mL, 80% sensitivity, and 72.2% specificity. These findings suggest the potential of IL-6, MDA, and SOD as prognostic biomarkers for T2DM in postmenopausal women, with IL-6 showing particularly promising sensitivity and specificity levels, as shown in Table 6. and Figs. 1 to 3. Chang et al. reported a significant alteration in the levels of IL-6, MDA and SOD in T2DM. [16] Both MDA and SOD are indicators of the redox state and

Table 6. ROC analysis outcomes.

Parameters	AUC	Standard error	*p*–value	Cut-off value	Sensitivity	Specificity
IL-6 (pg/dL)	0.941	0.018	<0.001	8.45	91.1%	80.1%
MDA (μmol/L)	0.990	0.005	<0.001	2.90	97.8%	90%
SOD (IU/mL)	0.816	0.032	<0.001	4.99	80%	72.2%

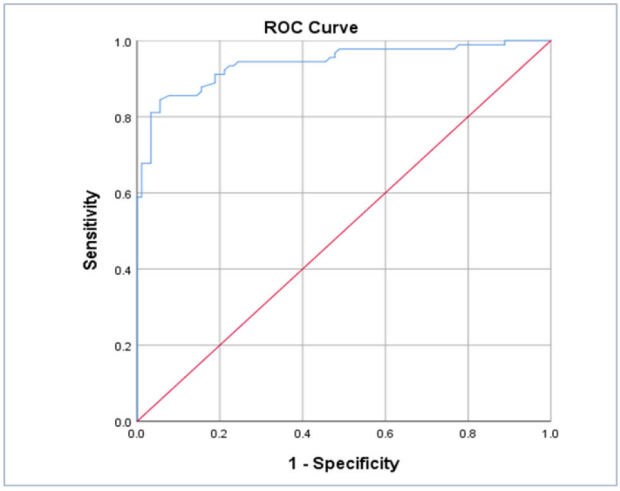

Fig. 1. The ROC curve of IL-6 in the prognosis of postmenopausal women with T2DM disease.

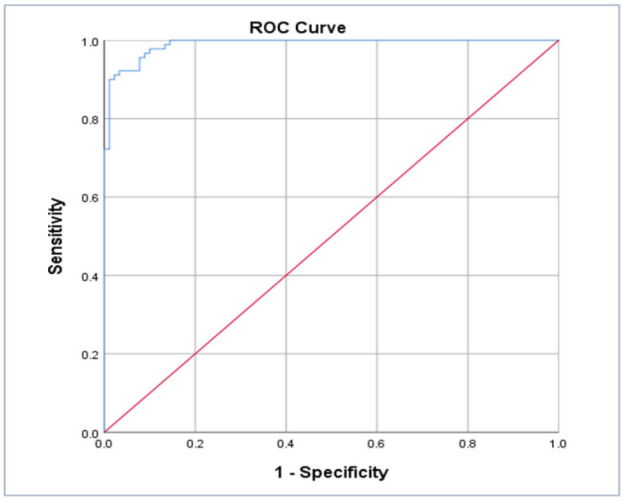

Fig. 2. The ROC curve of MDA in the prognosis of postmenopausal women with T2DM disease.

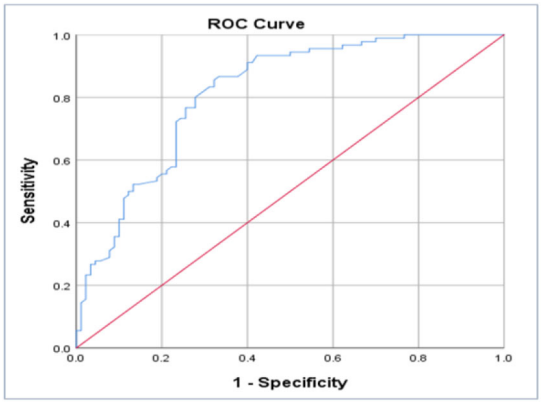

Fig. 3. The ROC curve of SOD in the prognosis of postmenopausal women with T2DM disease.

oxidative stress, which arises from high levels of ROS. There are many pathways in which ROS levels are elevated in TIIDM patients, most importantly high blood glucose levels and insulin resistance.[17] The electron transport system in the mitochondria is another route that contributes to oxidative stress. Adenosine triphosphate is a crucial organic energy source created in the mitochondrial inner membrane, where this system is found. Water molecules are formed in this electron transfer system by deoxidizing four oxygen molecule electrons; ROS is created as an intermediate product during this process, and some ROS escape from the system.[18]

Conclusion

Spexin levels are reduced significantly in postmenopausal women with T2DM disease, which reflects a state of dysregulation of glucose homeostasis and lipid metabolism. Targeting SPX in T2DM patients may improve the glycemic state of postmenopausal patients. Postmenopausal women with T2DM disease have shown a significant increase in MDA levels and a significant decrease in SOD, indicating a condition of oxidative stress.

Acknowledgment

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Thi-Qar.

Authors' contribution statement

M. S. S, A. B. R and B. C. designed and conducted the research; A. B. R. and M .S. S performed the data

management and statistical analyses; M .S. S and A. B. R wrote the manuscript. B. C., A. B. R and M. S. S reviewed/edited the manuscript for important intellectual content. A. B. R, M. S. S and B. C. read and approved the final manuscript.

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سبيكسين كمؤشر لدى العراقيات المصابات بالسكري في سن اليأس

مصطفى سليم شريف [1]، علي بدر رومي [2]، باسم شرف الدين [3]

[1] قسم الكيمياء الحيوية، كلية التقنيات الصحية والطبية، جامعة البيان، بغداد، العراق.
[2] قسم الكيمياء، كلية العلوم، جامعة ذي قار، ذي قار، 64001، العراق.
[3] قسم الكيمياء الحيوية، كلية الطب، جامعة سوسة، تونس.

الخلاصة

ببتيد سبكسين (SPX) ينظم نسبة الجلوكوز في الدم واستقلاب الدهون. ركزت على مسببات مرض السكري من النوع 2 (T2DM) لدى النساء بعد انقطاع الطمث، ودراسة علاقتها بمستويات SPX في الدم. الطريقة: دراسة الحالات والشواهد تطوعت 180 امرأة بعد انقطاع الطمث من ذي قار. تم تسجيلها في الدراسة الحالية. تم تشخيص إصابة 90 امرأة بالسكري في مرحلة ما بعد انقطاع الطمث، في حين أن الـ 90 الأخرى غير مصابات بالسكري بعد انقطاع الطمث. تم جمع عينات الدم لقياس HbA1c و SPX و MDA و SOD و CRP ، جلوكوز الدم الصائم (FSG))، الأنسولين، lipid Profile والإنترلوكين (IL-6). النتائج: ارتفاع بقيم FSG، وHbA1c، والأنسولين، مقاومة الأنسولين (HOMA-IR) بشكل ملحوظ (P <0.001) في النساء بعد انقطاع الطمث من النوع T2DM. كما أن مستويات MDA وCRP وIL-6 كانت مرتفعة بشكل كبير، كما انخفضت مستويات SPX وSOD بشكل ملحوظ (P<0.001). وقد لوحظت تغييرات كبيرة في الدهون في النساء بعد انقطاع الطمث وT2DM .أظهر الارتباط في T2DM لدى النساء بعد انقطاع الطمث علاقة سلبية بين SPX مع نسبة الخصر إلى الطول، SPX مع FSG، SPX مع HOMA-IR، MDA مع SPX، وجود علاقة إيجابية بين SPX وMDA، SPX والعمر، CRP وIL-6، CRP والعمر، IL-6 وFSG، SOD والأنسولين. الاستنتاج: لقد ثبت أن انخفاض SPX مهم في التسبب في مرض T2DM لدى النساء بعد انقطاع الطمث. ، قد يوفر استهداف SPX استراتيجية جديدة للحفاظ على التحكم في الجلوكوز.

الكلمات المفتاحية: بروتين سي التفاعلي، مالونديالدهيد، سبيكسين، سوبروكسيد ديسموتاز ومرض السكري من النوع الثاني.