WITHDRAWN:
Spexin Levels are Associated with Painful Diabetic Peripheral Neuropathy

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Abstract

Background: Spexin is implicated in multiple functions of energy metabolism and glucose homeostasis regulation. Diabetic peripheral neuropathy (DPN) is the most common complication of diabetes and approximately half of the patients with DPN suffer from neuropathic pain. Recent experimental studies in mice have shown that Spexin has an antinociceptive effect, but there are no relevant reports in clinical studies. This study aimed to evaluate Spexin levels in people with painful DPN and controls and assess the correlation between serum Spexin levels and painful DPN.

Methods: This is a cross-sectional study including 20 patients with diabetes but without DPN (non-DPN) as a control group, 24 patients with painless DPN, and 16 patients with painful DPN. Questionnaires and laboratory surveys were conducted to collect demographic and clinical data. The existence and severity of DPN were assessed using neurological symptom score, neurological examination, and electromyography. Serum Spexin levels were measured by ELISA.

Results: Serum Spexin levels of patients with painful DPN were significantly lower than those of non-DPN patients ($p<0.001$) and painless DPN patients ($p=0.035$). Serum Spexin levels were negatively correlated with neuropathic pain score. Compared with individuals with higher levels of Spexin, the prevalence rate of painful DPN in those with lower levels of Spexin was significantly higher. Binary logistic regression analysis showed that the odds ratios for painful DPN were significantly elevated along with decreasing Spexin levels even after adjusting for age, sex, BMI, diabetes duration, HbA1c, 2hPBG, hypertension and smoking or drinking status. serum Spexin levels have a sensitivity of 84.1% and a specificity of 56.2% for predicting painful DPN.

Conclusions: Decreased serum Spexin levels were strongly associated with painful DPN, suggesting a possible role of this peptide in pain-related pathogenesis.

Background

Diabetic peripheral neuropathy (DPN) is the most common cause of disability and mortality in diabetes [1, 2], and its prevalence increases with age and diabetes duration [2]. Approximately half of the patients with DPN suffer from neuropathic pain [3]. Chronic persistently painful DPN has profound negative effects on quality of life, sleep, and mood. Glucose control effectively halts the progression of painful DPN, however, patients also often need pharmacologic agents to relieve pain. Currently, the main lines of approach are tricyclic antidepressants (TCAs) (ex. amitriptyline), anti-convulsants (ex. gabapentin or pregabalin), serotonin–norepinephrine reuptake inhibitors (SNRIs) (ex. duloxetine or venlafaxine), opioids, opioid-like substances, and topical medications (ex. capsaicin cream) [4 5 6]. However, none of these methods can normalize the sensory disturbance of painful DPN. A treatment that provides pain relief by targeting specific pathways of neuropathic pain in DPN is therefore desirable. To develop such a treatment strategy, there is an urgent need to understand the pathological basis of painful DPN.

Spexin is a novel peptide with a wide spectrum of expression, both centrally and peripherally, such as in the hypothalamus, cerebral cortex, skin, ovary, liver, pancreatic islets and stomach, etc. [7,8 9]. Spexin plays important roles in the regulation of energy and glucose metabolism [8 10]. The circulating Spexin levels were lower in human obese patients compared to non-obese patients [11]. Treatment with Spexin reduces body weight gain by inhibiting food intake and gastrointestinal motility [9 12 13]. Spexin mRNA expression decreased in adipose tissue in obese patients and Spexin reduced fat accumulation by inhibiting free fatty acid uptake by adipocytes [14]. Circulating Spexin levels were also measured in type 1, type 2 and gestational diabetes patients [8 15 16].

Recent studies have shown that Spexin attenuates pain sensitivity in ovariectomized rats and produces antinociceptive effects against inflammatory pain by inducing central Fos activation [17, 18]. However, the role of Spexin in painful DPN remains unclear. Therefore, we conducted a cross-sectional study to investigate whether serum Spexin levels are associated with painful DPN.

Methods

Study design

We included 60 diabetic patients in this cross-sectional study, and all patients were classified into the following three groups: diabetes without DPN (non-DPN), painless DPN and painful DPN. All patients were recruited from the Huashan Hospital in Shanghai from 2015 to 2020.

The inclusion criteria were (1) signed informed consent, (2) diagnosis of type 2 diabetes according to the Standards of Medical Care in Diabetes issued by the ADA in 2021, (3) electrophysiological evidence of DPN, (4) complaints of neuropathic sensory pain (prickling or stabbing, burning or aching pain), and (5) age ≥ 18 years.

The exclusion criteria included (1) peripheral neuropathy of nondiabetic origin, (2) a concurrent serious mental illness that cannot cooperate with the study normally, (3) pregnancy, and (4) neurological diseases.

Demographic and clinical data

All patients completed a questionnaire and underwent a detailed anthropometric assessment to collect demographic and clinical data, including age, sex, diabetes duration, medical history of chronic diseases, smoking and alcohol history, blood pressure, and body mass index (BMI). BMI = kg/m^2.

Biochemical measurements

Levels of serum creatinine (sCr), blood urea nitrogen (BUN), high sensitivity C-reactive protein (hsCRP), HbA1c, fasting glucose, and fasting insulin were analyzed at the Institute of Endocrinology and Metabolism of Huashan Hospital (Shanghai, China). The homeostatic model assessment insulin

resistance (HOMA-IR) index was calculated as: HOMA-IR = fasting serum glucose (mmol/L) × fasting insulin (mcIU/mL)/22.5.

The homeostatic model assessment β cell function (HOMA-β) index was calculated as:

HOMA-β= (20 × fasting insulin (mcIU/mL)) / (fasting serum glucose (mmol/L)-3.5)

Assessment of diabetic peripheral neuropathy

The neuropathic symptoms and the clinical signs were evaluated by using a Neuropathy Symptom Score (NSS), Michigan neuropathy screening instrument (MNSI), and Neuropathy Disability Score (NDS) [19,20 21]. The NSS is in the form of a questionnaire, which includes: acupuncture-like pain, knife-cutting pain, abnormal hot and cold, and burning sensation. One of the above symptoms in the lower limbs and back of the foot is scored 1 point, or 2 points are aggravated at night, and a total score more than 3 points is abnormal. [20]. The MNSI score mainly includes foot appearance, ankle reflex, and big toe vibration score. Foot appearance: 0 points for normal, 1 point for abnormal (deformity, dryness, calluses, infection, cracking), plus 1 point if there is an ulcer. Ankle reflex and big toe vibration scoring rules: 0 points for normal, 0.5 points for decrease, 1 point for disappearance. MNSI > 2 is classified as abnormal[21]. The NDS involves sensations of touch, pain, and tremor in both lower limbs of the patient. NDS ≥ 5 is indicative of the existence of moderate or severe neuropathy[20].

The diagnosis of DPN was made when electromyography (EMG) test indicated abnormal. The diagnosis of painful DPN includes painful neurological symptoms, as well as evidence of DPN.

Serum Spexin measurement

The concentration of Spexin was determined in 50 μL aliquots of plasma samples, using an enzyme-linked immunosorbent assay kit (cat. no. EH4349; Wuhan Fine Biotech, China) following the manufacturer's instructions.

Statistical analysis

All statistical analyses were performed using SPSS (version 23.0; SPSS Inc., Chicago, Illinois, USA). All data were tested for normality using the Shapiro-Wilk test before statistical analysis. Non-normally distributed, continuous variables were expressed as median, 25th percentile, and 75th percentile (median [P25, P75]). Normally distributed variables were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare normally distributed continuous data. The nonparametric Kruskal-Wallis test was used to compare variables without a normal distribution or equal variance. Categorical variables were expressed as frequencies and proportions, and the Chi-square test or Fisher's exact test was used to explore the statistical significance of these categorical variables. Spearman's rank correlation analysis was performed for Spexin and the remaining variables. Binary logistic regression analysis was used to evaluate the association between painful DPN and Spexin after adjusting for other related variables. All statistical tests used the two-tailed method and considered a P-value < 0.05, statistically significant.

Results

Demographics and diabetic characteristics

Table 1 summarizes the demographic and diabetic characteristics performed for each group. There were no differences in age, sex, or BMI between the three groups. Compared with the control group, in terms of diabetic characteristics, patients with painful DPN (10.00 [6.75, 19.50]) vs. 6.50 [1.25, 17.25], $p = 0.016$) and those with painless DPN (14.50 [4.75, 20.25] vs. 6.50 [1.25, 17.25], $p = 0.02$) had a longer diabetes duration. However, there was no significant difference between patients with painful DPN and those with painless DPN. Patients with painless DPN had higher 2hPBG levels than those in the control group (13.12 ± 4.22 vs. 10.16 ± 3.81, $p = 0.024$) and the patients with painful DPN (13.12 ± 4.22 vs. 9.32 ± 3.19, $p < 0.001$), but there was no significant difference between the patients with painful DPN and control group. Most importantly, patients with painful DPN had lower levels of Spexin than those in the control group (0.09 [0.07, 0.16] vs. 0.15 [0.11, 0.25], $p < 0.001$) and patients with painless DPN (0.09 [0.07, 0.16] vs. 0.12 [0.08, 0.21], $p = 0.035$), but there was no significant difference in the Spexin levels between the patients in the control group and those with painless DPN (Fig. 1(a)). No significant differences were observed among the three groups in terms of blood pressure, sCr, BUN, FPG, HbA1c, fasting insulin, fasting insulin/FPG, hsCRP, HOMA-IR and HOMA-β ($p > 0.05$).

Table 1
Comparison of clinical and laboratory parameters among three group.

	Non-DPN (N = 20)	Painless DPN (N = 24)	Painful DPN (N = 16)	P value
Age (years)	55.53 ± 13.76	66.00 ± 9.99	65.57 ± 15.45	0.149
DM duration (years)	6.50[1.25,17.25]	14.50[4.75,20.25]	10.00[6.75,19.50]	0.033
Male N (%)	16(80%)	20(83.3%)	9(56.3%)	0.125
BMI (kg/m^2)	25.11 ± 2.19	24.39 ± 3.31	23.48 ± 4.21	0.145
Smoking N (%)	10(50%)	13(54.2%)	5(31.3%)	0.287
Alcohol intake N (%)	3(15%)	3(12.75%)	0(0%)	0.304
SBP (mmHg)	128.84 ± 13.70	131.53 ± 16.24	139.79 ± 20.51	0.063
DBP (mmHg)	79.47 ± 10.39	81.12 ± 11.45	82.29 ± 14.40	0.532
Scr (μmol/L)	64.42 ± 17.22	71.18 ± 22.24	79.79 ± 42.76	0.335
BUN (mmol/L)	5.38 ± 1.41	6.31 ± 2.09	7.41 ± 3.33	0.072
FPG (mmol/L)	7.20[5.90,9.33]	8.70[6.50,10.70]	8.55[6.93,10.75]	0.224
2hPBG (mmol/L)	10.16 ± 3.81	13.12 ± 4.22	9.32 ± 3.19	0.001
HbA1c (%)	8.44 ± 1.68	9.31 ± 2.30	8.75 ± 1.84	0.437
Fasting insulin (mmol/L)	10.70[8.40,17.00]	9.30[4.50,16.30]	13.70[5.78,41.05]	0.101
HOMA IR	3.92[2.45,5.33]	3.83[1.89,12.09]	4.76[1.70,15.92]	0.716
HOMA β	74.12[40.38,117.27]	44.11[18.14,126.67]	59.20[33.57,80.36]	0.368
hsCRP (mg/ml)	1.81[0.54,3.60]	0.99[0.59,1.77]	1.45[0.44,2.31]	0.722
Hypertension N (%)	5(25%)	12(50%)	7(43.8%)	0.227
CHD N (%)	3(15%)	4(16.7%)	2(12.5%)	1
CVA N (%)	0(0%)	3(12.5%)	2(12.5%)	0.249
Data are shown as means ± SD or median (interquartile range).				

Neuropathy parameters

Table 2 shows all measurements of neuropathy for the different groups. As expected, all measurements of neuropathy were higher in the painful DPN group compared with the other two groups. However, in

comparison between the painless and painful DPN groups, the only observed difference was NSS, which was higher in the painful DPN group (P 0.001), indicating well-established painful DPN.

Table 2
Neuropathy parameters

	Non-DPN (N = 20)	Painless DPN (N = 24)	Painful DPN (N = 16)	P-value (painful vs painless DPN)
NSS	0[0,0]	0[0,0]	6.00[4.25,6.75]	< 0.001
MNSI	0[0,0]	0[0,1.00]	1.00[0,2.00]	0.229
NDS	0[0,2.00]	2.5[0,4.75]	4.00[0.25,6.00]	0.261

Data are shown as median (interquartile range). NSS, Neuropathy Symptom Score; MNSI, Michigan neuropathy screening instrument; NDS, Neuropathy Disability Score.

Association of serum Spexin levels and clinical characteristics

As shown in Table 3, serum Spexin levels were negatively correlated with age (Spearman r =-0.341, P < 0.001), fasting insulin (Spearman r=-0.299, P = 0.002), and HOMA-IR (Spearman r=-0.231, P = 0.015). Spexin levels were significantly higher in male than in female patients (Spearman's r = 0.227, P = 0.014). Patients with a history of smoking had higher levels of Spexin (Spearman r = 0.294, P = 0.001). There was also a significant negative correlation between serum Spexin levels and NSS (Spearman's r=-0.27, P = 0.037; Fig. 1(b)). However, there was no significant correlation between serum Spexin levels and MNSI or NDS.

Table 3
Correlation analysis between all characteristics and Spexin in all participants.

	Spexin, ng/mL	
	r	P
Age (years)	-0.341	< 0.001
DM duration (years)	-0.128	0.172
Male N(%)	0.227	0.014
BMI (kg/m2)	0.041	0.66
SBP (mmHg)	-0.182	0.050
DBP (mmHg)	0.039	0.681
Scr1(umol/L)	-0.033	0.724
BUN (mmol/L)	-0.081	0.389
FPG (mmol/L)	-0.068	0.469
2hPBG (mmol/L)	-0.004	0.968
HbA1c (%)	-0.086	0.368
Fasting insulin (mmol/L)	-0.299	0.002
Fasting insulin/FPG	-0.176	0.072
HOMA IR	-0.231	0.015
HOMA β	-0.182	0.064
hsCRP (mg/ml)	-0.009	0.935
Smoking N (%)	0.294	0.001
Alcohol intake N (%)	0.079	0.401
Hypertension N (%)	-0.093	0.323
CHD N(%)	-0.041	0.661
CVA N(%)	-0.110	0.240
NSS	-0.270	0.037
MNSI	-0.049	0.709
NDS	-0.037	0.779

To further examine the relationship between serum Spexin levels and painful DPN, we divided the 60 enrolled patients into three groups based on the tertiles of Spexin levels. The tertiles of Spexin were tertile 1, 0.093 ng/mL; tertile 2, 0.093–0.152 ng/mL, and tertile 3 > 0.152 ng/mL. As predicted, the incidence of painful DPN in the T1 group was 4.1 times that of the T2 group and 3.1 times that of the T3 group (Fig. 1(c)). Then, binary logistic regression analysis was performed to calculate the odds ratio of painful DPN. We found that the odds ratios for painful DPN were significantly elevated along with decreasing Spexin tertiles, even after adjusting for age, sex, BMI, diabetes duration, HbA1c, 2hPBG, hypertension, and history of smoking or drinking, indicating that serum levels of Spexin were strongly associated with the presence of painful DPN (Table 4).

Table 4
Risk of painful DPN according to serum levels of Spexin

	Model 1		**Model 2**		**Model 3**	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Tertile 1	5.79(1.34–25.07)	0.019	4.32(0.80-23.36)	0.089	11.29(1.06-120.24)	0.045
Tertile 2	0.71(0.14–3.62)	0.681	0.46(0.07–2.95)	0.415	0.96(0.07–13.29)	0.978
Tertile 3	1		1		1	
Data are odds ratios (95% confidence interval).						
Model 1: unadjusted.						
Model 2: adjusted for age, gender and BMI.						
Model 3: model 2 further adjusted for duration of DM, HbA1c, 2hPBG, SBP, DBP and status of smoking or drinking						

Receiver operating characteristic analysis for painful DPN

To further expound the relationship between Spexin and painful DPN, we performed ROC curve analysis of serum Spexin levels for predicting painful DPN. The area under ROC curf was 0.699 (P = 0.019) with a sensitivity of 84.1% and specificity of 56.2% for predicting painful DPN (Fig. 1(d)). The best cut-off value for Spexin to predict painful DPN was 0.0939 ng/mL.

Discussion

Spexin, first discovered in 2007, is also known as the neuropeptide Q [12]. In the CNS of rodents, Spexin was detected in the brainstem, trigeminal ganglia, and brain cortex [7], which is closely related to nociceptive transmission. This study provided evidence of a negative correlation between serum Spexin levels and painful DPN after adjusting for age, sex, BMI, diabetes duration, HbA1c, 2hPBG, hypertension and smoking or drinking status.

Multiple studies have shown that Spexin plays a role in central pain syndrome. Intraventricular injection of Spexin produced antiinjury effects in a warm water tail withdrawal test in mice [22]. Intra hippocampal CA3 (IHCA3) injection of Spexin reduced pain sensitivity in both the early and late phases of the formalin test in rats [17]. Centrally administered Spexin also produced antinociceptive effects against acute inflammatory pain in the formalin test and visceral pain in the writhing test [18]. All these results indicate the central analgesic effect of Spexin. Mechanically, Spexin activated dynorphin/KOR by Fos pathway [18]. As mentioned before, the pathogenesis of painful DPN is still largely unknown. For the first time, our study suggests that Spexin might play a role in modulating painful DPN, and further research is needed to explore these mechanisms.

Pain as a sensory experience includes nociceptive, inflammatory, and neuropathic pain. Harmful stimuli such as cold, heat, and mechanical stimuli through specific receptors or ion channels, or the release of chemical mediators from locally damaged tissues and inflammatory immune cells, can be converted into electrical activity at the terminals of nerve fiber nociceptors. This electrical activity is transmitted to the dorsal root ganglion and spinal cord, and finally transmitted to the cortex after passing through the central path, and produces the sensation of pain [23]. Functional defects or damage of the peripheral or central nervous system can cause neuropathic pain [24]. M1 macrophages are significantly increased in patients with T2DM and DPN [25]. Macrophages in peripheral or central nerve injury sites or dorsal root ganglia have been shown to be involved in the initiation and maintenance of pain after nerve injury [26,27 28] through releasing mass proinflammatory mediators and sensitizing sensory neurons. A recent study reported that Spexin reduced M1 macrophage polarization in adipose tissue [29]. It can be postulated that reduced serum Spexin in painful DPN might facilitate the accumulation of M1 macrophages around injury nerve sites.

Although the specific Spexin receptor has not been identified, it is suggested that Spexin could bind and activate galanin receptors [30]. The effects of Spexin on bowel mobility and food intake can be partially blocked by Galanin receptor antagonists [9, 13]. Macrophages express Galanin receptors [31 32 33], which participate in the inflammatory response mediated by galanin and its related family members (GALP) [34 35 36]. We speculate that Spexin may directly act on nervous system macrophages via binding Galanin receptors to reduce the polarization of macrophages, thereby improving nervous system inflammation and reducing pain sensitivity in patients with DPN, which needs to be further explored.

One of the limitations of this study is the lack of more comprehensive neurophysiological examinations in the diagnosis of DPN, such as quantitative sensory tests and skin biopsy, both of which can be used to diagnose and evaluate small fiber neuropathy. Painful symptoms were assessed applying only NSS, because it well displays the symptoms of specific for painful DPN. Therefore, we believe that NSS is more appropriate in our study. Another limitation is the relatively small cohort size and cross-sectional design. To minimize the impacts of other factors, we had well matched the age and BMI of the three groups of patients. Although the duration of diabetes in the three groups was not matched and was shorter in the group of painful DPN when compared with the group of painless DPN, it should be pointed out that the duration of diabetes is rather long in the subgroup of DPN (P 0.05), and Spexin indeed reduced in the

group of painful DPN. In summary, we accurately stratified the presence of DPN and painful DPN through detailed clinical and neurophysiological assessments of all participants. Further long-term prospective cohort studies or intervention studies are needed to clarify the specific relationship.

Conclusions

For the first time, our results revealed that serum Spexin levels decreased in patients with painful DPN, and lower serum Spexin levels were strongly associated with the presence of painful DPN, suggesting the possible role of Spexin in the pain-related pathogenesis, and Spexin can potentially be a circulating biomarker to predict the risk of painful DPN.

Abbreviations

DPN, Diabetic Peripheral Neuropathy; DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; sCr, serum creatinine; BUN, blood urea nitrogen; FPG, fasting plasma glucose; 2hPBG, blood glucose 2h postprandial; HbA1c, glycated hemoglobin; HOMA-IR, Homeostatic model assessment insulin resistance; HOMA-β, Homeostatic model assessment β cell function; hsCRP, high sensitivity C-reactive protein; CHD, Coronary atherosclerotic heart disease; CVA, Cerebrovascular accident.

Declarations

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Availability of data and materials

The data sets in this study are available from the corresponding authors on reasonable request.

Authors’ contribution

LZ analyzed the data and wrote the manuscript. HPZ collected the data and revised the manuscript. YJJ analyzed the data. QZ collected the data. BL revised the study. YML conducted the study design and quality control. RL conducted the study design, collected and analyzed the data, and revised the manuscript. All authors have read and approve of the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study was approved by the Human Investigation Ethics Committee of Huashan Hospital of Fudan University. All methods were performed in accordance with the relevant guidelines and regulations. The patients provided their written informed consent to participate in this study.

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Figures

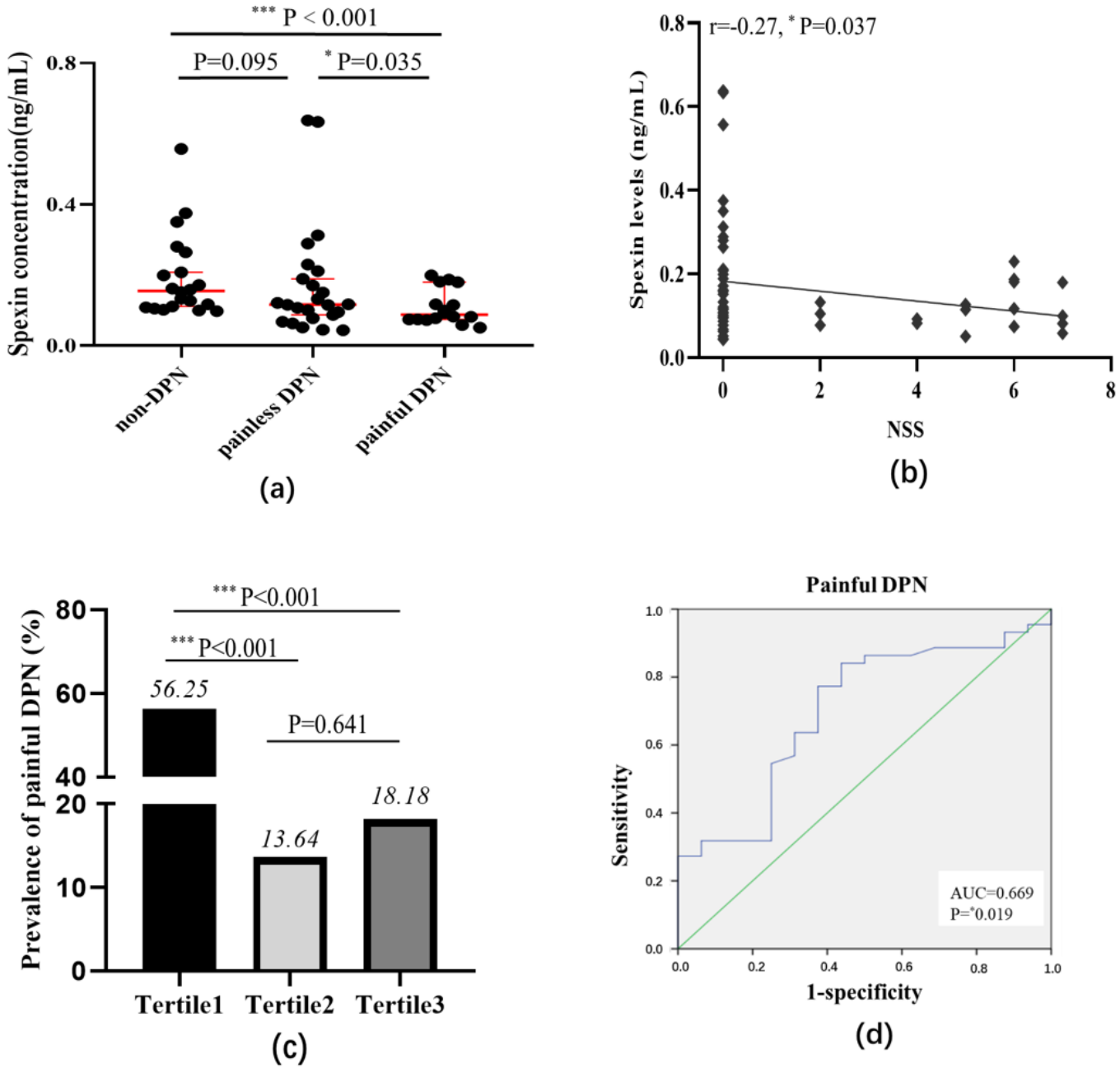

Figure 1

(a) Serum Spexin levels in patients with non-DPN, painless DPN and painful DPN. Individual data points are shown for all study patients. The median value of each group is shown as a thick red line. (b) Scatter plots showing the correlation of serum Spexin levels with NSS. (c) Prevalence of painful DPN in different tertiles of Spexin (%): tertile 1, <0.093 ng/mL; tertile 2, 0.093-0.152 ng/mL; tertile 3, >0.152 ng/mL. (d) ROC curve analyses for the prediction of painful DPN according to serum Spexin levels. (∗P<0.05, ∗∗P<0.01, ∗∗∗P<0.001).