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Association of Serum Level of Substance P with Glycemic Control Indices and Lipids Profile in Non-Obese Type 2 Diabetic Patients

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Abstract

Hyperglycemia and hyperinsulinemia which are associated with type 2 diabetes mellitus, are the main causes of metabolic abnormalities that result in a wide range of complications, such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy. Substance-P is an 11-amino acid neuropeptide that is highly conserved and secreted by sensory nerve endings as well as different types of non-neural cells. It was believed that substance P would reduce inflammation triggered by diabetes, interrupting the development of complications associated with this disease. The current study aims to investigate whether serum levels of substance P are associated with glycemic measures and /or lipids profile in non-obese type 2 diabetes mellitus patients. This case-control study involved eighty-five adult subjects (46 males and 39females), aged (30-60) years, and were divided into two groups; the first group involved 53 non-obese type 2 diabetic patients, and the second group involved 32 apparently healthy individuals of matching age, sex, and body mass index to the patients' group. The fasting serum glucose, insulin, homeostatic model assessment-insulin resistance (HOMA-IR), total cholesterol, triglycerides, and glycated hemoglobin A1c exhibited significantly higher levels in diabetic patients in comparison with the control group, (P =0.001). Whereas, the high-density lipoprotein levels were markedly lower in diabetic patients compared to the levels in the control group, (P =0.001). Serum substance P levels were significantly lower in the non-obese diabetic patients than those in the control subjects [181.49(79.93) pg/ml, and 445.40(136.24) pg/ml; respectively, (P < 0.001). Furthermore, neither the glycemic control indices nor the lipid profile components, that were examined, demonstrated any noteworthy associations with serum substance P levels, (P>0.05). In conclusion, the lower serum substance P levels in non-obese type 2 diabetic patients compared to control subjects, suggest a potential role of substance P in the pathophysiology of type 2 diabetes mellitus.

Keywords: HOMA -IR, Insulin sensitivity, Lipid profile, Substance P, Type 2 Diabetes Mellitus

Introduction

Diabetes mellitus (DM) is a metabolic condition characterized by a persistent elevation in blood glucose levels and different degrees of disruption in metabolizing carbohydrates, lipids, and proteins [1–3]. Prolonged hyperglycemia leads to severe sequelae in different organs including the retina resulting in vision impairment, nephropathy leading to kidney failure, vascular problems contributing to cardiovascular conditions, and peripheral neuropathy[4,5]. Individuals diagnosed with type-2 diabetes are more prone to developing cardiovascular disease due to a condition called atherogenic dyslipidemia. Coronary artery disease, particularly myocardial infarction, is the primary cause of illness and death of diabetics on a global basis[6] .

Sensory nerve endings and a variety of non-neural cells secrete the 11-amino acid neuropeptide known as Substance-P (SP). It is present in all parts of the neurological system, including the central nervous system and the peripheral nervous system. SP utilizes the G-protein coupling receptor pathway to send out signals upon binding strongly to the neurokinin receptor-1[7] .Conditions such as spinal cord injury, diabetic ulcers, and rheumatoid arthritis have demonstrated notable reduction when treated with SP[8].

Moreover, SP has been utilized in the management of several conditions, including depression, anxiety, stress, chemotherapy-induced nausea, and inflammatory bowel disease [9] . It has been shown that SP expression is dysregulated in both type 1 and type 2 diabetes. Researches have shown that SP can influence glucose levels by influencing insulin signaling in adipocytes and at a systemic level[10] . Moreover, SP can prevent endothelial dysfunction in the context of hyperglycemia and may have antioxidant properties that could be beneficial for treating diabetic complications [11].

This study aims to evaluate the serum levels of SP in non-obese T2DM patients in comparison with healthy individuals; and to investigate the potential correlations between serum SP levels with the glycemic control indices and with the lipid profile.

Materials and Methods

This case-control study was carried out at the National Center of Diabetes Treatment and Research/ Al-Mustansryia University – College of Medicine; from October 2023 to January 2024. The study included fifty-three non-obese T2DM patients who were selected under the supervision of a specialized endocrinologist; along with thirty- two healthy subjects of matching age, sex and body mass index (BMI) to the patients' group to serve as the control group.

Inclusion criteria

The selected patients were adult T2DM patients with a minimum disease duration of one year, a BMI (18.6-29.9 kg\m^2), and were receiving oral hypoglycemic medications only, for the treatment of diabetes and never received insulin therapy.

Exclusion criteria

T2DM patients with the following conditions were excluded from the study: alcohol consumption, dyslipidemia, autoimmune disease, chronic inflammatory diseases, chronic kidney and liver diseases, malignancy, pregnancy or lactation, and any other endocrinopathies or other types of diabetes mellitus.

Ethical concerns

The study was approved by the Research Ethics Committee at the University of Baghdad - College of Pharmacy (no. RECAUBCP6102023K). Verbal consent was obtained from participants prior to their enrollment in the study.

Assessment of biomarkers

Five milliliters of fasting venous blood were collected from the participants. Serum insulin and SP were measured using ELISA kits provided by Elabscience , USA, and Cloud-clone Corp., USA. respectively, Glycated hemoglobin (HbA1c) was measured by boronate affinity assay using the NycoCard Reader II, Sweden. The colorimetric test was used to evaluate fasting serum glucose (FSG), total cholesterol (TC), triglyceride(TG), and high-density lipoprotein cholesterol (HDL) levels; using the corresponding kits from Linear chemicals, Spain. Low-density lipoprotein (LDL) was estimated by the the Friedewald formula which is TC(mg/dl) minus HDL(mg/dl) minus TG(mg/dl) divided by 5) [12], The homeostatic Model Assessment of Insulin Resistance(HOMA-IR), which is simplified by the formula for fasting insulin (μU/ml) multiplied by fasting glucose (mg/dl) divided by 405 [13].

Statistical analysis

The Statistical Package for Social Sciences (SPSS) (version 26 for Windows) was employed to perform the statistical analysis. The assessment of data distribution normality was conducted using the Shapiro-Wilk test. The P-values for the measured data were less than 0.05, indicating that the continuous variables were not normally distributed; thus, non-parametric tests were used for data analysis. The results for both the patients and the controls were compared using the Mann-Whitney U test, with descriptive statistics presented as median and interquartile range (IQR). Categorical variables were expressed as numbers and percentages, and differences were assessed using the Chi-Squared test. Additionally, Spearman's correlation test was utilized to analyze the correlation between parameters. A P-value less than 0.05 was considered as a threshold for statistical significance.

Results

The selected group of T2DM patients and healthy control group in this study were comparable considering their anthropometric measures (age, sex, and BMI), as illustrated in "Table 1".

The range of age of the participants who were enrolled in this study was (35-60) years in the patients' group and the median (IQR) was [52 (13)], while in the control group, the age range was (30 to 51) years and the median (IQR) were [40 (7)]; (P =0.062). Furthermore, out of 53 T2DM patients, 33 (62%) were male and 20 (38%) were female; while; out of 32 individuals in the control group, 13 (41%) were male and 19 (59%) were female; so the sex distribution in both of the study groups were not significantly different (P = 0.073). Similarly, there was no significant difference between the T2DM patients and the control individuals with regard to BMI; 26.20 (3) kg/m^2 and 26.20 (4) kg/m^2, respectively; (P =0.068).

Table 1. Demographic information regarding the individuals involved

Variables		Control	Diabetes Patients	*P- Value*
		n=32	n=53	
Age(year)		40(7)	52(13)	0.062
Gender	Male	13(41%)	33(62%)	0.073
	Female	19(59%)	20(38%)	
BMI (kg/m^2)		25.53(3)	26.20(4)	0.068

Where p was significant if p< (0.05)

As demonstrated in "Table 2", the T2DM patients had higher FSG levels compared to the controls, 219.13 (31.18) vs. 90.94 (13.57), respectively; (*P*= 0.001). Furthermore, T2DM patients showed significantly higher levels of HbA1c compared to the control group, 8.10 (1.70) vs. 5.00 (0.7), respectively; (*P* =0.001). Similarly, serum insulin levels and HOMA-IR index showed significantly higher levels in the T2DM patients than in the control group; (*P*= 0.001).

Table 2. Glycemic Parameter of Participants

Variables	Control	Diabetes Patients	*P- Value*
	n=32	n=53	
FSG (mg/ dL)	90.94(13.57)	219.13(31.18)	0.001*
Insulin(µU/ml)	1.99(0.38)	3.03(1.27)	0.001*
HOMA-IR	0.45(0.60)	1.687(0.704)	0.001*
HbA1c (%)	5.00 (0.7)	8.10(1.70)	0.001*

* Significant when $p<0.05$

Lipid profile parameters revealed statistically significant differences in the levels of TC, TG, LDL and HDL between T2DM patients and the control group (*P* = 0.001). Specifically, serum levels of TC, TG, and LDL were higher, while HDL levels were lower in T2DM patients compared to the control group, as shown in "Table 3".

Table 3. Evaluation of lipid profiles in the studied groups

Variables (mg/dl)	Control	Diabetes Patients	*P -Value*
	n=32	n=53	
TG	139.49(14.54)	209.48(13.20)	0.001*
TC	138.31(17.93)	176.50(17.80)	0.001*
LDL	51.85(16.59)	76.62(19.47)	0.001*
HDL	59.14(5.069)	52.64(2.23)	0.001*
VLDL	27.89(2.91)	41.89(2.64)	0.001*

* Significant when $p<0.05$

The serum levels of SP were significantly lower in the T2DM patients than that in the control group, 181.49 (79.93) *p*g/ml vs. 445.40(136.24) *p*g/ml, respectively; (*P* < 0.001); as demonstrated in "Figure 1".

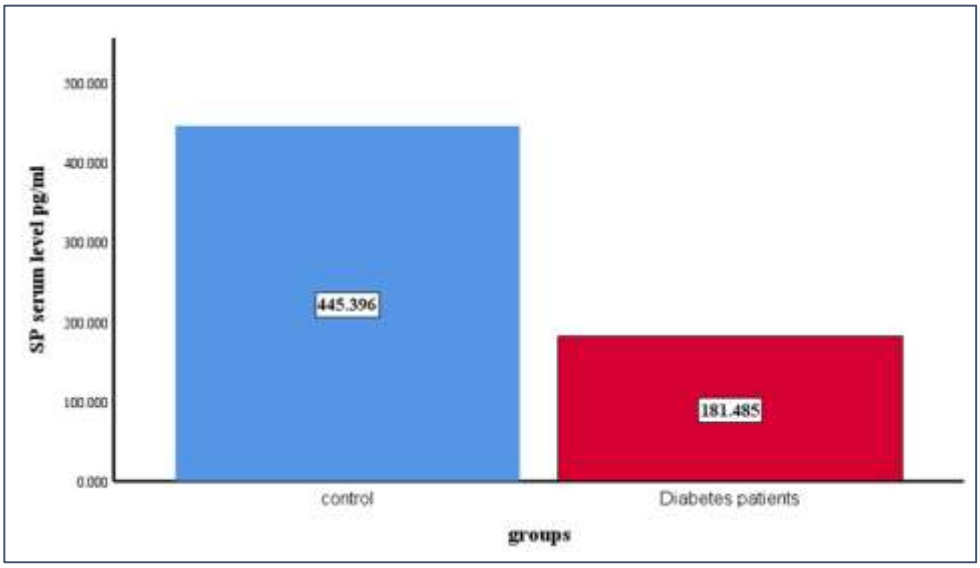

Figure 1. Substance P serum level in study groups

SP = substance P

The serum SP levels in the T2DM patients did not show any significant correlation with the glycemic control indices, lipid profile or with the other studied variables, *(P*>0.05); as presented in "Table 4".

Table 1. Spearman's Correlations of Serum SP Level with the Studied Variables in Diabetics

Variable		SP
Age	*P*-value	0.068
	rho	-0.253
BMI	*P*-value	0.769
	rho	0.041
HbA1c%	*P*-value	0.959
	rho	0.007
Insulin	*P*-value	0.165
	rho	-0.194
FSG	*P*-value	0.439
	rho	-0.109
HOMA-IR	*P*-value	0.176
	rho	-1.898
TG	*P*-value	0.247
	rho	-0.162
TC	*P*-value	0.166
	rho	-0.193
HDL	*P*-value	0.632
	rho	0.067
LDL	*P*-value	0.257
	rho	-0.159
VLDL	*P*-value	0.247
	rho	-0.162

Discussion

In this study, the T2DM patients and the control group were of comparable age, sex, and BMI to eliminate the potential effect of these factors on the measured variables. The T2DM patients had higher FSG, than healthy controls, which is highly anticipated and consistent with literature [14–16] .Furthermore, the T2DM patients had a more than 50% higher fasting insulin levels and a more than 250% higher HOMA-IR index than these of the control group; this occurs in accordance with the pathophysiology of T2DM [17,18].

In this study, the insulin levels and HOMA-IR values in non-obese T2DM patients were not as high as expected "Table 2"; These findings are consistent with the results drawn in earlier studies [19,20], This is likely due to a combination of reduced insulin secretion and minimal or no insulin resistance. It has been observed that circulating insulin levels in non-obese diabetic patients are lower compared to their obese counterparts, these findings suggest a more severe beta-cell failure in the non-obese group. Importantly, this beta-cell failure appears to be functional rather than structural [21] .

There is a direct relationship between blood glucose levels and HbA1c levels [22]. The present study found that the HbA1c values were elevated by more than 60% compared to these in the control group. [23–25].

Patients with diabetes mellitus commonly exhibit dyslipidemia. Our data revealed elevated levels of TC, TG and LDL, as well as decreased levels of HDL in T2DM patients compared to the control group. Such abnormalities in lipid metabolism are considered as risk factors for a higher occurrence of cardiovascular complications associated with diabetes [26–28].

The link SP/NK1R system and diabetes has been examined previously in different contexts. There have been reports of dysregulated expression of SP in individuals with diabetes and its chronic complications [29]. This study showed marked lower serum levels of SP, approximately (59.25%), in T2DM patients compared to the levels in the control group. This finding occurs in accordance with Guo Z.[30] , Yan *et al.* [31] and Wang *et al.* [32] that the serum concentration of SP was shown to be significantly reduced in T2DM patients in comparison with healthy ones. However, a study by Fu *et al.* refuted this evidence in obese diabetes patients [33].

The study results revealed that no significant association was found between the serum SP levels and the glycemic control indices, the lipid profile parameters or the other variables that were investigated. Despite the non-significant correlation, the statistical data indicate a negative relationship of the serum SP levels with fasting insulin levels, HOMA-IR, lipid profile, except for HDL, and positively correlated with BMI, HbA1c and HDL. This finding aligns with a prior investigation carried out by Kunt *et a* [34]. According to which substance P levels did not correlate with any of the following: gender, type or duration of diabetes, age,TC, HDL LDL,TG, or HbA1c values.

Conclusion

The study revealed that non-obese type 2 diabetic patients exhibit lower serum substance P levels compared to control subjects. This finding suggests that substance P may play a significant role in the pathophysiology of type 2 diabetes mellitus. Further larger scale, longitudinal and mechanistic studies are recommended to investigate the temporal relationship between serum substance P levels and the development or progression of type 2 diabetes in non-obese individuals, and to elucidate how substance P affects metabolic processes such as insulin signaling, glucose uptake, and pancreatic function.

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Conflicts of interest

There are no conflicts of interest that the authors have to declare.

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Author contribution

Contribution of the first author: data collection, analysis, statistical analysis, and manuscript writing. Contribution of the second author: Have significantly contributed to the article's conception and layout; have created the framework for the analysis; have critically revised and approved the version to be published.

Ethics statements

The Ethical Committee of the College of Pharmacy, University of Baghdad, registered and approved the protocol of this study (the registration no. RECAUBCP6102023K). Before agreeing to participate, everyone was briefed on the study's goals and expected advantages.

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الارتباط بين مستوى المادة P في مصل الدم ومؤشرات التحكم في نسبة السكر في الدم ومستوى الدهون لدى الأشخاص المصابين بمرض السكري من النوع الثاني والذين لا يعانون من السمنة

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الخلاصة

فرط سكر الدم وفرط الأنسولين، المرتبطان بداء السكري من النوع الثاني، هما من الأسباب الرئيسية للاضطرابات الأيضية التي تؤدي إلى مجموعة واسعة من المضاعفات مثل الأمراض القلبية الوعائية، والاعتلال الكلوي، والاعتلال العصبي، واعتلال الشبكية. المادة P هي ببتيد عصبي يتكون من ١١ حمض أميني ومحفوظ بدرجة كبيرة ويتم إفرازها من النهايات العصبية الحسية وكذلك من أنواع مختلفة من الخلايا غير العصبية. كان يُعتقد أن المادة P يمكن أن تقلل من الالتهاب الناجم عن مرض السكري، مما يعوق تطور المضاعفات المرتبطة بهذا المرض. تهدف الدراسة الحالية إلى التحقيق فيما إذا كانت مستويات المصل للمادة P مرتبطة بمستويات السكر و / أو ملف الدهون في الأشخاص المصابين بمرض السكري من النوع الثاني والذين لا يعانون من السمنة. تضمنت الدراسة دراسة الحالات والتحكم والتي شملت خمسة وثمانين مشاركًا بالغًا (٤٦ ذكورًا و٣٩ إناثًا)، تتراوح أعمارهم بين (٣٠-٦٠) سنة، وتم تقسيمهم إلى مجموعتين. المجموعة الأولى: ٥٣ مريضًا بالسكري من النوع ٢ لا يعانون من السمنة، والمجموعة الثانية: تم اختيار أفراد صحيون ظاهريًا ومطابقة لعمر وجنس ومؤشر كتلة جسم المرضى، بإجمالي ٣٢ مشاركًا. أظهرت قيم الجلوكوز بالمصل عند الصيام، والإنسولين، ومؤشر تقييم نموذج الاستتباب ومقاومة الأنسولين (HOMA-IR)، والكولسترول الكلي، والدهون الثلاثية، وهيموغلوبين (HbA1c) ارتفاعًا بشكل كبير في مرضى السكري مقارنة بمجموعة السيطرة (p= ٠,٠٠١). بينما انخفضت مستويات المادة P في المصل والدهون عالية الكثافة بشكل ملحوظ عند مرضى السكري مقارنة بمجموعة السيطرة (p=٠,٠٠١)، كانت مستويات مادة P في المصل أقل بكثير لدى مرضى السكري الذين لا يعانون من السمنة مقارنة بالأشخاص الأصحاء [١٨١,٤٩ (٧٩,٩٣) بيكوغرام/مل، و٤٤٥,٤٠ (١٣٦,٢٤) بيكوغرام/مل؛ على التوالي] ,(p<٠,٠٠١). علاوة على ذلك، لم تظهر أي من مؤشرات التحكم في نسبة السكر في الدم أو ملف الدهون التي تم فحصها أي ارتباطات ملحوظة مع مستويات مادة P في المصل(p>٠,٠٥). ،في الاستنتاج، تشير المستويات المنخفضة لمادة Pفي المصل لدى مرضى السكري من النوع ٢ والذين لا يعانون من السمنة مقارنة بالأشخاص الأصحاء إلى دور محتمل للمادة P في الفيزيولوجية المرضية لمرض السكري من النوع ٢.

الكلمات المفتاحية: تقييم نموذج الاستتباب ومقاومة الأنسولين، حساسية الانسولين، مستوى الدهون، المادة P، داء السكري من النوع ٢