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Vitamin B12 and Brain-Derived Neurotrophic Factor in Diabetic Patients with Chronic Generalized Pruritus

Nancy Wadie Mikhael[1], Asmaa A. Elfallah[2], Alzahraa Mosa Ahmed[1], Karem Taha Khalil*[1]
Departments of [1]Dermatology, Venereology and Andrology and
[2]Clinical Pathology, Faculty of Medicine – Benha University, Egypt
***Corresponding author**: Karem Taha Khalil, **Mobile:** (+20) 01282559828, **E-Mail:** karem.nawar_76@yahoo.com

ABSTRACT

Background: A common and bothersome condition with a complicated pathophysiology, including neuropathy, is diabetes-related pruritus. The growth factor known as "brain-derived neurotrophic factor" (BDNF) affects how nerve cells survive, differentiate, and are maintained.

Objectives: To measure the serum levels of BDNF and vitamin B12 (vitB12) in diabetic patients with generalized Pruritus.

Patients and Methods: One hundred fifty participants were included in this case-control research. They were split into three equal groups: diabetes patients with chronic generalized pruritus (n=50), diabetic patients with no pruritus (n=50), and normal age-sex matched control subjects (n=50). Complete clinical information, including the duration of diabetes, the duration of pruritus, and a history of drugs, was gathered. Using the 12-Item Pruritus Severity Scale (12-PSS), the severity of the pruritus was evaluated. A thorough general and dermatological evaluation was performed. Lab tests included FBS, PPBS, and HbAIC. Additionally, ELISA was used to measure the serum levels of BDNF and vitB12.

Results: Serum vitB12 and BDNF levels were significantly different among the study groups (P=0.000, P=0.000, respectively) with higher levels in diabetics compared to controls. Diabetics with pruritus exhibited higher vitB12, BDNF levels than diabetic non-pruritic group (P1=0.005, P1=0.000, respectively). Both vitB12 and BDNF showed significant positive correlation with 12-PSS (r=0.499, P=0.000; r=0.513, P=0.000, respectively). BDNF was positively correlated with vitB12 (r=0.518, P=0.000). There was a significant positive correlation between BDNF and the duration of diabetes and FBS, PPBS, HbA1C (P=0.000).

Conclusion: Increased serum levels vitB12 and BDNF levels might explain the cause of chronic generalized pruritus in diabetic patients.

Keywords: Brain-derived neurotrophic factor, Diabetes mellitus, Pruritus, Vitamin B12.

INTRODUCTION

Among diabetes patients, pruritus is a typical symptom [(1)]. If the itching continues for more than six weeks, it is considered chronic. Diabetes mellitus (DM) is one of the systemic diseases that is frequently connected to pruritus [(2)]. Patients with type 2 diabetes (T2DM) may develop pruritus in the range of 18.4% to 27.5% [(3)].

The pathophysiology of itching in diabetic people is unclear, and antihistamine therapy has failed in many patients. Skin disorders, neuropathy, end-stage kidney disease, and subsequent to hypoglycemic medications are all linked to the onset of pruritus in diabetic patients [(4)]. Diabetic patients can sometimes feel pruritus for no obvious reason [(5)].

Neurotrophins are chemicals that enhance neuronal development, health, and survival [(6)]. Among them, brain-derived neurotrophic factor (BDNF) has emerged as an important regulator of synaptic plasticity, neuronal survival, and differentiation [(7)], as well as systemic or peripheral inflammatory diseases such T2DM [(8)]. BDNF maintains high levels of expression in the adult brain and modulates both excitatory and inhibitory synaptic transmission [(9, 10)]. BDNF is expressed in a variety of non-neuronal organs, with platelets being the primary source of peripheral BDNF [(11)]. BDNF can pass the blood-brain barrier [(12)], and its serum concentration can reflect the nervous system concentration [(13)].

Vitamin B12 (vitB12) is a vitamin that is required for proper hematopoietic and neurocognitive processes. Biochemical and clinical vitB12 deficiency has been shown to be extremely common in people with type 1 and type 2 diabetes mellitus [(14)].

The aim of the work was to measure the serum levels of (BDNF) and vitamin B12 (vitB12) in diabetic patients with generalized Pruritus.

PATIENTS AND METHODS

Study population and design

The current study is a case-control study. Participants were recruited from the Dermatology & Andrology Department, Faculty of Medicine, Benha University Hospitals, between October 2021 and July 2022. Three study groups were composed of: fifty diabetic patients with chronic generalized pruritus (group A), fifty diabetic patients with no pruritus (group B), and fifty age- and sex-matched healthy volunteers served as a control group (group C).

Inclusion criteria:

Diabetic patients older than 18 years complaining of chronic generalized itching with no primary skin

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lesions were candidate to participate. Chronicity was defined as itching for more than six weeks.

Exclusion criteria:

Patients with primary pruritic skin diseases, or other systemic diseases as chronic autoimmune diseases, neoplastic, infectious diseases, renal failure, liver cell failure, biliary disorders, as well as pregnant or lactating women were not eligible to share in the study.

METHODS

Each participant underwent careful history taking (age, duration of DM, manifestations of DM, therapies for DM, vitB12 supplementations, duration of pruritus) as well as thorough clinical examination(general and dermatological).

The 12-item Pruritus Severity Scale (12-PSS), which includes questions on pruritus intensity, extent, and duration, as well as the impact of it on patient mental health, concentration, and scratching as a result to pruritus stimuli, is used to assess the severity of pruritus (15). Following 12-PSS rating in respect to itching severity, pruritus severity was graded as: mild (3-6 points), moderate (7-11 points), and severe (> 12 points) (16).The 12-PSS is adequate in assessing not only the intensity of itching but also provides information about its the impact on quality of life.

Laboratory investigations were done, including fasting blood sugar (FBS), postprandial blood sugar (PPBS), and glycosylated hemoglobin (HbA1C). Serum level of BDNF was done using Human BDNF ELISA kit (Catalogue No. 201-12-1303 made in China), as well as Vit B12 was measured using Human Vitamin B12 (VB12) ELISA Kit (Catalogue No.201-12-1545 2, made in China)).

Ethical approval:

This research was carried out after receiving approval from the Ethics Committee for Human Research at the Faculty of Medicine at Benha University in Egypt (MS38-9-2021). Each subject gave written informed consent prior to being enrolled in the present research. The Declaration of Helsinki for human beings was followed during the conduct of this study.

Statistical Analysis

SPSS version 24 software was used to tabulate and analyze the gathered data (Spss Inc, Chicago, ILL Company). Categorical data were displayed as percentages and numbers. Category-specific factors were analyzed using the Chi Square Test (X2). In terms of quantitative data, the mean, standard deviation, median, and range were used. Two independent groups' normally distributed variables were examined using the student "t" test. The correlation between non-parametric variables was calculated using Spearman's correlation coefficient (rho). Risk of association was examined using regression analysis. The most sensitive and specific cutoff values were found using the ROC curve. P <0.05 was deemed significant.

RESULTS

There were no significant differences among the study groups regarding age, sex, and BMI (P=0.245, .086, .036, respectively).

The age of diabetic patients ranged from 22-77 years and duration of diabetes from 1-25 years. Mean duration of pruritus was 6.76± 4.23months with range 2-17 months. Mean 12-PSS score was 17.20± 2.35 with range 10-21 (moderate cases (n=16), severe cases (n=34)). There was no statistically significant difference between the studied diabetic groups regarding duration of DM (P=0.309) (Table 1).

Table (1): Comparison of the data between the study groups

	Group A (n.=50)	**Group B (n.=50)**	**Group C (n.=50)**	**test**	**P. value**
Age (years) Mean±SD	45.42± 15.43	47.86±15.25	43.08± 11.48	F =1.421	0.245
Sex Female(n,%) Male (n,%)	 25, 50% 25, 50%	 26, 52% 24, 48%	 16, 32% 34, 68%	X^2=4.909	0.086
BMI(kg/m^2) Mean±SD	29.25± 5.23	29.37± 6.46	26.80±4.79	F =3.414	0.036
Duration of DM (years) Mean±SD	5.88± 3.65	6.80± 5.21	-	t=1.046	0.309
Duration of pruritus (months) Mean±SD	6.76± 4.23	-	-	-	-
12-PSS Scale Mean±SD Moderate (7-11) n,% Severe (12-22) n,%	 17.20± 2.35 16, 32% 34, 68%				
FBS (mg/dL) Mean±SD	137.06± 30.17	118.94±9.43	86.82±4.05	F =34.258	.000 LSD P1=.004 P2=.000 P3=.000
PPBS (mg/dL) Mean±SD	230.10± 55.64	184.36±44.80	126.40±3.03	F =40.322	000 LSD: P1=.000 P2=.000 P3=.000
HbA1C (%) Mean±SD	8.52± 0.851	8.39±0.956	4.94±0.472	F=331.648	.000 LSD: P1=.403 P2=.000 P3=.000
VitB12 (pmol/L) Mean±SD	506.14± 123.66	426.61±103.78	172.03±41.61	t= 58.458	.000 LSD: P1=.005 P2=.000 P3=.000
Serum BDNF (ng/ml) Mean±SD	2.02± 0.42	1.17±0.12	0.473±0.114	t=32.833	.000 LSD: P1=.000 P2=.000 P3=.000

Group A: Diabetic patients with generalized Pruritus, Group B: Diabetic patients with no Pruritus, Group C: Control group. P1= between Group A and Group B, P2 = between Group A and Group C, P3 = between Group B and Group C, LSD= Least Significant Difference, BMI (body mass index), 12-PSS (12-item pruritus severity scale), FBS (fasting blood sugar), PPBS (postprandial blood sugar), HbA1C(glycosylated hemoglobin), vitB12 (vitamin B12), BDNF(brain-derived neurotrophic factor).

In consideration to the laboratory investigations, there was statistically significant difference between study groups regarding vitB12, BDNF (P=0.000, 0.000, respectively). Diabetic group with pruritus showed significant differences when compared to diabetics with no pruritus in terms of FBS, PPBS and HbA1C (P1=0.004, 0.000, 0.403, respectively) (Table 1). History collection from the participants in terms of vitB12 supplementation, there was significant difference among the three groups (P=0.000). Diabetic patients with pruritus showed variable frequency of vitB12 administration, with 58% (n = 29) receiving injections three times weekly and 38% (n = 19) receiving injections twice weekly (Table 2).

Table (2): Comparison between the studied groups regarding vitB12 supplementation

	Group A (n=50)	Group B (n==50)	Group C (n==50)	X^2	P. value
History of vitB12 supplementation (n, %)	50, 100%	50, 100%	0, 100%	150.000	0.000
Frequency of vit B12 injections (n, %)					
Four times/week	1, 2%	0, 0%	0, 0%	266.773	0.000
Three times/week	29, 58%	0, 0%	0, 0%		
Twice/week	19, 38%	6, 12%	0, 0%		
Once/week	1, 2%	44, 88%	0, 0%		
No	0, 0%	0, 0%	50, 100%		

Using Pearson correlation analysis, there was significant positive correlation between vitB12, BDNF and 12-PSS score (r= 0.499, p=0.000; r= 0.513, p= 0.000, respectively). As well as, there was significant positive correlation between vitB12 and BDNF (r= 0.518, p= 0. 000). BDNF showed significant positive correlation with duration of DM, duration of pruritus, FBS, PPBS, HbA1C (p=0.000) (Table 3, Figure 1).

Table (3): Correlation between serum level of BDNF and other data.

Correlation	Pearson's correlation	
	R	P
BMI	0.094	0.255
Duration of DM	0.286	0.000
Duration of pruritus	0.380	0.00
FBS	0.292	0.000
PPBS	0.389	0.000
HbA1C	0.452	0.000
VitB12	0.518	0.000
12-PSS	0.513	0.000

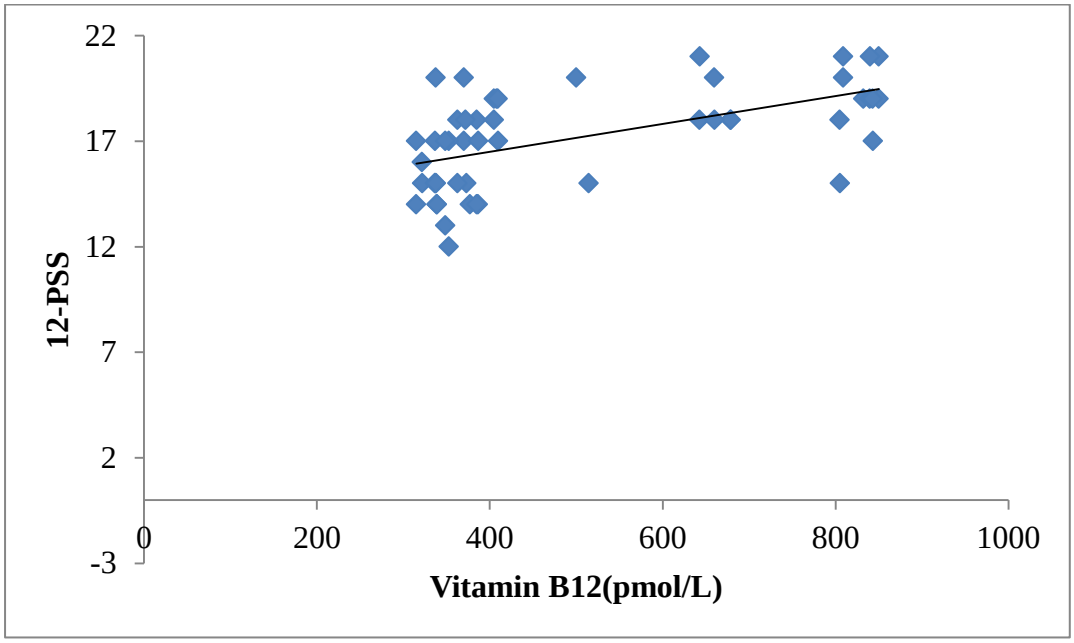

Figure (1): Correlation between vitB12 and severity of pruritus (12-PSS) (r=0.499, P=0.000).

As regards the levels of vitB12 and BDNF among group of pruritus, mean value of vitamin B12 was significantly lower among cases with moderate 12-PSS than severe cases. But, there was no significant difference in serum BDNF between all grades of 12-PSS (Table 4).

Table (4): Serum vitB12 and BDNF in relation to grading of 12-PSS

		Moderate (7-11) (n=16)	Severe (12-22) (n=34)	T-test	P. value
Serum BDNF (ng/ml)	Mean ± SD	1.75± 0.420	2.15±0.438	-1.041	0.303
Serum vitB12 (pmol/L)	Mean ± SD	389.32± 9.01	561.11±36.60	-3.054	0.004

Regarding diagnostic accuracy of serum BDNF in diagnosis of pruritus in diabetic patients, at cutoff point of 0.85ng/ml, AUC was 0.77 with sensitivity 94.0%, specificity 66.0%, PPV 73.4%, NPV 91.7% and accuracy 81% (Table 5, Figure 2).

Table (5): Diagnostic accuracy of serum BDNF in diagnosis of pruritus in diabetics.

	Cutoff point	AUC	Sensitivity	Specificity	+PV	-PV	Accuracy
Serum BDNF(ng/ml)	0.85	0.77	94.0%	66.0%	73.4%	91.7%	81%

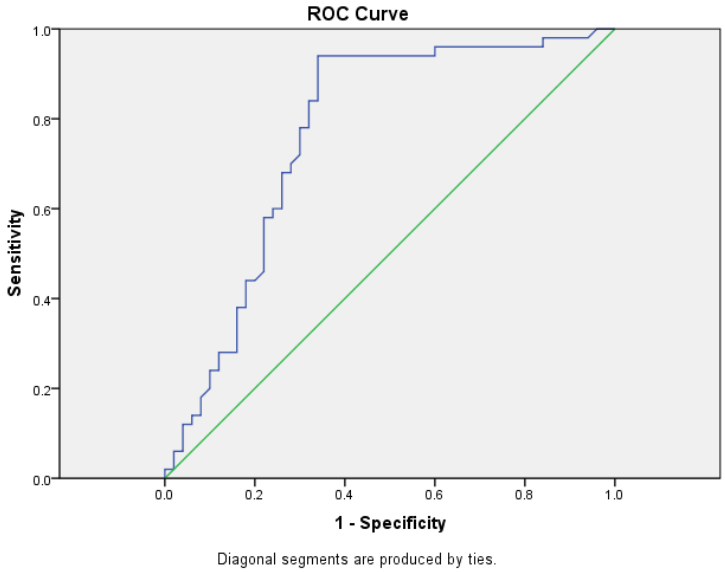

Figure (2): Roc curve for serum BDNF in diagnosis of pruritus in diabetics.

In the same context, the diagnostic accuracy of vitB12 in diagnosis of pruritus in diabetics, at cutoff point of 355 (pmol/L), AUC was 0.64 with sensitivity 64.0%, specificity 52.0%, PPV 57.1%, NPV 59.1% and accuracy was 58% (Table 6, Figure 3).

Table (6): Diagnostic accuracy of vitB12 in diagnosis of pruritus in diabetics.

	Cutoff point	AUC	Sensitivity	Specificity	+PV	-PV	Accuracy
vitB12 (pmol/L)	355	0.64	64.0%	52.0%	57.1%	59.1%	58%

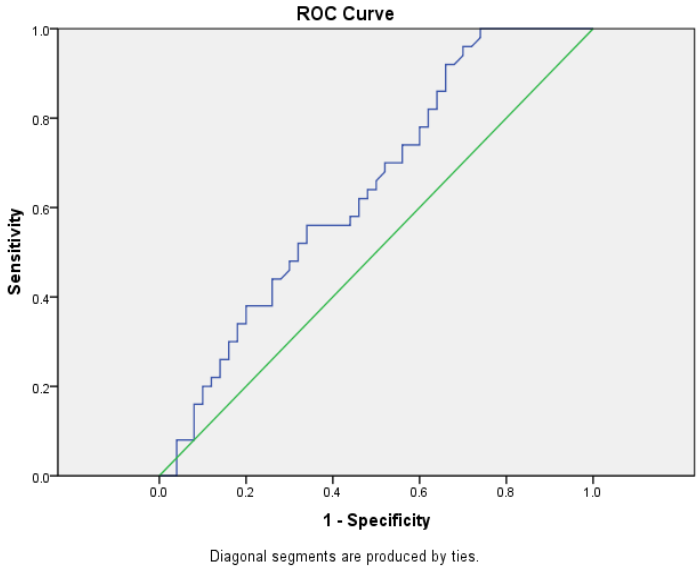

Figure (3): Roc curve for vitB12 in diagnosis of pruritus in diabetics.

DISCUSSION

Polyneuropathy is most frequently brought on by DM. Patients with diabetes may consequently have neuropathic itch. Truncal itch has been identified by **Yamaoka *et al.*** [5] as a typical clinical sign of neuropathy in diabetic individuals. Patients with diabetes who have widespread pruritus may have impaired peripheral sensory pathways, cortical oversensitivity, insufficient cortical inhibitory pathways, or an ineffective spinal cord regulating process[17].

In addition to its primary impacts on brain tissue, BDNF contributes to human energy homeostasis. Because of its effects on glycemia, lipid profiles, and energy balance, BDNF is also known as "metabokine." BDNF levels are decreased in type 2 diabetes[18], acute coronary syndrome, metabolic syndrome, and atherosclerosis [8].

Without proper dosage monitoring, vitB12 is frequently given to diabetic patients to prevent or treat peripheral nerve damage. Through the elevation of BDNF expression, **Sun *et al.*** [19] showed that vitB12 facilitates peripheral nerve healing in a rat model of sciatic nerve damage. According to studies, BDNF may play a role in atopic dermatitis-related pruritus [20], and chronic spontaneous urticarial itching [21]. In order to better understand the pathophysiology of chronic generalized pruritus in diabetic patients, we evaluated serum BDNF and vitB12 levels in this study.

Due to its hydrophilicity and ease of removal from the body in the event of an excess supply, vitB12 supplementation has long been regarded as safe. There are no definitive recommendations on how to detect vitB12 deficiency or how frequently people with diabetes should take vit B12 supplements. Supplementation of vitB12 is also not known to have an ideal dosage [14]. However, the results of the study by **Flores-Guerrero *et al.*** [22] that was published in the Journal of the American Medical Association have lately called into question this. Greater cobalamin levels were linked to higher mortality in the general population, according to these authors. Unexpectedly, when plasma cobalamin levels were within the guideline range, this connection became apparent. These findings led them to advise against taking vitB12 supplements unless a deficiency is proven.

The current study found that diabetic patients had higher amounts of vitB12 and BDNF than controls, and that patients with pruritus had higher levels than those without. Similarly, **Suwa *et al.*** [8] **and Boyuk *et al.*** [23] previously demonstrated that the serum of type 2 diabetes patients contained significantly higher amounts of BDNF than the serum of the control group. In contrast to past research that found lower levels in diabetics compared to non-diabetics, that found higher levels in diabetics [18, 24, 25]. The increased levels of BDNF in diabetic patients of the present study could be explained by the raised vitB12 levels in the participants; as supported by the synergism between vitB12 and BDNF that was documented by **Sun *et al.*** [19].

Through its numerous impacts on sensory nerves, BDNF may contribute to pruritus. It encourages sensory neurons to branch out [26], and synapse maintenance [27]. This shows how BDNF plays a crucial part in the initiation and/or preservation of hyperexcitability of the nervous system [28]. Additionally, BDNF can cause an allodynic condition that makes pruritus easier to experience [29].

The findings of this study exhibited significant positive associations between vitB12 and the 12-PSS score. Significant positive correlations between BDNF and 12-PSS, duration of pruritus, duration of diabetes, FBS, PPBS, HbA1C, and vitB12 were also observed. In contrast, **Krabbe *et al.*** [18] found that BDNF had an inverse relationship with FBS.

Limitations: The small size of study groups, the fact that both types of DM were not investigated separately, and the lack of neurological assessment were the main weak points in the current research.

CONCLUSION

Uncontrolled vitB12 supplementation, in conjunction with high serum BDNF in diabetic patients, may play a role in the aetiology of persistent widespread pruritus in diabetic patients.

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Competing interests: Nil.

REFERENCES

1. **Babakinejad P, Walton S (2016):** Diabetes and pruritus. Br J Diabetes, 16(4):154-5.
2. **Sabban E (2018):** Dermatoses most frequently related to diabetes mellitus. In: Sabban ENC, Puchulu FM, Cusi K, eds. Dermatology and diabetes. 1st ed. Cham: Springer International Publishing. Pp. 145-77. https://doi.org/10.1007/978-3-319-72475-1
3. **Stefaniak A, Chlebicka I, Szepietowski J (2021):** Itch in diabetes: a common underestimated problem. Adv Dermatol Allergol., 2: 177-83.
4. **Lima A, Illing T, Schliemann S *et al.* (2017):** Cutaneous manifestations of diabetes mellitus: a review. Am J Clin Dermatol., 18(4):541-53.
5. **Yamaoka H, Sasaki H, Yamasaki H *et al.* (2010):** Truncal pruritus of unknown origin may be a symptom of diabetic polyneuropathy. Diabetes Care, 33: 150-5.
6. **Bibel M, Barde Y (2000):** Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev., 14: 2919–2937.
7. **Binder D, Scharfman H (2004):** Brain-derived neurotrophic factor. Growth Factors, 22: 123–131.
8. **Suwa M, Kishimoto H, Nofuji Y *et al.* (2006):** Serum brain-derived neurotrophic factor level is increased and associated with obesity in newly diagnosed female

patients with type 2 diabetes mellitus. Metabolism, 55: 852–857.

9. **Tyler W, Perrett S, Pozzo-Miller L (2002):** The role of neurotrophins in neurotransmitter release. Neuroscientist, 8: 524–531.
10. **Wardle R, Poo M (2003):** Brain-derived neurotrophic factor modulation of GABAergic synapses by postsynaptic regulation of chloride transport. J Neurosci., 23: 8722–8732.
11. **Eyileten C, Kaplon-Cieslicka A, Mirowska-Guzel D *et al.* (2017):** Antidiabetic E_ect of Brain-Derived Neurotrophic Factor and Its Association with Inflammation in Type 2 Diabetes Mellitus. J Diabetes Res., 17: 2823671. doi: 10.1155/2017/2823671.
12. **Palma-Álvarez R, Ros-Cucurull E, Amaro-Hosey K *et al.* (2017):** Peripheral levels of BDNF and opiate-use disorder: literature review and update. Rev Neurosci., 28(5):499–508.
13. **Tekatas A, Arican O, Guler S *et al.* (2014):** Pruritus: do Aδ fibers play a role? J Dermatol., 41(1):98–101.
14. **Kibirige D, Mwebaze R (2013):** Vitamin B12 deficiency among patients with diabetes mellitus: is routine screening and supplementation justified?. Journal of Diabetes & Metabolic Disorders, 12: 17. doi: 10.1186/2251-6581-12-17
15. **Reich A, Bożek A, Janiszewska K *et al.* (2017):** 12-Item Pruritus Severity Scale: Development and Validation of New Itch Severity Questionnaire. Biomed Res Int., 17:3896423. doi: 10.1155/2017/3896423.
16. **Stępień K, Reich A (2020):** The 12-Item Pruritus Severity Scale – Determining the Severity Bands. Front Med (Lausanne), 7: 614005. https://doi.org/10.3389/fmed.2020.614005
17. **Ji Y, Lu Y, Yang F *et al.* (2010):** Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. Nat Neurosci., 13(3):302–309.
18. **Krabbe K, Nielsen A, Krogh-Madsen R *et al.* (2007):** Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. Diabetologia, 50: 431–438.
19. **Sun H, Yang T, Li Q *et al.* (2012):** Dexamethasone and vitamin B_{12} synergistically promote peripheral nerve regeneration in rats by upregulating the expression of brain-derived neurotrophic factor. Arch Med Sci., 8(5): 924–930.
20. **Hon K, Lam M, Wong K *et al.* (2007):** Pathophysiology of nocturnal scratching in childhood atopic dermatitis: the role of brain-derived neurotrophic factor and substance P. British Journal of Dermatology, 157: 922–925
21. **Rössing K, Novak N, Mommert S *et al.* (2011):** Brain-derived neurotrophic factor is increased in serum and skin levels of patients with chronic spontaneous urticarial. Clinical & Experimental Allergy, 41: 1392–1399.
22. **Flores-Guerrero J, Minovic I, Groothof D *et al.* (2020):** Association of plasma concentration of vitamin B12 with all-cause mortality in the general population in the Netherlands. JAMA Netw Open, 3(1): e1919274. doi: 10.1001/jamanetworkopen.2019.19274.
23. **Boyuk B, Degirmencioglu S, Atalay H *et al.* (2014):** Relationship between levels of brain-derieved neurotrophic factor and metabolic parameters in patients with type 2 diabetes mellitus. J Diabetes Res., 14:978143. doi: 10.1155/2014/978143.
24. **Fujinami A, Ohta K, Obayashi H *et al.* (2008):** Serum brain-derived neurotrophic factor in patients with type 2 diabetes mellitus: Relationship to glucose metabolism and biomarkers of insulin resistance. Clin Biochem., 41: 812–817.
25. **Li B, Lang N, Cheng Z (2016):** Serum Levels of Brain-Derived Neurotrophic Factor Are Associated with Diabetes Risk, Complications, and Obesity: A Cohort Study from Chinese Patients with Type 2 Diabetes. Mol Neurobiol., 53: 5492–5499.
26. **Vicario-Abejón C, Owens D, McKay R *et al.* (2002):** Role of neurotrophins in central synapse formation and stabilization. Nat Rev Neurosci., 3(12):965–974.
27. **Zhen Y, Liu X, Zhou D *et al.* (2018):** Cognition, serum BDNF levels, and BDNF Val66Met polymorphism in type 2 diabetes patients and healthy controls. Oncotarget., 9(3):3653–3662.
28. **Nijs J, Malfliet A, Ickmans K *et al.* (2014):** Treatment of central sensitization in patients with 'unexplained' chronic pain: an update. Expert Opin Pharmacother., 15(12):1671–1683.
29. **Vranken J (2012):** Elucidation of pathophysiology and treatment of neuropathic pain. Cent Nerv Syst Agents Med Chem., 12(4):304–314.