

The correlation between B12 and D3 with some Oxidative stress indicators in Diabetic Neuropathy Patients in Salah Al-Din Governorate

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ABSTRACT:

Diabetic neuropathy is the main cause of neuropathy and major complications of diabetes mellitus throughout the world. This may lead to incapacity and amputations due to severe diabetes. The morbidity and mortality rates increase in diabetes due to severe neuropathy. Diabetic neuropathy is one of the most common chronic complications of diabetes. The present study aimed to study the correlation between B12 and D3 with some Oxidative stress indicators in Diabetic Neuropathy Iraqi Patients. Method: 90 serum specimens for both sexes were collected from the consulting Clinic at Tikrit Teaching Hospital. Participants aged between 45 and 65 were enrolled in a control case study. The patients diagnosed by specialist doctors conduct diagnostic analyzes and subject patients to the mechanism of peripheral nerve planning, the groups are divided into three groups, the first group included (30) samples of people with diabetic neuropathy, the second group included (30) samples for both sexes for patients with diabetic, the Third Control group included (30) samples of healthy people who do not suffer from diseases and enjoy good health. Result: A statistically significant increase in the mean RBS level, Nitric oxide and Free amino, a statistically significant decrease in the mean vitamin B12 level and D3 level in the Neuropathy and diabetes compared with healthy controls. In the Neuropathy group and Diabetic group have no statistically significant correlation between Free amino and D3, B12 and between Nitric oxide and D3, B12. In the control group (G3), there is a statistically significant positive moderate correlation between Free amino and D3 ($r = 0.479$ $p = 0.007$), and there is a statistically significant positive moderate correlation between Nitric oxide and D3 ($r = 0.489$ $p = 0.006$), but there is no statistically significant correlation between Nitric oxide and B12, Nitric oxide and B12. there is no statistically significant correlation between B12 and D3 in the Neuropathy group, Diabetic group and control group.

Keywords: Diabetic Neuropathy, Oxidative stress, vitamin B12, vitamin D3

العلاقة بين B12 و D3 مع بعض مؤشرات الإجهاد التأكسدي لدى مرضى الاعتلال العصبي السكري في محافظة صلاح الدين

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مستخلص:

الاعتلال العصبي السكري هو السبب الرئيسي للاعتلال العصبي والمضاعفات الرئيسية لمرض السكري في جميع أنحاء العالم. هذا قد يؤدي إلى العجز وبتير الأطراف بسبب مرض السكري الحاد. تزداد معدلات المرض والوفيات في مرض السكري بسبب الاعتلال العصبي الحاد. الاعتلال العصبي السكري هو واحد من المضاعفات المزمنة الأكثر شيوعاً لمرض السكري. هدفت الدراسة الحالية إلى دراسة العلاقة بين B12 و D3 مع بعض مؤشرات الإجهاد التأكسدي لدى مرضى الاعتلال العصبي السكري العراقيين. طريقة العمل: تم جمع 90 عينة مصل لكلا الجنسين من العيادة الاستشارية في مستشفى تكريت التعليمي. تم تسجيل المشاركين الذين تتراوح أعمارهم بين 45 و 65 عاماً في دراسة حالة التحكم. أما المرضى الذين تم تشخيصهم من قبل أطباء مختصين فيقومون بإجراء التحاليل التشخيصية وإخضاع المرضى لآلية تخطيط الأعصاب الطرفية، حيث تم تقسيم المجموعات إلى ثلاث مجموعات، المجموعة الأولى ضمت (30) عينة من المصابين باعتلال الأعصاب السكري، والمجموعة الثانية شملت (30) عينة لكلا الجنسين لمرضى السكري، والمجموعة الثالثة ضمت المجموعة الضابطة (30) عينة من الأشخاص الأصحاء الذين لا يعانون من الأمراض ويتمتعون بصحة جيدة.

النتائج: هنالك زيادة ذات دلالة إحصائية في متوسط مستوى RBS وأكسيد النيتريك Nitric oxide والأمينية الحرة Free amino ، وانخفاض معتد به إحصائياً في متوسط مستوى فيتامين B12 ومستوى D3 في الاعتلال العصبي والسكري مقارنة مع الضوابط الصحية. في مجموعة الاعتلال العصبي ومجموعة السكري ليس لها علاقة ذات دلالة إحصائية بين الأمينية الحرة Free amino و B12 ، D3 وبين أكسيد النيتريك Nitric oxide و D3 ، B12. في المجموعة الضابطة (G3) ، هناك ارتباط معتدل إيجابي ذو دلالة إحصائية بين الأمينية الحرة Free amino و D3 ($r = 0.479$ $p = 0.007$) ، وهناك علاقة معتدلة إيجابية ذات دلالة إحصائية بين أكسيد النيتريك Nitric oxide و D3 ($r = 0.489$ $p = 0.006$) ، ولكن لا يوجد ارتباط ذو دلالة إحصائية بين Free amino و B12 وأكسيد النيتريك Nitric oxide و B12. لا يوجد ارتباط ذو دلالة إحصائية بين B12 و D3 في مجموعة الاعتلال العصبي ومجموعة السكري ومجموعة السيطرة.

الكلمات المفتاحية: اعتلال العصب السكري، جهد الاكسدة، فيتامين بي 12، فيتامين دي 3 .

Introduction:

Neuropathy is a nerve injury that starts with the longest nerves that innervate the toes and progresses proximally [1]. Common symptoms are numbness, tingling, pain, and weakness in the distal lower extremities. Diabetes is well established as the most important metabolic risk factor for neuropathy, but treatment of hyperglycaemia is not enough to prevent neuropathy in those with type 2 diabetes [2]. The presence of diabetic neuropathy determines a high risk of death, development of pain symptoms, foot ulcers and amputation of the lower limbs [3]. Data on peripheral nervous system damage in diabetes vary from 15 to 90% (4,236), depending on the diagnostic methods used to confirm the diagnosis of DPN and the patient population examined [4]. The prevalence of neuropathy is 8–45% in those with type 2 diabetes, with about a quarter of patients experiencing pain [5]. Cobalamin, also known as vitamin B12, is an indispensable water-soluble molecule. As far as it is known, its function is restricted to cofactorial activity for only two enzymes. However, it is crucial for the body's anabolic and catabolic processes, with more empha-

sis on strong cell replication events like hematopoiesis and tissue expansions [6,7]. Vitamin D is a group of fat-soluble vitamins traditionally recognized for its role in maintaining calcium and phosphorous homeostasis. Vitamin D commonly occurs in two forms: vitamin D2 and vitamin D3 [8]. Vitamin D3, also known as cholecalciferol, is synthesized *de novo* in the skin on exposure to ultraviolet-B radiation, and it is also available from animal-source foods [9,10].

Patient and Methods:

Ninety serum specimens for both sexes were collected from the Consulting Clinic at Tikrit Teaching Hospital. Participants aged between 45 and 65 were enrolled in a control case study. The study was carried out between November 2023 and April 2024, following approval from the Ethical Committee at the Faculty of Education for Women, University of Tikrit. Informed consent was obtained from all participants, and the study received the necessary permissions from the Consulting Clinic at Tikrit Teaching Hospital.

The patients diagnosed by specialist doctors conduct diagnostic analyses and subject patients to the mechanism of peripheral nerve planning, the

groups are divided into the following: The first group (G1): includes (30) samples of people with diabetic neuropathy medically diagnosed, the second group (G2): included (30) samples for both sexes for patients with diabetes (diabetic). and The Third Control group (C): included (30) samples of healthy people who do not suffer from diseases and enjoy good health.

Data collection included anthropometric measurements such as age, gender, and body mass index (BMI). Approximately 5 ml of blood was collected from each patient group and control group to measure the concentration level of parameters (vitamin B12, D3, Nitric oxide, Free amino)

Inclusion criteria: Patients aged 45 to 65 years, Type 2 diabetes medical history, HBA1c-based diagnostic criteria for DM2 from 1 to 5 years. A control group of volunteers was formed using the following criteria: Clinically healthy, Negative for clinical indicators of systemic illnesses, and Negative for diabetes.

Exclusion criteria: Patients/individuals were removed from the research, including those with physiological conditions such as pregnancy and breastfeeding, behaviors such as

smoking, drinking, and chewing tobacco, Subjects under the age of 45, and those who have had radiation treatment and chemotherapy.

Statistical analysis was performed using SPSS version 25.0. Descriptive statistics, including mean, range, standard deviation and frequency. The ANOVA test was employed to evaluate differences in mean levels of numeric data across groups. The Chi-square test assessed associations between quantitative variables. Pearson correlation regression was used to evaluate correlations between numeric variables, with r values interpreted as follows: $r < 0.2$ indicates a weak correlation, $0.2-0.8$ indicates a moderate correlation and $r > 0.8$ indicates a strong correlation. Scatter plots were utilized to illustrate the correlations between variables. A p -value of ≤ 0.05 was considered statistically significant.

Results:

A total of 90 patients participated in the study, there is no statistically significant difference in the mean BMI was 25.320 ± 5.060 kg/m² in the first group of Neuropathy, 27.498 ± 4.189 kg/m² in the second group of diabetes, and 27.245 ± 2.881 kg/m² in the third group

of healthy controls. A statistically significant increase in the mean RBS level was 165.9 ± 13.5 mg/dL in the first group of Neuropathy, 169.0 ± 17.1 mg/dL in the second group of diabetes, and 121.6 ± 19.9 mg/dL in the third group of healthy controls ($p \leq 0.05$). In addition, there was a statistically significant decrease in the mean vitamin D3 level between the groups; were 36.59 ± 5.96 in the first group of Neuropathy, 37.27

± 5.68 in the second group of diabetes, and 40.68 ± 6.36 in the third group of healthy controls ($p \leq 0.05$). Also, a statistically significant decrease in the mean vitamin B12 level between the groups; were 129.8 ± 26.7 in the first group of Neuropathy, 132.2 ± 21.5 in the second group of diabetes, and 165.7 ± 26.1 in the third group of healthy controls ($p \leq 0.05$). as shown in Table 1.

Table 1: Clinical characteristics of the included participant

Groups	BMI	RBS (mg/dl)	D3 (ng/ml)	B12 (ng/l)
G1 Neuropathy	25.320 ± 5.060 a	165.9 ± 13.5 a	36.59 ± 5.96 b	129.8 ± 26.7 b
G2 Diabetic	27.498 ± 4.189 a	169.0 ± 17.1 a	37.27 ± 5.68 b	132.2 ± 21.5 b
G3 Control	27.245 ± 2.881 a	121.6 ± 19.9 b	40.68 ± 6.36 a	165.7 ± 26.1 a
P-value	Ns	0.05*	0.05*	0.05*

* A p-value of ≤ 0.05 was considered statistically significant.

Table 2 shows there is a statistically significant increase in the mean level of Nitric oxide was 24.93 ± 2.91 in the first group of Neuropathy, 38.02 ± 8.56 in the second group of diabetes, compared with the third group of healthy controls 13.33 ± 3.61 ($p \leq 0.01$). Also, there is a statistically significant increase in the mean level of Free amino

was 92.92 ± 15.92 in the first group of Neuropathy, 99.69 ± 17.96 in the second group of diabetes, compared with the third group of healthy controls 79.25 ± 17.2 ($p = 0.01$).

Table 2: shows the mean difference in Nitric oxide and Free amino in the groups

Groups	Nitric oxide	Free amino
G1 Neuropathy	24.93±2.91 B	92.92±15.92 A
G2 Diabetic	38.02±8.56 A	99.69±17.96 A
G3 Control	13.33±3.61 C	79.25±17.2 B
P-value	**0.01	**0.01

** A p-value of ≤ 0.05 was considered statistically significant.

The correlation between D3, B12, Free amino, and Nitric oxide in the three groups was assessed using the Pearson correlation coefficient.

The Neuropathy group (G1) and Diabetic group (G2) have no statistically significant correlation between Free amino and D3, B12 and between Nitric oxide and D3, B12.

In the control group (G3), there is a

statistically significant positive moderate correlation between Free amino and D3 ($r=0.479$, $p=0.007$), and there is a statistically significant positive moderate correlation between Nitric oxide and D3 ($r= 0.489$ $p= 0.006$), but there is no statistically significant correlation between Free amino and B12, Nitric oxide and B12. as presented in table 3.

Table 3: The correlation between D3, B12, Free amino and Nitric oxide in the three groups

Type		parameter	D3	B12
Neuropathy G1	Pearson Correlation	Free amino	0.236	0.015 -
	p-value		0.209	0.581
	Pearson Correlation	Nitric oxide	0.225	0.076
	p-value		0.232	0.689
Diabetic G2	Pearson Correlation	Free amino	-0.133	-0.061
	p-value		0.483	0.747
	Pearson Correlation	Nitric oxide	-0.075	0.023
	p-value		0.694	0.903
control G3	Pearson Correlation	Free amino	**0.479	0.026
	p-value		0.007	0.892
	Pearson Correlation	Nitric oxide	**0.489	0.232
	p-value		0.006	0.218

** Correlation is significant at the 0.01 level (2-tailed).

The correlation between vitamin B12 and D3 in the Neuropathy group, Diabetic group and control group. as presented in Table 4.

Table 4: The correlation between vitamin D3 and B12 in three groups.

Type			D3
Neuropathy G1	Pearson Correlation p-value	B12	-0.086 0.650
Diabetic G2	Pearson Correlation p-value	B12	0.050 0.795
control G3	Pearson Correlation p-value	B12	-0.212 0.262

The ROC test was used to evaluate the presence of vitamin B12 and D3 in the control and diabetic groups and the control and neuropathy groups. between the Control and diabetic groups, B12 and D3 had small AUC 0.397, 0.368, low sensitivity 56%, 52%, and very low specificity 40%, and 36%, respectively. between the Control and neuropathy groups, B12 and D3 had small AUC 0.344, 0.373, low sensitivity 64%, 56%, and very low specificity 24%, and 36%, respectively, as presented in Table 4.

Table 4: ROC test in the detection of D3 and B12 in the Control and diabetic group and the Control and neuropathy group.

Control and diabetic group					
Parameters	AUC	Cut off	% Sensitivity	% Specificity	P-value
D3 ng/ml	0.397	37.506	56	40	0.211
B12 ng/l	0.368	124.9395	52	36	0.109
Control and neuropathy group					
Parameters	AUC	Cut off	% Sensitivity	% Specificity	P-value
D3 ng/ml	0.344	35.25	64	24	0.059
B12 ng/l	0.373	117.31	56	36	0.123

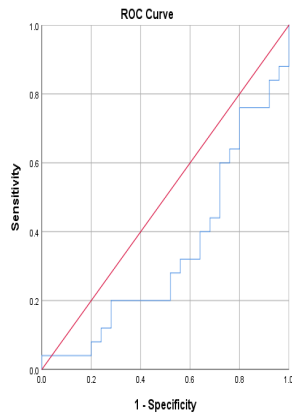


Figure 1: ROC diagram of vitamin D3 between the Diabetic Neuropathy group and control group.

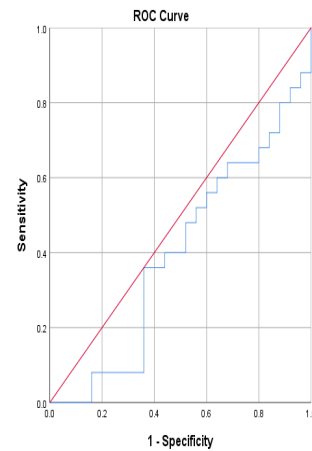


Figure 2: ROC diagram of vitamin D3 between the Diabetes mellitus group and control group.

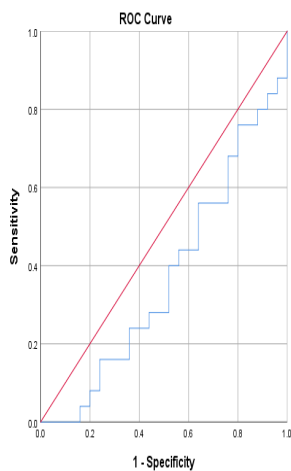


Figure 3: ROC diagram of vitamin B12 between the Diabetic Neuropathy group and control group.

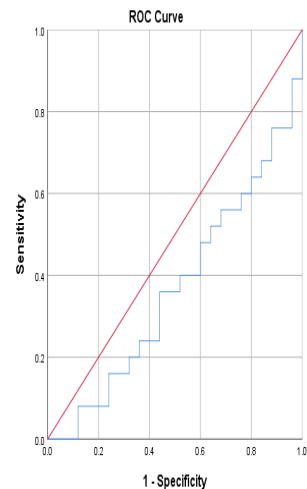


Figure 4: ROC diagram of vitamin B12 between the Diabetic Neuropathy group and control group.

Discussion:

Diabetes is a major cause of morbidity and mortality worldwide. The increase in the prevalence of diabetes mellitus inevitably leads to an increase

in microvascular and macrovascular complications. These complications lead to serious health problems like neuropathy. Neuropathy is a nerve injury that starts with the longest nerves

that innervate the toes and progresses proximally. This study aims to find out the correlation between B12 and D3 with some Oxidative stress indicators in Diabetic Neuropathy.

In Table 1 shows a statistically significant increase in the mean RBS level in the group of Neuropathy and diabetes compared with the group of healthy controls ($p < 0.01$). This corresponds with the Puri study which found a statistically significant increase in the mean RBS level in the group of Neuropathy compared with control [11].

Several studies have examined the relationship between a low vitamin D level and neuropathy. In this study a statistically significant decrease in the mean vitamin D3 level between the groups; in Neuropathy and diabetes compared with healthy controls ($p < 0.05$). this corresponds with Abdel-sadek *et al.* (2018) and Marton (2023) reported that vitamin D deficiency had a significant role in the development and severity of Diabetic neuropathy in Egyptian patients with type 2 diabetes mellitus (T2DM). Patients with diabetes and Diabetic neuropathy, as well as healthy control subjects, were included. The mean serum 25(OH)D level was decreased in patients with Diabet-

ic neuropathy compared to the control group. On regression analysis, vitamin D deficiency was an independent risk factor for Diabetic neuropathy [12, 13].

Neuropathy, being a relative health abnormality occurring owing to vitamin B12 deficiency affects about 30% of diabetic patients who are over forty years of age and state around having a decreased sensory perception in their feet [14]

Unfortunately, signs and symptoms of diabetic neuropathy and paraesthesia are somewhat identical, with decreased vibration sense and reduced proprioception related to vitamin B12 deficiency [15]

In this study there is a statistically significant decrease in the mean vitamin B12 level between the groups; Neuropathy and diabetes compared with healthy controls group ($p < 0.05$). Diabetic neuropathy symptoms overlap with deteriorated vibration sensation and proprioception, and paraesthesia, which has also been shown to be linked with vitamin B12 deficiency [15]

Recent studies showed a high prevalence of vitamin B12 deficiency from 28 to 41% of diabetic patients [16-19]. However, a study by Alvarez et al re-

vealed that the prevalence of vitamin B12 deficiency in diabetic patients with neuropathy was 17%. Also, they found inverse correlation between diabetic neuropathy and vitamin B12 levels and a significant difference in vitamin B12 concentrations. [20]

Conclusion:

In this study reached in the Neuropathy group and Diabetic group have no statistically significant correlation between Free amino and D3, B12 and between Nitric oxide and D3, B12. In the control group (G3), there is a statistically significant positive moderate correlation between Free amino and D3 ($r=0.479$ $p=0.007$). There is a statistically significant positive moderate correlation between Nitric oxide and D3 ($r=0.489$ $p=0.006$), but there is no statistically significant correlation between Free amino and B12, Nitric oxide and B12. there is no statistically significant correlation between B12 and D3 in the Neuropathy group, Diabetic group and control group.

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