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Is Vitamin D Status a Factor Influencing the Onset of Neuropathy in Type 2 Diabetics?

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Abstract

Introduction: A growing body of evidence suggests that the role of vitamin D (vit D) is not limited to phosphocalcic homeostasis, but other target organs have also been discovered, justifying a growing interest in this hormone. Although a drop in vit D levels is very common in type 2 diabetes mellitus (T2DM), no study has shown that this is a major factor in the onset of diabetic neuropathy. The aim of this study was to determine the prevalence of vit D deficiency in patients with T2DM, to identify factors influencing vit D status and to study the association between vit D deficiency and the development of neuropathy in patients with type T2DM. ***Methods:*** A cross-sectional study was conducted at the Farhat Hached University Hospital Centre (CHU) in Sousse, among type 2 diabetic patients aged between 25 and 70 years. All patients presenting a physiological or pathological situation likely to lead to hypovitaminosis D were excluded. ***Results:*** A total of 155 patients were included. There were 62 men (40%) and 93 women (60%), giving a sex ratio of 0.66. Nearly 2/3 (64%) were over 50 years of age, with a mean age of 53.4±10.8 years. Diabetic peripheral neuropathy was diagnosed in 40% (62 patients) of cases with a mean DN4 score of 2.8 ± 2.67. The most common vegetative neuropathy was cardiovascular autonomic neuropathy (29.23%), followed by gastrointestinal digestive neuropathy (26.15%). Only one patient with neuropathy had normal vitamin status and almost all (60 patients) had vit D levels below 20 ng/ml. The mean vit D level was significantly lower in diabetics with neuropathy than in those without this complication ($p \leq 10\text{-}3$). There was a statistically significant negative correlation between mean vit D level and DN4 score (p=10-3). The univariate study showed that a vit D level < 20 ng/ml was one of the factors associated with the onset of neuropathy ($p=10^{-3}$). After multiple binary logistic regression, including the variables of interest, vit D level appeared to be significantly associated with neuropathy (p=0.012) with an adjusted OR=0.904. ***Conclusion:*** Our results show that vit D deficiency is a risk factor directly and independently associated with the development of peripheral neuropathy.

Keywords: type 2 diabetes mellitus, diabetic neuropathy, microangiopathy, complications of diabetes, vitamin D

1. Introduction

In recent years, there has been increasing evidence to suggest that the role of vitamin D (vit D) is not limited to phosphocalcium homeostasis but has been discovered to have other target organs (Mitri J., 2014). Indeed, the

recent discovery of its physiological role in neuroprotection, immunity and cell differentiation and proliferation has led to a growing interest in this hormone. As a result, it is increasingly seen as one of the main environmental factors that can limit the risk of certain diseases (Muscogiuri G, Mitri J, Mathieu C, Badenhoop K, Tamer G et al., 2014).

Recently, vit D has been presented as a protective agent against the development of type 2 diabetes mellitus (T2DM) (Mitri J., 2014). The theory that vit D is involved in the pathogenesis of diabetes is also based on immunohistological findings, in which the presence of the vit D receptor on pancreatic β cells has been demonstrated (Mitri J., 2014). In addition, this vitamin-hormone is thought to have parameters that influence the expression of diabetes, such as involvement in the modulation of insulin secretion, peripheral insulin sensitivity and the phenomena of systemic inflammation (Mitri J., 2014). Vit D deficiency (VDD) may therefore be associated with poor glycaemic control and may also be a risk factor for the development or progression of degenerative complications associated with T2D, such as diabetic neuropathy (DN) (Joergensen C, Hovind P, Schmedes A & Parving RP., 2011).

It appears that low levels of vit D may be linked to the onset of neuropathy and other neurodegenerative disorders, suggesting that treatment with vit D corrects this problem of predisposing metabolic disturbance (Fernandes DA, Eyles D & Fe´ron F. Vitamin D, 2009). In animal models, studies have shown that treatment with a vit D analogue stimulates the production and prevents the depletion of nerve growth factors, which are necessary for the development and survival of neurons (Riaz S, Malcangio M, Miller M & Tomlinson DR., 1999).

Hypovitaminosis D(HVD) is very common in patients with T2DM (Isaia G, Giorgino R & Adami S., 2001) and may be a prelude to the development of DN.

The aims of this study are to determine the prevalence of VDD in T2DM and study the association between VDD and the occurrence of ND in patients with T2DM.

2. Patients and Methods

It's across-sectional and analytical prospective study conducted from 1st May 2015 until 27th February 2017 among T2DM patients consulting the endocrinology department of CHU Farhat Hached in Sousse.

2.1 Inclusion Criteria

All patients between 25 and 70 years of age, with known T2DM at least 6 months duration and who had given informed consent to participate in the study were included.

2.2 Exclusion Criteria

Patients with other types of diabetes or with progressive cancer or a serious pathology endangering short-term vital prognosis, or with any physiological or pathological situation likely to lead to hypovitaminosis D such as pregnancy/breastfeeding, recent fracture with immobilisation during the last 6 months, cardiovascular event during the last 3 months, hepatic insufficiency, chronic renal disease, nephrotic syndrome, renal insufficiency with renal clearance of less than 60 ml/min, known thyroid and/or parathyroid pathology, lymphoma, sarcoidosis, tuberculosis, rickets, osteomalacia and osteoporosis, malabsorption syndromes (Crohn's disease, cystic fibrosis, coeliac disease), bariatric surgery, patients taking medication that may interfere with phosphocalcic metabolism for less than two years: vit D or calcium, anticonvulsants, glucocorticoids, rifampicin, immunosuppressants, HIV treatments and antifungals.

2.3 Data Collection

Data were collected using a form that included:

- epidemiological Data,
- family and personal history,
- lifestyle habits,
- sun exposure,
- clothing habits and whether a protective screen is used,
- time spent outdoors and timetable,
- type of accommodation (house with or without garden, flat with or without balcony),
- phototype (the classification used for phototypes is that of Fitzpatrick (Mithal P., 2004), which is an international reference. It is made up of 6 groups, classified in ascending order according to skin pigmentation),
- data on dietary intake, rich in vit D: Consumption of foods rich in vit D: dairy products, oily fish, eggs

or foods containing them. We used the questionnaire of Mrs M. Garabédian (Garabedian M, Menn S, Walrant-Debray O, Teinturier C & Delaveyne R RA., 2005) and Amena Sadiya (Amena Sadiya SMA, 2014) validated in their studies for the items relating to diet and sun exposure.

- characteristics of diabetes: duration, treatment and degenerative complications of diabetes.
- General clinical examination
- Anthropometric measurements:
    - Weight (only one weighing was done). All measurements were taken on subjects wearing light clothing and no shoes.
    - Height: only one measurement was taken. This was done on subjects with their shoes off, feet flat on the floor, heels together, backs against the wall and heads placed horizontally.
    - Body Mass Index (BMI) defined as the ratio of weight in kg to height in metres squared and expressed in kg/m². The obesity class is determined by reference to the classification adopted by the WHO in 1998 (Organization World Health, 2000).
    - Waist circumference: a single measurement was taken. This was done on scantily clad subjects using a non-stretchable tape measure, taking the minimum diameter between the iliac crest and the lower costal margin. Androïd fat distribution was defined by a waist circumference ≥ 80 cm in women and ≥ 94 cm in men according to the European standards of the International Diabetes Federation (International Diabetes Fideration, 2009).
- Blood pressure: Statistical Methods
- A neurological examination including:
    - Assessment of osteotendinous reflexes.
    - Assessment of deep and superficial sensitivity.
    - DN4 questionnaire: the diagnosis of neuropathic pain (DN) is made if the patient's score is equal to or higher than 4/10 (Bouhassira D., 2005).

- All patients underwent the following biological investigations:
    - Fasting blood glucose, glycated haemoglobin.
    - Lipid profile, including total cholesterol, triglycerides and HDL-cholesterol.
    - LDL cholesterol was calculated using the FRIDEWALD (Friedewald WT & Levy RI FD., 1972) formula.
    - A phosphocalcic work-up: serum calcium, serum phosphorus, serum albumin and calculation of corrected serum calcium.
    - A creatinine assay with calculation of creatinine clearance using the simplified MDRD formula.
    - Measurement of 25 hydroxy vitamin D (25(OH) D): performed on the 'Liaison Diasorin' analyser, using the direct competitive immunoassay technique with chemiluminescence revelation (CLIA: Chimiluminecent Immuno Assay Technology). Blood samples were taken in dry tubes and sent to the laboratory the same day for centrifugation. Patient sera were identified and then frozen at -80°C until the day of the assays.

3. Statistical Analysis of Data

The data were entered and analysed using SPSS version 20 software. For qualitative variables, relative frequencies were calculated. For quantitative variables, means and standard deviations were calculated and extreme values were determined.

Comparisons of two means on independent series were carried out using Student's t-test for independent series. Comparisons of percentages on independent series were made using Pearson's chi-square test. Links between 2 quantitative variables were studied using Pearson's correlation coefficient. To identify the risk factors associated with the occurrence of diabetic neuropathy, we conducted a multivariate analysis using stepwise logistic regression. The multivariate analysis was used to calculate adjusted odds ratios, measuring the specific role of each factor. The significance threshold for the tests used was $p<0.05$.

4. Ethical Considerations

This study required the agreement of the ethics committee and the informed consent of the patients. All patients with vitamin D deficiency were invited to participate and were given supplements. There are no conflicts of interest in this work.

5. Results

The study population comprised 155 diabetics, 62 men (40%) and 93 women (60%), giving a sex ratio of 0.66. The mean age of the participants was 53.4±10.8 years.

Smoking and alcohol consumption were observed in 24.5% and12.3% of patients.

The average age of diabetes was 9.32 years, with extremes ranging from 1 to 30 years. The mean age at onset of diabetes was 44.12 years, ranging from 15 to 69 years.

Diabetes treatment was oral antidiabetics alone in 88.4% of cases (including 80% of patients on metformin, 41.3% on sulfonamides, 26.5% on acarbose, 5.8% on glinides and 3.2% on IDPP4) and insulin therapy in 45.2% of cases, 37.1% of whom were on a Bed time regimen.

These diabetic patients were complicated by microangiopathy other than neuropathy, such as diabetic retinopathy in 26.4% of cases and diabetic nephropathy in 25.2% of cases. Retinopathy was proliferative in 21 cases (51.21%) and nephropathy was at the stage of macroalbuminuria with correct renal function in 35.1% of cases.

The presence of macroangiopathy was noted: ischemic heart disease in 13.5% of patients, 66.7% of whom were in heart failure, stroke or transient ischemic attack (TIA) in 12.9%, arteritis of the lower limbs (AOMI) in 17.4%.

Diabetic neuropathy is the most frequently encountered complication of diabetes. It manifests as peripheral diabetic neuropathy and autonomic neuropathy. Peripheral diabetic neuropathy was diagnosed in 40% of patients, with an average DN4 score of 2.8 ± 2.67. The most common forms of autonomic neuropathy were as follows: cardiovascular autonomic neuropathy (29.23%), gastrointestinal autonomic neuropathy (26.15%), vasomotor autonomic neuropathy (16.92%), urinary incontinence (16.92%) and erectile dysfunction was present in 11.3% of men.

The biological assessment showed that the mean fasting blood glucose was 9.9±3.5mmol/l and the mean HbA1c was 9.12±2.01%. More than 50% of patients have Hb1Ac>9%.

The lipid profile showed hypercholesterolemia in 20.6% of cases, hypertriglyceridemia in 43.9% and low levels of HDL in 33.5%.

All patients were tested for vitamin D. The mean level was 12.8±6.9 ng. We found that 85.2% of patients had vit D levels < 20 ng/ml.

Vit D levels became lower with increasing age. There was a statistically significant negative correlation between age and vit D levels (p=10-3, r=-1.56).

Vit D levels were lower in women with a statistically significant difference ($p= 10^{-3}$). Table 1 summarises the various factors influencing vitamin status.

Only one patient (1.6%) with diabetic peripheral neuropathy had normal vitamin status.

There was a statistically significant negative correlation between mean vit D levels and the DN4 score ($p=10^{-3}$).

The univariate study identified factors associated with the development of diabetic neuropathy. A fall in vit D levels of less than <20ng/ml increased the risk of developing diabetic neuropathy almost 9-fold ($p \leq 10^{-3}$) (see Table 2).

6. Discussion

Through this cross-sectional study of 155 individuals with type 2 diabetes in a tertiary care centre in Sousse, we identified a high prevalence of vitamin D deficiency (VDD) within this population. Notably, our findings revealed that vitamin D deficiency is significantly associated with the development of diabetic neuropathy, independent of other influencing factors.

Vitamin D deficiency affects populations worldwide, yet its epidemiological characteristics vary significantly due to geographic and socio-economic factors (Isaia G, Giorgino R & Adami S., 2001). The discrepancies in prevalence rates across countries can be attributed to the heterogeneity of the studied populations (including race and ethnicity), differences in sample sizes, geographic location, climate variations, as well as the criteria used to define deficiency, and the assay techniques employed (Isaia G, Giorgino R & Adami S., 2001). In our study, the prevalence of vitamin D deficiency among participants with type 2 diabetes, defined as levels below 20 ng/ml, was 85.2%. This finding aligns with existing literature, indicating that VDD is prevalent among diabetics in our region, regardless of age (Mithal P., 2004). For instance, prevalence rates are approximately 70% in Iran (Mithal P., 2004), 80% in Saudi Arabia (Garabedian M, Menn S, Walrant-Debray O, Teinturier C & Delaveyne R RA., 2005), 56% in Kuwait (Amena Sadiya SMA, 2014), and between 60% and 65% in Lebanon and Jordan, where a deficiency threshold of 10 ng/ml was applied (Mithal P., 2004). In North Africa, reports indicate an alarming 98.1% deficiency rate among Moroccans, with a threshold set at 30 ng/ml (Organization World Health, 2000).

The variability in reported vitamin D deficiency prevalence is influenced by the specific thresholds used to define it (International Diabetes Fideration, 2009). Nevertheless, VDD is considered widespread globally, irrespective of the definition employed (Bernard Waeber GW., 2013). In our study, we opted for a threshold of 20 ng/ml, as only four participants had vitamin D levels above 30 ng/ml. This threshold may be more representative of our Mediterranean population (Bouhassira D., 2005).

Currently, there is no consensus on what constitutes optimal serum vitamin D concentrations (Friedewald WT & Levy RI FD., 1972). However, it is established that levels below a certain threshold can lead to elevated parathyroid hormone (PTH) levels, resulting in increased bone remodelling and decreased bone mass. Moreover, discrepancies in reported vitamin D levels can often be traced back to variations in assay methods, which lack standardization (Friedewald WT & Levy RI FD., 1972; Masakazu H, Kazunori U, Daisuke K, Tetsuya B & Tatsumi Moriya HM., 2015).

Distinguishing between physiological variations and clinically significant deficiencies can be challenging, especially since severe bone impairment typically presents only at very low serum vitamin D concentrations (ADA, 2017). Consequently, linking serum levels, even when below normative ranges, to specific clinical manifestations remains complex. Recent evidence suggests that the relationship between vitamin D and PTH is too variable to be a reliable biological indicator of insufficiency (ADA, 2017).

Several factors contribute to the observed decline in vitamin D levels. The increased prevalence of VDD may be partially attributed to public health campaigns that emphasize the risks of skin cancer from sun exposure, leading to reduced sunlight exposure and more indoor activities (Puel J, Valensi P VG et al., 2004).

Our results are consistent with findings from other studies (Andronikof M., 2010; Benhamou C-L, Souberbielle J-C, Bernard C, Patrice F & Jean-Bernard Gauvain TT., 2011), which suggest that a 1 kg/m² increase in body mass index (BMI) correlates with a 0.52 ng/ml decrease in serum 25(OH)D levels (Mithal A, Wahl DA, Bonjour JP, Burckhardt P & Dawson-Hughes, 2009), while a 1 cm increase in waist circumference results in a reduction of approximately 0.11 ng/ml (Andronikof M., 2010). The association between obesity and vitamin D deficiency is partly due to the sequestration of vitamin D in adipose tissue, as its fat-soluble nature facilitates storage in fat (Mithal A, Wahl DA, Bonjour JP, Burckhardt P & Dawson-Hughes, 2009; Al-Daghri NM., 2018) and can lead to dilution effects (Safi S, Ouleghzal H, Khaldouni I, Hassikou H, Ballouch L, Bamou Y et al., 2015).

Additionally, relatively low dietary intake of vitamin D — primarily synthesized endogenously — contributes to the deficiency. Despite abundant sunlight, many Middle Eastern and African countries report some of the highest rates of rickets globally. This paradox may be explained by cultural practices that limit sun exposure, such as the wearing of covering clothing, and by the darker skin pigmentation prevalent in these populations, which reduces vitamin D synthesis. Notably, older participants demonstrated a significant correlation with higher rates of VDD, particularly among those with diabetes. Aging is associated with decreased dietary intake, reduced sun exposure, and diminished capacity for vitamin D synthesis in the skin.

Abbreviation

BMI: Body Mass Index

HVD: Hypovitaminosis D

Vit D: Vitamin D

VDD: Vitamin D deficiency

T2DM: Type 2 diabetes mellitus

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Appendix

Table 1. Factors influencing vitamin D status

		Vit D ng/ml (m±SD)	*p*
Gender	**Men n (%)**	16,2±7,4	**10^{-3}**
	Women n (%)	10,5±5,5	
Menopause	Yes (n=57)	10,0±5,3	0,4
	No (n=34)	11,1±5,8	
Residence	**East Center n (%)**	12,8±7,0	0,52
	Central West n (%)	9,7±4,1	
	North East n (%)	15,8±8,3	
	North West n (%)	12,7±5,0	
	South East n (%)	14,8	
Season	**Spring n (%)**	12,3±4,3	**0,04**
	Autumnn (%)	18,5±3,8	

Duration of exposure (h/d)		-	**0,02**
Sun protection	Yes (n=26)	10,6±4,8	0,07
	No (n= (129)	13,2±7,2	
Wearing the veil n (%)	Yes (n=65)	10,1±	**0,01**
	No (n= (43)	13,1±6,7	
Type of housing	**Apartment**	12,2±6,5	0,54
	House	13,0±7,1	
Profession	**In the open air**	18,7±8,3	**10^{-3}**
	In an enclosed space (%) n(%)	13,7±6,7	
Sports activity n (%)	Yes (n=11)	17,89,6	**0,01**
	No (n= (144)	12,4±6,5	
Phototype	**Type I n (%)**	12,7±5,0	0,32
	Type II n (%)	12,3±7,5	
	Type III n (%)	14,5±8,9	
	Type IV n (%)	12,2±6,0	
	Type V n (%)	11,1±3,9	
Nutrition	**<1 ration Dp/d**	9,4±3,7	**10^{-3}**
	1-2 rations Dp/d	14,2±7,8	0,574
	>2 rations Dp/d	16,3±6,3	0,321
Alcohol	Yes (n=19)	18,6±7,1	**10^{-3}**
	No(n=136)	11,9±6,5	
Tobacco	Yes (n=38)	16,2±7,1	**10^{-3}**
	No (n=117)	11,7±6,5	

Note: Vit D: vitamin D, m: mean; n: number; SD: standard deviation; h/d: hour/day; Dp/d: dairy product/ day p<0.05.

Table 2. List of factors associated with the development of peripheral neuropathy

		Peripheral neuropathy		***p***	***OR[CI95%] (5IC95%)***
		Yes (n=62)	**No (n=93)**		
Age (years) (m±SD)		57,23±9,45	52,17±11	**0,011**	**1,04[1,01-1,08]**
Gender	**Men n (%)**	17(27,4)	45 (48,4)	**0,009**	**2,48 [1,24-4,9]**
	Women n (%)	45 (72,6)	48 (51,6)		
Tobacco n (%)		8 (12,9)	30 (32,3)	**0,006**	**0,31[013-0,74]**
Alcohol n(%)		5 (8,1)	14 (15,1)	0,19	
Sports activity n (%)		2 (3,2)	9 (9,7)	0,22	
Personal history of hypertension n (%)		40 (64,5)	37 (39,8)	**0,003**	**2,75 [1,41-5,35]**
Personal history of dyslipidaemia n (%)		39 (62,9)	29(31,2)	**≤10^{-3}**	**3,74 [1,9-7,3]**
Duration of diabetes (years)		13,46±8,25	9,68±7,45	**≤10^{-3}**	**1,09 [1,03-1,14]**
BMI (Kg/m^2) (m±SD)		34,78±6,08	31,99±6,02	**0,014**	**1,02 [0,97-1,08]**
Waist circumference (cm) (m±SD)		104,5±11,7	101,34±11,3	0,137	
SBP (mmHg) (m±SD)		135,9±16,01	133,8±15,4	0,466	

DBP (mmHg) (m±SD)	80,5±9,1	77,8±10,3	0,146	
Fasting blood glucose (m±SD)	10,6±3,7	9,7±3,5	0,146	
Hb1Ac (%) (m±SD)	9,32±2,1	9,05±1,96	0,471	
(mmol/l) (m±SD)	1,62±0,3	1,68±0,39	0,376	
HDL cholesterol (mmol/l) (m±SD)	0,94±0,23	1,07±0,21	**≤10^{-3}**	**0,14 [0,02- 0,52]**
LDL Cholesterol (mmol/l) (m±SD)	2,77±0,72	2,42±0,55	**≤10^{-3}**	**1,68 [0,99-2,84]**
Cholesterol total (mmol/l) (m±SD)	4,58±0,72	4,45±0,65	0,347	
Albuminurie 24h(m±SD)	227,92±234,07	25,4,±66,4	**≤10^{-3}**	**1,01[1,007-1,18]**
Vit D<20ng/ml n (%)	60 (96,8)	72 (77,4)	**≤10^{-3}**	**8,75[1,97-38,83]**
Vit D (ng/ml) (m±SD)	9,16±3,98	14,01±7,28	**≤10^{-3}**	**0,88 [0,82-0,94]**
Hb1Ac ≥8% n (%)	40 (64,5)	52 (55,9)	0,285	
Metformin n (%)	44 (71)	93(60)	**0,02**	**0,39 [0,17-0,88]**

Note: Vit D: vitamin D, SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; m: mean; n: number; SD: standard deviation; h/d: hour/day; Dp/d: dairy product/ day; p<0.05.

Table 3. List of factors associated with the development of autonomic neuropathy

		Neuropathie autonome		***p***	***CI95%[OR]***
		Yes (n=19)	**No (n=136)**		
Age (ans) (m±SD)		58,6±7,6	52,7±11	**0,006**	**1,06[1,001-1,12]**
Gender	Men n (%)	8 (42,1)	54 (39,7)	0,84	
	Women n (%)	11 (57,9)	82 (60,3)		
Tobacco n (%)		3 (15,8)	35 (25,7)	0,51	
Alcohol n (%)		3 (15,8)	16 (11,8)	0,7	
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Sports activity n (%)		0 (0)	11 (8,1)	0,36	
Personal history of hypertension n (%) 555(%) 55ù°(%)		14 (73,7)	63 (46,3)	**0,025**	**4,25 [1,4-12,5]**
Personal history of dyslipidaemia n (%)		14 (73,7)	54 (39,7)	**0,005**	**3,4 [1,42-6,45]**
Duration of diabetes (years)(m±SD)		13,6±8,9	8,9±6,7	**0,023**	**1,08 [1,02-1,15]**
BMI (Kg/m^2) (m±SD)		34,9±4,9	32,3±6,2	**0,05**	**1,07 [0,99-1,16]**
Waist circumference (cm) (m±SD)		103,9±10,1	101,9±11,7	0,506	
SBP (mmHg) (m±SD)		134,2±12,1	134,3±15,9	0,97	
DBP (mmHg) (m±SD)		78,9±7,3	78,4±10,4	0,83	
Fasting blood glucose (m±SD)		10,2±3,4	9,9±3,5	0,72	
Hb1Ac (%) (m±SD)		9,33±2,1	9,09±2	0,63	
Triglyceride (mmol/l) (m±SD)		1,67±0,43	1,67±0,36	0,99	
HDL cholesterol (mmol/l) (m±SD)		0,93±0,29	1,05±0,2	**0,01**	**0,09 [0,01- 0,7]**
LDL Cholesterol (mmol/l) (m±SD)		2,77±0,78	2,47±0,59	**0,05**	**1,91 [0,97-3,77]**
Total cholesterol (mmol/l) (m±SD)		4,58±0,78	4,47±0,65	0,47	

Uric acid (mmol/l) (m±SD)	286,4±54,4	298,6±60	0,4	
Albuminuria 24h(m±SD)	202,6±194,1	58,7±143	**10^{-3}**	**1,004[1,001-1,006]**
creatinine level µmol/l (m±SD)	76,3±11	71,2±11,5	0,042	
VitD<20ng/ml n (%)	19 (100)	113 (83,1)	0,07	
Vit D (ng/ml) (m±SD)	8,2±3,37	13,4±7	**≤10^{-3}**	**0,83 [0,74-0,94]**
Hb1Ac ≥8% n (%)	12 (63,2)	80 (58,8)	0,719	
Metformin n (%)	6 (31,6)	27 (81,8)	**0,004**	**0,21 [0,08-0,59]**

Note: Vit D: vitamin D, SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure (SBP m: mean; n: number; SD: standard deviation; h/d: hour/day; Dp/d: dairy product/ day; p<0.05.

Table 4. Direct Factors Related to Diabetic Neuropathy

	p	***Adjusted OR***	***[CI95%]***
Peripheral neuropathy			
History of dyslipidaemia	0,01	**2,81**	**1,2-6,57**
HDL cholesterol	0,03	**0,11**	**0,01-0,85**
24h microalbuminuria	0,00	**1,01**	**1,00-1,02**
VitD (ng/ml)	0,01	**0,90**	**0,83-0,97**
Metformin	0,02	**0,30**	**0,10-0,86**
Autonomic neuropathy			
creatinine level	**0,04**	**1,05**	**1,00-1,10**
24h microalbuminuria	**0,02**	**1,00**	**1,00-1,00**
Vit D (ng/ml)	**0,00**	**0,81**	**0,70- 0,94**
Metformin	**0,00**	**0,20**	**0,06-0,66**

Note: Vit D: vitamin D, OR: Odds Ratio; CI: Confidence interval; p<0.05.