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PANCREATIC STONE PROTEIN/REGENERATING PROTEIN AS A BIOMARKER OF DIABETIC NEUROPATHY

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Diabetic neuropathy (DN) is considered the most common complication of type 2 diabetes mellitus (T2DM). There is an unmet need for potential biomarkers that can be used to diagnose diabetic neuropathy and to halt its progression. Chronic low-grade inflammation plays a key role in the development of DN. It was shown that secretory lectin-binding pancreatic stone protein/ regenerating protein (PSP/reg) is raised in the blood during inflammatory and infectious conditions. The aim of this study was to investigate the relationship between the serum level of PSP/reg and DN in patients with type 2 diabetes mellitus. A total of 250 participants with T2DM aged 40-70 years were divided into two groups: patients without DN and DN patients. The presence of T2DM and DN was confirmed clinically. The serum PSP/reg protein level was determined using ELISA. It was shown that serum PSP/reg level was significantly higher in DN patients compared with diabetic patients without DN and was positively correlated with glycated hemoglobin and blood glucose level. ROC analysis revealed that the optimal cut-off point of PSP/reg was 10.15 ng/ml to indicate DN with a sensitivity of 92%. These results indicate the potential role of serum PSP/reg level as a supplementary diabetic neuropathy marker which may be useful in identifying T2DM patients who are at a high risk of nerve damage.

K e y w o r d s: pancreatic stone protein/regenerating protein, biomarker of diabetic neuropathy, type 2 diabetes mellitus.

Diabetes mellitus (DM) is among the most prevalent metabolic diseases worldwide. According to the International Diabetes Federation, the global number of people with diabetes will increase to 783 million by 2045 [1]. Diabetes mellitus is a major contributor to disability since it causes a number of chronic macrovascular and microvascular complications. Among these complications, diabetic neuropathy is ranked as the third most common neurological condition throughout the world. It is known as one of the most mysterious and painful complications of diabetes, which lowers the quality of life and, as a result, places a financial strain on society and healthcare systems [2]. The pathophysiology of diabetic neuropathy is complicated and involves both somatic and autonomic components of the nervous system. Nearly half of the population with type 2 diabetes mellitus (T2DM) suffer from diabetic neuropathy [3]. There is a lack of clarity about the mechanisms behind diabetic neuropathy development, which are often complex and multifactorial. A growing body of evidence suggests that chronic low-grade inflammation contributes to T2DM and neurodegenerative complications [4]. Researchers have shown that inflammatory cytokines are involved in the pathogenesis of diabetic neuropathy (DN), and increased cytokine concentrations have been reported in T2DM patients with large nerve damage [5]. Pancreatic stone protein/ regenerating protein (PSP/reg) is a lectin-binding protein that is secreted by pancreatic acinar cells into pancreatic juice as well as by subsets of intestinal and gastric cells. It has been studied mainly in the pancreas and is prominently up-regulated in acute or chronic pancreatitis [6, 7]. PSP/reg has been proposed to act as an acute-phase protein and it is considered to be regulated by IL-6, tumor necrosis factor-a, and other cytokines released during inflammation and infection. It was also found to be elevated in sepsis [8, 9], ventilator-associated pneu-

monia (VAP) [10], chronic obstructive pulmonary disease (COPD) exacerbation [11], and diabetes mellitus [12-15]. These observations indicated that PSP/reg might respond to infectious conditions and organ failure. Given that inflammation is linked to diabetic microangiopathy and plays a significant role in the development of diabetic neuropathy. In this study, we investigated the possibility that serum PSP/reg levels are associated with the disease and may predict future risk. The purpose of this study was to examine the relationship between PSP/reg and diabetic neuropathy in individuals with type 2 diabetes mellitus.

Materials and Methods

Study population and design. This cross-sectional study was carried out at AL-Faiha Teaching Hospital, Basrah, Iraq, from May 2024 to September 2024. The study was approved by the Ethics Committee of Basrah Medical College. All subjects provided informed consent to participate in the study. A total of 250 patients with T2DM (89 males, 161 females; age range 40-70 years) were enrolled in this study. The subjects were divided into two groups according to the presence of diabetic neuropathy; diabetic patients with DN (DN group) and diabetic subjects without DN (non-DN group). Al Faiha Teaching Hospital consultants confirmed the diagnosis of neuropathy, which was recorded in the electronic medical records. For diagnosis of neuropathy, the following guidelines were followed: (i) all patients with T2DM were assessed for distal symmetric polyneuropathy, (ii) the evaluation included a detailed history, temperature or pinprick sensation (small-fiber function), and vibration sensation using a 128-Hz tuning fork (large-fiber function) and (iii) A 10-g monofilament test was conducted annually on patients at risk for ulceration and amputation. Furthermore, electrophysiological testing or referral to a neurologist were rarely required for screening, except in cases with atypical clinical features, an unclear diagnosis, or an alternative etiology is suspected. Atypical features included motor neuropathy that was greater than sensory neuropathy, rapid onset, and asymmetrical presentation. Exclusion criteria were: (1) type 1 DM; (2) acute complications of DM including hyperosmolar coma, ketoacidosis, lactic acidosis, hypoglycemic coma; (3) active or chronic infection or inflammatory disorders; (4) neoplastic disorders; (5) severe liver dysfunction; (6) on medication that affects blood or urine glucose levels.

Data collection. A standardized questionnaire was used to gather a comprehensive clinical assessment. Anthropometric measurements, including height, weight, were obtained. BMI was calculated as weight (kg) divided by the square of the height (m). Age, gender, smoking, drinking, family history of diabetes, and duration of diabetes were also recorded. In addition, vital signs were also measured and recorded, including systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Sampling. Peripheral blood (PB) samples were collected on ethylene diamine tetra-acetic acid (EDTA) (1.2 mg/mL) for analysis of HbAlc. Serum obtained from clotted samples by centrifugation for 15 min at 1000g was used for chemical analysis and stored at - 20°C till subsequent use in ELISA.

Laboratory assays. Hemoglobin Alc (HbA1c, %), fasting blood glucose (mmol/l), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), were measured based on standard methods. The serum PSP/reg protein level was determined using enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology Co., Ltd) in accordance with manufacturer's instructions.

Statistical analysis. Data analysis was performed using the IBM SPSS 26 (Released 2019; IBM Corp., Armonk, New York, USA). Data were presented as a mean ± standard deviation or as percentages for normal distribution. The means between groups were compared using independent Student's *t*-test. Correlations of PSP/reg and clinical parameters were performed using spearman's rank correlations.

Ethics approval. The study was approved by the Institutional Review Board of Basrah Medical College, under approval number 68 on 11/5/2024.

Results

Participants and clinical analysis at baseline. A total of 250 subjects (89 males, 161 females; age range 40-70 years) were enrolled in the study, and were divided into two groups: T2DM patients without DN, and DN patients. The main clinical and biochemical characteristics of these two study populations are summarized in Table 1 and Table 2. Diabetic neuropathy patients had significantly longer duration of diabetes, higher systolic and diastolic blood pressure, HbA1c and random blood glucose levels than non-neuropathy patients.

Table 1. Clinical characteristics of subjects

Parameters	T2DM without DN	T2DM with DN	*P*-value
N (M/F)	100 (59/41)	150 (102/48)	–
Age, years	56.75 ± 8.30	60.69 ± 7.70	0.23
BMI, kg/m^2	32.21 ± 6.10	31.32 ± 5.10	0.18
SBP, mmHg	133.30 ± 18.09	142.67 ± 22.20	0.001
DBP, mmHg	79.7 ± 10.7	84.13 ± 13.20	0.001
Duration, years	4.8 ± 3.2	8.67 ± 5.90	< 0.001

Note. Data are expressed as mean ± SD or as n. BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure.

Table 2. Biochemical parameters of subjects

Parameters	T2DM without DN	T2DM with DN	*P*-value
RBG, mg/dl	195.29 ± 84.52	248.38 ± 126.41	< 0.001
HbA1c, %	8.72 ± 1.50	9.95 ± 1.99	0.02
Total cholesterol, mg/dl	174.19 ± 44.76	170.15 ± 44.64	0.7
HDL-cholesterol, mg/dl	40.70 ± 11.2	41.94 ± 13.46	0.1
LDL-cholesterol, mg/dl	122.74 ± 45.80	116.92 ± 40.87	0.3
Triglycerides, mg/dl	176.08 ± 113.47	184.85 ± 89.03	0.9
PSP, ng/ml	4.73 ± 3.17	23.85 ± 11.85	< 0.001

Note. Data are expressed as mean ± SD. RBG – random blood glucose; HbA1c – hemoglobin A1c; HDL – high-density lipoprotein; LDL – low-density lipoprotein; PSP – pancreatic stone protein.

Elevated PSP/reg levels were detected in DN patients. PSP/reg levels were significantly higher in T2DM patients with DN (23.85 ± 11.85 ng/ml) as compared to T2DM patients without DN (4.73 ± 3.17 ng/ml, $P < 0.001$).

Correlation of serum PSP/reg levels with diabetes-related parameters. A significant positive correlation between PSP/reg level and glycated hemoglobin (HbAlc Spearman's rank correlation coefficient 0.372, $P < 0.001$, Fig. 1, in all the participants was noted.

Meanwhile, blood glucose also correlated positively with PSP/reg levels (blood glucose Spearman's rank correlation coefficient 0.217, $P = 0.001$). A correlation between PSP/reg and systolic blood pressure (Spearman's rank correlation coefficient 0.222, $P < 0.001$) and diastolic blood pressure (Spearman's rank correlation coefficient 0.163, $P < 0.01$) was also found. However, there was no association between PSP/reg and gender, BMI and duration of type 2 DM.

Relationship between PSP/reg and DN. A receiver operating characteristic (ROC) analysis was used to assess the predictive value of PSP/ reg in T2DM. The area under the curve (AUC) of PSP/reg for the presence of DN was 0.971 (95% CI: 0.949-0.992) (Fig. 2). Furthermore, ROC analysis revealed that the optimal cut-off point of PSP/reg was 10.15 ng/ml to indicate DN (sensitivity, 92%; specificity, 93 %).

Discussion

The present study analyzed PSP/reg levels in diabetic neuropathy. A number of interesting findings have been revealed. First, T2DM patients with DN presented significantly higher PSP/reg levels than those without diabetic neuropathy. Second, there is a positive correlation between PSP/reg and major parameters of T2DM. Complex biochemical mechanisms and processes, including increased oxidative stress, activation of the polyol and protein kinase C pathways, decreased nerve blood supply and neuronal apoptosis, contribute to the development of diabetic neuropathy. These processes typically occur in conjunction with chronic low-grade inflammation, which has also been found to be a major pathogenic factor causing chronic diabetes complications, in-

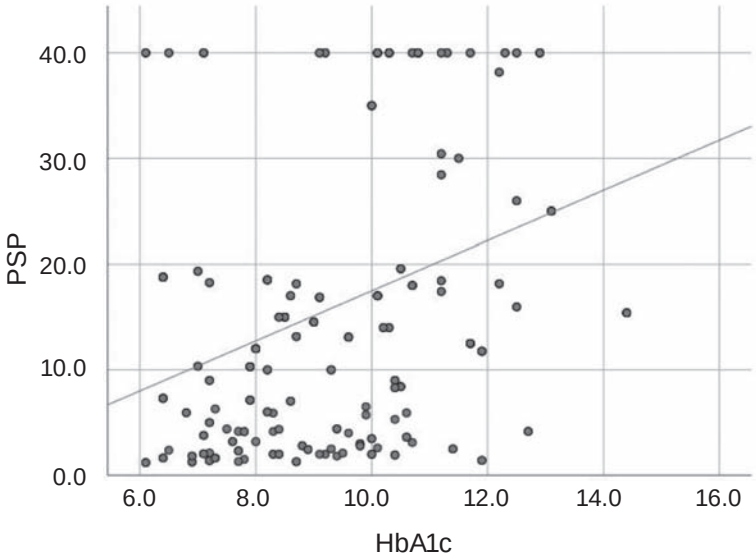

Fig. 1. Correlation of PSP/reg with HbA1c (Spearman's rank correlation coefficient 0.372, P < 0.001)

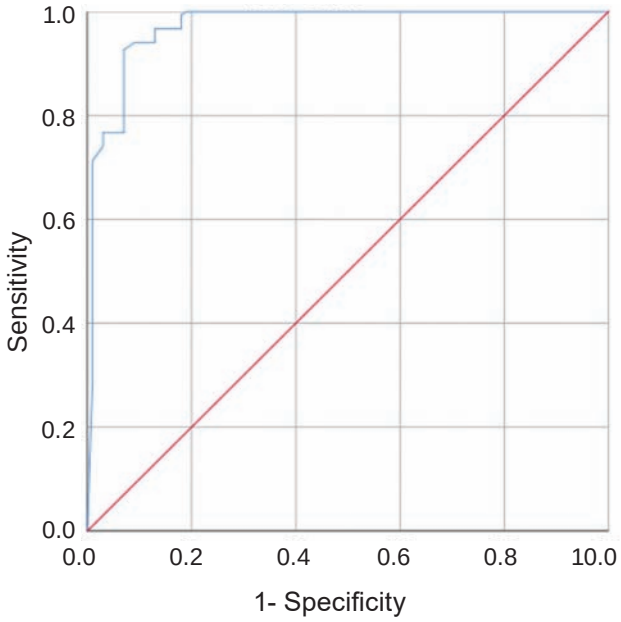

Fig. 2. ROC curve analysis. PSP/reg predicts the incidence of DN. The 10.15 ng/ml cut-off reveals a sensitivity of 92% and a specificity of 93% to predict the incidence of DN

cluding diabetic neuropathy [5, 16]. Although these pathways were not directly assessed in our study, our results in particular, the higher PSP/reg levels in neuropathy patients may be indicative of these underlying mechanisms. These events have been well established in the literature and constitute a valid biological context for interpretation.

According to available data, one of the main factors influencing the etiology and development of diabetic complications is inflammation. PSP/reg is one of the biomarkers that has recently brought more attention in this context. Patients with T2DM, especially those with chronic complications, have been found to have significantly greater circulating PSP/reg concentrations than control participants. These results raise the prospect that PSP/reg may play a part in the inflammatory processes underlying diabetic complications as well as serving as a marker of disease activity [15, 17]. The present study investigated PSP/reg levels in diabetic neuropathy patients and found a close association between PSP/reg and DN. The serum PSP/reg was significantly higher in the DN group than in patients without DN. This further suggests that inflammation may play an important role in the development and progression of DN.

In this study, the observed differences between groups in biochemical markers confirm the potential involvement of poor glycemic control and inflammation in diabetic neuropathy pathogenesis. Although these findings do not establish causality, they suggest that PSP/reg and glycemic markers could serve as early indicators of neuropathic risk, which warrants further investigation.

We also found positive correlations between PSP/reg levels and HbA1c and blood glucose levels, suggesting that elevated PSP/reg levels may be closely linked to beta cell dysfunction. This finding is consistent with recent evidence that suggests

elevated extracellular glucose concentrations promote PSP/reg gene expression, indicating an important feedback loop for the regulation of beta-cell mass [18]. Moreover, a correlation was observed between PSP/reg and both systolic and diastolic blood pressure. A major factor in the pathophysiology of hypertension is endothelial dysfunction, which is influenced by persistent low-grade inflammation, and PSP/reg has been linked to pro-inflammatory processes [19]. Therefore, elevated PSP/reg levels could be indicative of a persistent inflammatory state that encourages vascular abnormalities and elevated blood pressure. Also, autonomic modulation of cardiovascular function is known to be impaired by autonomic neuropathy, a typical feature of DN. Blood pressure can increase in a labile or persistent manner when the sympathetic and parasympathetic nervous systems are out of balance. Thus, autonomic dysregulation may be the mediating factor in this relationship between PSP/reg and blood pressure [20, 21].

While some of the correlations seen were rated as "weak" by traditional standards (Spearman's $\rho < 0.3$), they were statistically significant and potentially still have biological or clinical significance in the multifactorial context of diabetic neuropathy, where there are many overlapping mechanisms.

Interestingly, we did not find a significant correlation between the duration of diabetes and circulating PSP/reg levels in our study. Contrary to the findings of Yang et al. [16], who reported a positive correlation between PSP/reg levels and diabetes duration, which suggests that chronic inflammation and long-term metabolic dysregulation have a combined effect on PSP/reg expression. There may be differences in study populations, sample sizes, or different levels of diabetic complications in our cohort, resulting in a lack of correlation.

For future risk assessment, the AUC of PSP/reg for the presence of DN was calculated to be 0.971. In the process of this analysis, we identified a cut-off value for PSP/reg at 10.15 ng/ml in T2DM patients. This was the most significant parameter associated with the occurrence of DN. The present study provides clinical evidence that serum levels of PSP/reg are significantly higher in individuals with DN and are positively correlated with HbA1c levels and blood glucose levels. As a result of these data, it appears that PSP/reg may be associated with nerve injury in T2DM patients. This study has certain limitations, to begin with, this study's sample size is relatively small for a preliminary analysis, and because of the cross-sectional design, larger study populations will be needed in future prospective studies to ascertain whether PSP/reg can be utilized as a potential biomarker for DN diagnosis and development.

In addition, even though our findings demonstrate a significant correlation of elevated PSP/reg levels with diabetic neuropathy, one should realize that PSP/reg is not a diabetic-specific protein. Elevated levels have also been reported in other inflammatory conditions. Therefore, we would recommend PSP/reg not be considered a standalone marker, but as part of a broader panel of inflammatory and metabolic biomarkers to increase diagnostic efficacy and risk stratification in diabetic neuropathy.

Conclusion. The present study has shown that PSP/reg levels is significantly up-regulated in T2DM patients with DN. Moreover, a significant correlation was found between PSP/reg and HbA1c. This preliminary data suggests that PSP/reg may be useful in identifying T2DM patients who are at a high risk of nerve damage. Therefore, it is worth considering the potential role of serum PSP/reg level as a supplementary indicator of DN. These results may provide an important first step toward discovering DN biomarkers in T2DM patients, which aids in the patient group's screening and stratification for possible treatment targets and the application of intervention techniques.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ПРОТЕЇН ПАНКРЕАТИЧНОГО КАМЕНЮ/РЕГЕНЕРУЮЧИЙ ПРОТЕЇН PSP/REG ЯК БІОМАРКЕР ДІАБЕТИЧНОЇ НЕЙРОПАТІЇ

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Діабетична нейропатія (ДН) вважається найбільш поширеним ускладненням цукрового діабету 2 типу (ЦД2). Існує недостатність у потенційних біомаркерах, які можна було б використовувати для діагностики діабетичної нейропатії та запобігання її прогресуванню. Хронічне запалення низького рівня відіграє ключову роль у розвитку ДН. Було показано, що секреторний лектин-зв'язувальний PSP/reg (протеїн панкреатичного каменю/регенеруючий протеїн) підвищується в крові при запальних процесах. Метою даного дослідження було вивчення взаємозв'язку між сироватковим рівнем PSP/reg і діабетичною нейропатією у пацієнтів з ЦД2. Загалом 250 учасників із ЦД2 у віці 40-70 років були розділені на дві групи: пацієнти без ДН і пацієнти з ДН. Наявність ЦД2 і ДН підтверджували клінічно. Рівень протеїну PSP/reg визначали за допомогою ELISA. Було показано, що рівень PSP/reg у сироватці крові був значно вищим у пацієнтів із ДН порівняно з пацієнтами без ДН і позитивно корелював із глікованим гемоглобіном та рівнем глюкози в крові. ROC-аналіз показав, що оптимальна точка відсікання PSP/reg становила 10,15 нг/мл, що свідчить із чутливістю 92% про наявність ДН. Ці результати вказують на потенційну роль рівня PSP/reg у сироватці крові як додаткового маркера діабетичної нейропатії, що може бути використаний для виявлення пацієнтів із ЦД2, які схильні до високого ризику нервових уражень.

Ключові слова: протеїн PSP/reg, біомаркер діабетичної нейропатії, цукровий діабет 2 типу.

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