The Review of DIABETIC STUDIES

RESEARCH

The Association between Spexin Levels, Lipid Profiles, Glucose Metabolism, and Immune Function in Newly Diagnosed Diabetes in Postmenopausal Iraqi Women

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

[1]College of Health and Medical Techniques, AL-Bayan University, Baghdad, Iraq. [2]Department of Biochemistry, Faculty of Medicine, University of Sousse, Tunisia. [3]Department of Quality Assurance, University of Thi-Qar, Thi-Qar, 64001, Iraq. Address correspondence to: Mustafa S. Shareef, Email: mustafa123saleem@gmail.com

Manuscript Submitted November 19, 2023; Resubmitted December 02, 2023; Accepted December 19, 2023

■ Abstract

Background: Type 2 diabetes mellitus (TIIDM) is a metabolic disorder in which the level of glucose is elevated in the blood as a consequence of insulin resistance. Spexin (SPX) is a peptide involved in the regulation of blood glucose and lipid metabolism. **Aims:** The study aims were targeting the pathophysiology of TIIDM in postmenopausal women (PMW), where it focused on the evaluation of SPX levels in the serum of TIIDM-PMW. **Results:** There was a significant (P<0.001) difference in waist circumference and waist-to--to- height ratio between TIIDM and non-diabetic PMW, which indicates more central fat in patients. FSG, HbA1c, insulin, and HOMA-IR levels were elevated significantly in TIIDM PMW, indicating poor glycemic control. SPX and SOD levels were significantly reduced in TIIDM patients, while MDA, CRP, and IL-6 levels were significantly increased, reflecting oxidative stress and inflammation. The lipid profile was changed significantly in TIIDM PMW. Moreover, the correlation in TIIDM PMW indicated a positive association between SPX and MDA, SPX and age, CRP and IL-6, CRP and age, IL-6 and FSG, SOD and insulin, and a negative association between SPX and waist-to-height ratio, SPX and FSG, SPX and HOMA-IR, and MDA and insulin. At last, SPX has shown excellent sensitivity in the prognosis of TIIDM in PMW. **Conclusion:** SPX has been shown to play an important role in the pathophysiology of TIIDM in PMW. Targeting SPX may provide a novel technique to maintain good glycemic control in diabetic patients.

Keywords: Type 2 Diabetes Mellitus, Lipid Profile, MDA, CRP, IL-6.

1. Introduction

Spexin (SPX), is a newly identified neuropeptide hormone encoded by the Ch12orf39 gene, which is located on the q short arm of chromosome 12 in humans. The Spexin gene contains six exons and five introns. Spexin is co-evolved with the galanin/Kisspeptin family [1]. Glucose homeostasis improves glucose tolerance & insulin resistance in type 2 diabetes (TIIDM), and decreases serum SPX level in TIIDM [2, 3]. Spexin is also likely to be involved in reproduction, cardiovascular/renal function and harmful effects. The precise function of SPX in these activities, despite the widespread expression of SPX, is unknown due to a lack of knowledge about the SPX receptor in human tissues indicates its potential involvement in many still-to-be-established physiological functions [4]. Spexin is well-known in TIIDM, but its relation to cardiovascular problems such as insulin resistance and dyslipidemia needs to be demonstrated. Since cardiovascular morbidity and mortality are more likely to develop in people with metabolic syndrome. Therefore, it is interesting to examine the relationship between plasma insulin levels and the LDL/HDL cholesterol ratio in diabetes mellitus [5]. Chronic conditions such as insulin resistance, obesity, and TIIDM were reported to have reduced amounts of circulating SPX [6-9]. The study aims were targeting the pathophysiology of TIIDM in postmenopausal women (PMW), where it focused on the evaluation of SPX levels in the serum of TIIDM PMW, predicting its role in the pathophysiology of the disease, and the possibility of using it as a biomarker for predicting the diabetic complications. Moreover, the correlation of SPX with sugar profile, lipid profile, oxidative stress including malondialdehyde (MDA) and superoxide dismutase (SOD), inflammatory indicators including C-reactive protein (CRP) and interleukin-6 (IL-6) was aimed in this study.

2. Materials and Methods

Sample collection: Samples were taken from women

from diabetes and endocrinology centres in the Thi-Qar Governorate, while controls were collected from Al-Hussein Teaching Hospital in the same governorate. The Ethical Committee of Al-Hussein Teaching Hospital granted the study approval (IRB number: 01/2023). The samples were obtained from (2/28 to 4/17/2023), taking into account the name, age, and family history at the time of the search mechanism. The obtained samples are placed in clean, plain test tubes and allowed to clot for at least 15 minutes at room temperature. Following this, the samples are centrifuged at (3600 rpm) for 10 minutes to separate the serum for biochemical analysis. Hemolysis samples were discarded, and the recovered serum was divided into various tubes and refrigerated at -20 °C for analysis later. The tests indicated below will be performed in line with the procedure (kit user handbook) laid out by the product's manufacturer. ELISA kits (catalogue numbers KBH3507 and L-4260, Krishgen Biosystems India) were used to measure the serum SPX levels in accordance with the manufacturer's protocol.

Methods: The study included 180 PMW from Thi-Qar, Iraq. Ninety women were recently diagnosed with TIIDM disease, while the other 90 were non-diabetic PMW. The age, weight, height, and waist circumference of each woman were registered, and the vein blood was withdrawn. The whole blood was used to analyze glycated haemoglobin (HbA1c) while the rest of the blood was left to clot, then centrifuged to collect the serum which was used for the analyses of SPX, MDA, SOD, CRP, fasting blood glucose (FSG), insulin, lipid profile, and IL-6. The levels of FSG and insulin were used to evaluate the HOMA-IR level. All obtained data were processed statistically for mean comparison using an independent sample t-test, correlation using Pearson's analysis, and receiver operating characteristic (ROC) for predicting the sensitivity of the tests as biomarkers for disease prognosis.

Included criteria: Women with new diabetes were diagnosed by the doctors of the above-mentioned centre. This criterion was based on the causes of TIIDM in the menopausal stage, which are frequent urination, obesity, autoimmunity, and insulin resistance.

Statistical analysis: The gathered data were displayed as mean±SD. One-way analysis of variance to determine the difference between more than two groups, Bonferroni's analysis was employed. The parameters are correlated using the Pearson correlation (r) coefficient. For the investigation, an SPX parameter's diagnostic viability was evaluated using ROC curve analysis, or receiver operating characteristic curve analysis. Statistics were judged significant at $p<0.001$. All statistical analysis was carried out utilising IBM's SPSS software, version 24.

3. Results

Anthropometrics: The anthropometric indications of healthy and diseased PMW are shown in Table 1. The average age of postmenopausal participants was in the mid-fifties, where the differences were non-significant (P<0.001) between control (54.97±4.31 years) and TIIDM patients (56.21±4.37 years. The waist circumference of healthy PMW (78.98±5.21 cm), and PMW with newly diagnosed TIIDM (80.70±5.77cm) were statistically significant (P<0.001) where the patients showed higher waist circumference than control. The differences in the BMI were non-significant (P<0.001) between postmenopausal healthy women (25.70±1.63kg/m^2) and postmenopausal women with TIIDM (26.11±1.64kg/m^2). PMW with a new diagnosis of TIIDM disease (0.494±0.039) has shown significant (P<0.001) differences of waist to waist-to-height ratio (WHtR) compared to postmenopausal healthy women (0.483±0.032).

Table 1: The Basic Anthropometric Criteria of the Study Subgroups.

Parameter (means±SD)	Health PMW (N=90)	PMW/newly Diagnosed TIIDM (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	0.483±0.032	0.494±0.039	0.030

The sugar profile included fasting serum glucose (FSG), glycated haemoglobin (HbA1c), insulin, and HOMA-IR, all are shown in Table 2. The level of FSG was significantly higher (P<0.001) in PMW with newly diagnosed TIIDM (10.25±2.15 mmol / L) compared to postmenopausal healthy women (5 .09±0.33mmol/L). The level of HbA1c was significantly higher (P<0.001) in PMW with newly diagnosed TIIDM (8.30±1.39 %) compared to postmenopausal healthy women (5 .16±0.37%). The level of insulin was significantly higher (P<0.001) in PMW with newly diagnosed TIIDM (15.77±2.43 µIU / mL) compared to postmenopausal healthy women (8.65±2.75µIU / mL). The level of HOMA-IR was significantly higher (P<0.001) in PMW with newly diagnosed TIIDM (7.21±2.04 mmol / L×µIU / mL) compared to postmenopausal healthy women (1 .96±0.64 mmol / L×µIU / mL).

Table 2: Sugar Profile Levels in the Study Participants.

Parameter (means±SD)	Health PMW (N=90)	PMW/newly Diagnosed TIIDM (N=90)	p value
Serum Fasting glucose (mmol/L)	5.09±0.33	10.25±2.15	<0.001*
HbA1c (%)	5.16±0.37	8.30±1.39	<0.001*
Serum Insulin (µIU/mL)	8.65±2.75	15.77±2.43	<0.001*
HOMA-IR (mmol/L × µIU/mL)	1.96±0.64	7.21±2.04	<0.001*

The level of SPX was reduced significantly (P<0.001) in postmenopausal women with newly diagnosed TIIDM (38.64±11.70pg/mL) compared to postmenopausal healthy women (64.26±15.43 pg/mL), as shown in Table 3.

Table 3: The Level of SPX in the Study Participants.

Parameter (means±SD)	Health PMW (N=90)	PMW/newly Diagnosed TIIDM (N=90)	p value
SPX (pg/mL)	64.26±15.43	38.64±11.70	<0.001*

The results of lipid profile parameters including; TGs, TC, HDL, LDL, and VLDL are shown in Table 4. The level of TGs was increased significantly (P<0.001) in PMW with newly diagnosed TIIDM (137.75±27.63mg/dL) compared to postmenopausal healthy women (120.15±20.83mg/dL). The level of TC was increased non-significantly (P<0.001) in PMW with newly diagnosed TIIDM (167.17±27.11mg/dL) compared to postmenopausal healthy women (159.82±23.80mg/dL). The level of HDL was reduced significantly (P<0.001) in PMW with newly diagnosed TIIDM (39.00±6.01mg/dL) compared to postmenopausal healthy women (40.92±5.66mg/dL). The level of LDL was increased non-significantly (P<0.001) in PMW with newly diagnosed TIIDM (100.62±25.90 mg/dL) compared to postmenopausal healthy women (94.87±23.77mg/dL). The level of VLDL was increased significantly (P<0.001) in PMW with newly diagnosed TIIDM (27.55±5.53mg/dL) compared to postmenopausal healthy women (24.03±4.17mg/dL).

Table 4: The Level of Lipid Profile Parameters in the Study Participants.

Parameter (means±SD)	Health PMW (N=90)	PMW/newly Diagnosed TIIDM (N=90)	p value
TGs (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*

The ROC test of SPX indicated an excellent sensitivity (AUC=0.909) of using SPX as a biomarker for the prognosis of TIIDM disease in PMW. Moreover, the cut-off value was evaluated as 46.33 pg/mL with 85.6% sensitivity and 77.8% specificity (Table 5, Figure 1). The ROC test of CRP was indicated as good sensitivity (AUC=0.853) of using CRP as a biomarker for the prognosis of TIIDM disease in PMW. Moreover, the cut-off value was evaluated as 8.87 mg/L with 78.8% sensitivity and 80% specificity (Table 5, Figure 2).

Table 5: ROC Analysis Outcomes.

Parameters (means ± SD)	AUC	Standard error	*p*-value	Cut-off value	Sensitivity	Specificity
SPX (pg/mL)	0.909	0.020	<0.001	46.33	85.6%	77.8%
CRP (mg/L)	0.853	0.029	<0.001	8.87	78.8%	80%

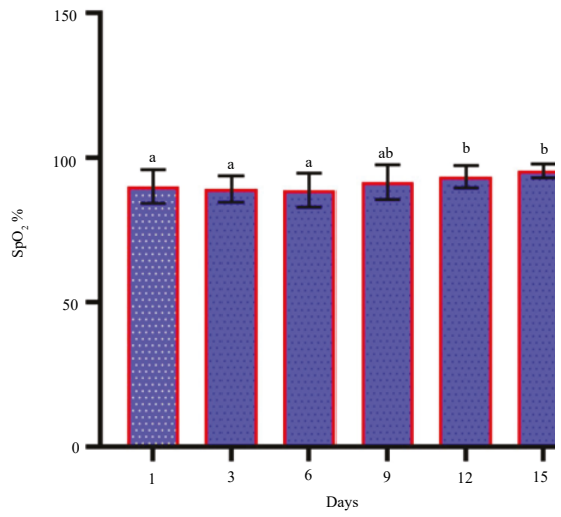

Figure 1: The ROC Curve of SPX in the Prognosis of PMW with TIIDM Disease.

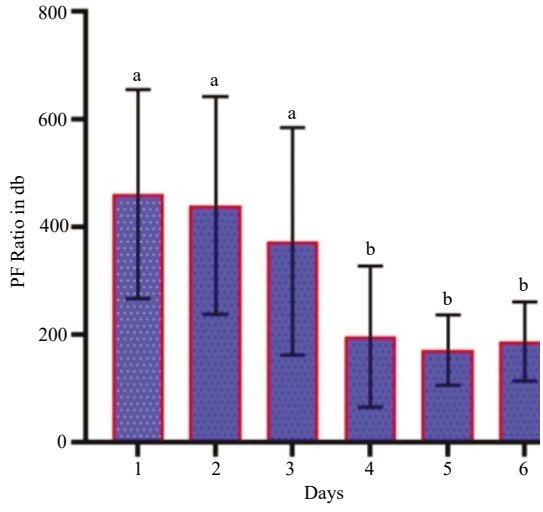

Figure 2: The ROC Curve of CRP in the Prognosis of PMW with TIIDM Disease.

4. Discussion

The average age of postmenopausal participants was in the mid-fifties, where the differences were non-significant (P <0.001) between control (54.97±4.31years) and TIIDM patients (56.21±4.37years). Age is a crucial risk factor for many health disorders, including TIIDM disease. Since this study was conducted on PMW, the age of the participants was above 45, but it was restricted upon the selection of the study participants to maintain the differences between control and patients in the non-significant region. This would result in the elimination of age involvement in the fluctuations of the studied parameters. Moreover, the BMI was also treated similarly to avoid any influence of obesity/ overweight on the levels of the studied parameters. Yet, there was a significant difference in the values of waist circumference and WHtR between controls and patients [10, 11]. The sugar profile included fasting serum glucose (FSG), glycated haemoglobin (HbA1c), insulin, and HOMA-IR (Table 2). The level of FSG was significantly higher (P<0.001) in PMW with newly diagnosed TIIDM (10.25±2.15 mmol / L) compared to postmenopausal healthy women (5.09±0.33mmol/L).

The diagnosis of TIIDM depends on the levels of FSG and HbA1c, while insulin resistance is indicated by the level of HOMA-IR. The levels of FSG and HbA1c indicated poor glycemic control (HbA1c>8%) of the postmenopausal patients with TIIDM disease [12]. Poor glycemic control can lead to the deterioration of patients' health by increasing the risk of infection, the risk of cancer recurrence, symptom severity, pain severity, hospitalization, and mortality, and it can cause a reduction in the quality of life [13]. Karaca et al. (1019), have reported a significant increase in FSG and HbA1c in patients with TIIDM disease [14]. Tejaswi et al. (2021), have reported a significant increase in HbA1c and insulin levels in TIIDM patients, indicating the presence of insulin resistance in patients [15]. Gowdu et al. (2021) have reported a significant increase in FSG and HOMA-IR in TIIDM patients [16].

The level of SPX was reduced significantly (P<0.001) in PMW with newly diagnosed TIIDM (38.64±11.70pg/mL) compared to postmenopausal healthy women (64.26±15.43 pg/mL) (Table 3) Karaca et al. (2019), have reported a significant reduction in the serum level of SPX in patients with TIDM and TIIDM diseases. They have concluded that the expression of SPX is reduced in the pancreas of patients regardless of their glycemic control [14]. Kolodziejski*et al.* [17], have indicated that the administration of SPX in obese and diabetic mice has improved the glycemic state and metabolic functions during 30 days of administration [17]. They have concluded that SPX exhibited anti-inflammatory, hormonal regulating, and glycemic control effects [17-19].

The results of lipid profile parameters including; TGs, TC, HDL, LDL, and VLDL (Table 4) show the level of TGs was increased significantly (P<0.001) in PMW with newly diagnosed TIIDM (137.75±27.63mg/dL) compared to postmenopausal healthy women (120.15±20.83mg/dL), Tejaswi*et al.* [15], have reported significant alteration in the levels of lipid profile parameters, where the levels of TGs, TC, LDL, and VLDL were elevated while the level of HDL was reduced in TIIDM patients [15].

This means that when the level of SPX drops below 46.33 pg/mL in the serum of PMW, there would be a chance of 85.6% that the women would have TIIDM disease. This means that when the level of CRP rises above 8.87mg/L in the serum of PMW, there would be a chance of 78.8% that the women would have TIIDM disease. In addition to these aforementioned impacts of diabetes on SPX levels other proinflammatory cytokines released by cells into localized milieu [20-22] should be also considered.

5. Conclusion

PMWs with TIIDM disease have shown low-grade inflammation caused by ageing, hyperglycemia, and insulin resistance. Spexin level was reduced significantly in PMW with TIIDM disease, which reflects a state of regulation of glucose homeostasis and lipid metabolism. Targeting SPX in TIIDM patients may improve the glycemic state of postmenopausal patients. PMW with TIIDM disease has shown a significant increase in MDA levels with a significant decrease in SOD, indicating a condition of oxidative stress. Abdominal fat in PMW with TIIDM disease may be a major contributor to poor glycemic control and severe symptoms. The fluctuation in lipid profile parameters in PMW with TIIDM disease is a major responsible for the development of diabetic complications.

Acknowledgements: This research has been conducted in diabetes and endocrinology centres in Thi-Qar Governorate and Al-Hussein Teaching Hospital . We especially thank all the staff of the Al-Hussein Teaching Hospital and all the people involved in building the study.

Conflict of Interest Disclosures: There are no material financial or non-financial interests to disclose for the authors.

Funding Statement: Self-Funded

Authors' Contributions: MSS, BC and ABR designed and conducted the research; MSS and ABR performed the data management and statistical analyses; MSS and BC wrote the manuscript. MSS, BC and ABR reviewed/edited the manuscript for important intellectual content. MSS, BC and ABR read and approved the final manuscript.

■ References

1. **Mills EG, Izzi-Engbeaya C, Abbara A, Comninos AN, Dhillo WS**. Functions of galanin, spexin and kisspeptin in metabolism, mood and behaviour. *Nature Reviews Endocrinology* 2021. 17(2):97-113.
2. **Klimontov V, Mavlianova K, Semenova J, Orlov N**. Circulating peptides of the tnf superfamily and tnf receptor superfamily in subjects with type 1 diabetes: Relationships with clinical and metabolic parameters. *Georgian Medical News* 2023. (340-341):243-8.

3. **Ahmadov G**. Analysis of clinical and laboratory parameters children with diabetes mellius type 1 using different types of insulin preparations. *Georgian Medical News* 2023. (336):63-5.
4. **Reyes-Alcaraz A, Lee Y-N, Son GH, Kim NH, Kim D-K, Yun S, et al.** Development of spexin-based human galanin receptor type II-specific agonists with increased stability in serum and anxiolytic effect in mice. *Scientific reports* 2016. 6(1):21453.
5. **Mohammad JA, Fathi FH, Almulathanon AA, Merkhan MM**. Hyperlipidemia connoted vitiation of serum adipokines and redox imbalances. *Military Medical Science Letters/Vojenské Zdravotnické Listy* 2023. 92(2).
6. **Chen T, Wang F, Chu Z, Sun L, Lv H, Zhou W, et al.** Circulating spexin decreased and negatively correlated with systemic insulin sensitivity and pancreatic β cell function in obese children. *Annals of Nutrition and Metabolism* 2019. 74(2):125-31.
7. **Khadir A, Kavalakatt S, Madhu D, Devarajan S, Abubaker J, Al-Mulla F, et al.** Spexin as an indicator of beneficial effects of exercise in human obesity and diabetes. *Scientific Reports* 2020. 10(1):10635.
8. **Kumar S, Hossain J, Nader N, Aguirre R, Sriram S, Balagopal PB**. Decreased circulating levels of spexin in obese children. *The Journal of Clinical Endocrinology & Metabolism* 2016. 101(7):2931-6.
9. **Kumar S, Mankowski RT, Anton SD, Babu Balagopal P**. Novel insights on the role of spexin as a biomarker of obesity and related cardiometabolic disease. *International Journal of Obesity* 2021. 45(10):2169-78.
10. **Darsini D, Hamidah H, Notobroto HB, Cahyono EA**. Health risks associated with high waist circumference: A systematic review. *Journal of public health research* 2020. 9(2):jphr. 2020.1811.
11. **Piqueras P, Ballester A, Durá-Gil JV, Martinez-Hervas S, Redón J, Real JT**. Anthropometric indicators as a tool for diagnosis of obesity and other health risk factors: a literature review. *Frontiers in Psychology* 2021. 12:631179.
12. **Anetor JI, Uche CZ, Ayita EB, Adedapo SK, Adeleye JO, Anetor GO, et al.** Cadmium level, glycemic control, and indices of renal function in treated type II diabetics: implications for polluted environments. *Frontiers in public health* 2016. 4:114.
13. **Younis HY, Thanoon IA, Fadhil NN, Merkhan MM**. Effect of zinc as an add-on to metformin therapy on glycemic control, serum insulin, and c-peptide levels and insulin resistance in type 2 diabetes mellitus patient. *Research Journal of Pharmacy and Technology* 2022. 15(3):1184-8.
14. **Karaca A, Bakar-Ates F, Ersoz-Gulcelik N**. Decreased spexin levels in patients with type 1 and type 2 diabetes. *Medical Principles and Practice* 2019. 27(6):549-54.
15. **Tejaswi G, Dayanand C, Prabhakar K**. Insulin resistance and decreased spexin in Indian patients with type 2 diabetes mellitus. *Bioinformation* 2021. 17(9):790.
16. **Gowdu T, Balakrishna S, Kamarthi P**. Spexin gene polymorphism in type 2 diabetes mellitus patients of South Indian population. *Romanian Journal of Diabetes Nutrition and Metabolic Diseases* 2021. 28(4):407-17.
17. **Kolodziejski PA, Leciejewska N, Chmurzynska A, Sassek M, Szczepankiewicz A, Szczepankiewicz D, et al.** 30-Day spexin treatment of mice with diet-induced obesity (DIO) and type 2 diabetes (T2DM) increases insulin sensitivity, improves liver functions and metabolic status. *Molecular and Cellular Endocrinology* 2021. 536:111420.
18. **Nori W, Hamed RM, Roomi AB, Akram W**. Alpha-1antitrypsin in pre-eclampsia; from a clinical perspective. *J Pak Med Assoc* 2021. 71(12):S53-6.
19. **Roomi AB, AL-Salih R, Ali SA**. The effect insulin therapy and metformin on osteoporosis in diabetic postmenopausal Iraqi women. *Indian Journal of Public Health* 2019. 10(4):1479.
20. **Shephard MT, Merkhan MM, Forsyth NR**. Human Mesenchymal Stem Cell Secretome Driven T Cell Immunomodulation Is IL-10 Dependent. *International Journal of Molecular Sciences* 2022. 23(21):13596.
21. **Merkhan MM, Shephard MT, Forsyth NR**. Physoxia alters human mesenchymal stem cell secretome. *Journal of Tissue Engineering* 2021. 12:20417314211056132.
22. **Chen L, Merkhan MM, Forsyth NR, Wu P**. Chorionic and amniotic membrane-derived stem cells have distinct, and gestational diabetes mellitus independent, proliferative, differentiation, and immunomodulatory capacities. *Stem cell research* 2019. 40:101537.