

*Full Length Research paper*

# Association of serum vitamin D level with diabetic polyneuropathy

Leila Chaychi\*, Todd Mackenzie, Diane Bilotta, Matthew Lynch, Jeffrey Cohen and Richard Comi

Dartmouth-Hitchcock Medical Center, Lebanon.

Accepted 21 February, 2020

There is an association between serum vitamin D level and diabetic polyneuropathy. Topical vitamin D has been proposed for treatment of neuropathic pain in diabetics. The relationship between the serum 25-hydroxyvitamin D level and diabetic neuropathy has never been evaluated. The objective of the study was to evaluate a quantitative and qualitative relationship between the serum 25-hydroxyvitamin D level and diabetic polyneuropathy. This was a case - control study and the study was conducted on an outpatient basis. Twenty two subjects were recruited from the Diabetes Clinic at Dartmouth Hitchcock Medical Center. All subjects had type-2 diabetes (male and female, 50-80 years old). The Michigan Neuropathy Screening Instrument (15 point questionnaire) was used to identify patients with neuropathy (score  $\geq 7/15$ ). 11 patients did not have neuropathy and were designated the control group. 11 patients did have neuropathy ("study group") and these patients underwent a detailed quantitative neuropathy evaluation by a neurologist using the 46-point Michigan Diabetic Neuropathy Scale (MDNS) and nerve conduction studies. We measured the serum 25-hydroxyvitamin D concentrations with liquid chromatography-tandem mass spectrometry (LC/MS) chromatography in all patients. These values were compared between the control and study group, and were correlated with the detailed neuropathy score in the study group. Patients with diabetic polyneuropathy had a lower mean serum 25-hydroxyvitamin D level (21.36 ng/ml with the SD of: 8.56) in comparison to the controls (36.18 ng/ml with the SD of 7.53). However, there was no correlation between the vitamin D level and the detailed quantitative neuropathy score in the study group. There is an association between serum 25-hydroxyvitamin D level and diabetic polyneuropathy. The quantitative association of serum vitamin D level with the severity of diabetic polyneuropathy requires further investigation and possible larger number of patients.

**Key words:** Serum vitamin D, diabetic polyneuropathy, neuropathy, 25-hydroxyvitamin D.

## INTRODUCTION

Investigations into the molecular and biochemical pathophysiology of diabetic neuropathy have focused mainly on glucose metabolic pathways (<http://www.diabetes.org/diabetes-statistics/prevalence.jsp>; Sahin et al., 2008; Boulton et al., 2005; Franklin et al., 1990; Thomas, 1999; Kong et al., 1999; Lasker, 1993; Watkins, 1998; Leininger et al., 2006; Edwards et al., 2008), some animal and human studies suggest

25-hydroxyvitamin D may be involved in peripheral and central nervous system development (Chabas et al., 2008; Garcion et al., 2002; Eyles et al., 2004). 25-hydroxyvitamin D deficiency is common in patients with diabetes (Mattila et al., 2007; Scragg et al., 2004; Pittas et al., 2007). We hypothesized that low 25-hydroxyvitamin D levels are associated with sensory neuropathy in diabetes and that Vitamin D concentrations might be correlated with severity of the neuropathy. Mechanisms and pathophysiology of neuropathy have all been tied to glucose via microvascular injury or direct neuronal metabolic injury. Previously reported pathways relevant

\*Corresponding author. E-mail: [leilachaychi@gmail.com](mailto:leilachaychi@gmail.com).

to diabetic neuropathy include: 1. Glucose flux: polyol pathway (Aldose Reductase, sorbitol) 2. The hexosamine pathway (Fructose6P). 3. Excess/inappropriate activation of protein kinase C isoforms (DAG). 4. Accumulation of advanced glycation end products. 5. Poly (ADP-ribose) polymerase pathway (PARP) found in: Schwann, endothelial cells and sensory neurons. These pathways are known to be injurious alone or collectively as a cause of an imbalance in the mitochondrial redox state of the cell which can possibly lead to excess formation of reactive oxygen species (Leininger et al., 2006; Edwards et al., 2008). Neuropathy may occur from hyperglycemia-induced damage to nerve cells per se and /or from neuronal ischemia caused by decreases in neurovascular flow (Leininger et al., 2006; Edwards et al., 2008).

According to the American Diabetes Association, there are 20.8 million children and adults in the United States, or 7% of the population, who have diabetes. While an estimated 14.6 million have been diagnosed, unfortunately, 6.2 million people (one-third) are undiagnosed. Fifty-four million people are in pre-diabetes status (1). Peripheral Neuropathy is a common and costly complication of type 1 (T1DM), type 2 (T2DM) and pre-diabetes (Sahin et al., 2008; Boulton et al., 2005; Franklin et al., 1990). The prevalence of neuropathy is estimated to be about 8% in newly diagnosed patients and greater than 50% in patients with long-standing disease (Boulton et al., 2005). There is increasing evidence that pre-diabetic conditions are also associated with some forms of neuropathy (Franklin et al., 1990). An estimated 15% of all patients with diabetes will develop foot ulcers (Thomas, 1999) and diabetic neuropathy is the leading cause of non-traumatic limb amputation (Kong et al., 1999). Therefore sensory neuropathy is common and debilitating, underlies ulcer and amputation and is reported even in prediabetes based on previous studies (DCCT, UKPDS, (Lasker, 1993; Watkins, 1998) progression of sensory neuropathy can be related to glucose levels, however onset in pre-diabetes suggests that marked hyperglycemia is either not necessary or other factors may be involved.

## PATIENTS AND METHODS

We recruited men and women, 50 to 80 years old, with type-2 diabetes as defined by American Diabetes Association (ADA) criteria. Patients with diseases other than diabetes known to be associated with peripheral neuropathy and pain such as symptomatic peripheral vascular disease, abnormal liver function tests, evidence of renal failure, hypo- or hyperparathyroidism, rheumatoid arthritis, gout, fibromyalgia and Charcot foot as well as patients on narcotics or chronic NSAID use were excluded from our study. The following data were collected from the study patients and control group: Age, gender, duration of diabetes, HbA1c

average for the last 12 months (at least 2 values), BUN, creatinine, presence of retinopathy, presence of microalbuminuria, using medications with neurological effects or side effects such as: amitriptyline, gabapentin, or vitamin D intake. We did not measure PTH and calcium since all these patients were under the primary care physician care with no signs or symptoms of hyperparathyroidism. Recruitment was closed after 11 patients were identified. Patients with low 25-hydroxyvitamin D level were referred to their primary care provider at the end of the study. All subjects gave their witnessed informed consent before entering the study, which was approved by local investigational research board in our center.

We recruited patients with Type 2 diabetes from the Diabetes Clinic at Dartmouth Hitchcock Medical Center, using posters that described the study in the clinic. More than 50 patients volunteered for the study. After obtaining a careful history and applying our inclusion and exclusion criteria, we identified 32 subjects suitable for the study (The sample size was calculated for a significant p value with the standard deviation of 10 for vitamin D level). All these patients took the Michigan Neuropathy Screening Instrument (MNSI), which has been validated to identify diabetic neuropathy in multiple clinical trials (Feldman et al., 1994). The MNSI consists of initial 15 questions and a positive response to  $\geq 7$  is diagnostic of diabetic peripheral neuropathy (Feldman et al., 1994). 11 patients had scores high enough to qualify for diabetic neuropathy ("study group"); We randomly chose another 11 patients among the 21 remaining, who had scores under 7 and those were designated the control group (Feldman et al., 1994). 25-hydroxyvitamin D concentrations in serum were measured in all subjects using the LC-TMS methodology (Lensmeyer et al., 2006).

All the samples were collected in 4 week period (mid September to mid October). Patients with neuropathy were subsequently evaluated in detail by a neurologist, using the Michigan Diabetic Neuropathy Scoring System. The total MDNS score comes to 46 points (Feldman et al., 1994) and the score  $\geq 7$  are usually considered positive. The MDNS score was combined with 5 nerve conduction studies, which consisted of: sural, peroneal motor, median sensory and motor as well as ulnar sensory (Feldman et al., 1994). The NCS assessed the number of nerves with abnormal conduction velocities and amplitudes. The NCS in combination with the 46 points of the Neurological exam could quantitate the severity of the nerve pathology (Feldman et al., 1994). Each of these scores was analyzed against vitamin D independently and as a composite of both scores added together ("Total neuropathy score") versus 25-hydroxyvitamin D level alone.

## Statistical analysis

25-hydroxyvitamin D, whose distribution did not deviate appreciably from normality, was compared between polyneuropathy patients and controls using a t-test, as well as Wilcoxon on-Mann-Whitney test. Other continuous variables were compared using a t-test or Wilcoxon on-Mann-Whitney as appropriate. Gender and other categorical characteristics were compared using a Fisher's test. The association of 25-hydroxyvitamin D and severity in patients with diabetic neuropathy was assessed using a Pearson correlation with permutation test calculated p-value.

## RESULTS

### Clinical characteristics

Clinical characteristics of the patient with diabetic

**Table 1.** Clinical characteristics of diabetic neuropathy patients and control subjects.

	<b>Diabetic neuropathy subjects (n = 11)</b>	<b>Control subjects (n = 11)</b>	
Age (yr)	58.00 ± 8.21 (50 - 77)	58.36± 5.73 (51 - 69)	P = 0.90
BMI (kg/m <sup>2</sup> )	33.8 ± 6.58 (25.5 - 45.0)	31.1± 3.4 (25.0 - 35.0)	P = 0.24
Females/males	7/4	6/5	P = 1.00
HbA1c (%)	7.7 ± 0.8 (6.5 - 9.1)	7.6± 0.7 (6.1 - 8.2)	P = 0.73
Medication <sup>1</sup>	2/11	0/11	
GFR	>60 (all 11 patients)	>60 (all 11 patients)	
Urine Albumin/ Cr (mcg/mg Cr)	10.8 ± 12.3 (5.5 - 46)	9.1 ± 5.5 (4.5 - 18)	P = 0.89
Retinopathy	1/11	0/11	

Data are mean ± SD with range in parenthesis or absolute number of patients. Medication<sup>1</sup>: Gabapentin, lyrica.

**Table 2.** Serum vitamin D levels in diabetic neuropathy patients and control subjects (ng/dl).

	<b>Diabetic neuropathy subjects (n = 11)</b>	<b>Control subjects (n = 11)</b>	
Male	23.2 ± 7.8 (13-34)	35.0 ± 6.8 (30-45)	P = 0.007
Female	19.8 ± 9.6 (11-32)	36.9 ± 8.4 (22-46)	P = 0.11
Total	21.4 ± 8.6 (11-34)	36.2 ± 7.5 (22-46)	P = 0.0001

Data are mean ± SD with range in parenthesis for the male and female patients separately and combined.

polyneuropathy and controls are reported in Table 1. Age, BMI, prevalence of male and female, HbA1c within the last 3 months, prior medical treatments for diabetic neuropathy, renal function, presence of microalbuminuria or retinopathy were comparable among patients with diabetic polyneuropathy and controls. Patients with clinically proven diabetic neuropathy had remarkably lower serum 25-hydroxyvitamin D levels compared to the controls, which were statistically significant. Female patients had slightly lower 25-hydroxyvitamin D levels than male patients in diabetic group (Table 2) as was expected based on prior studies (Mattila et al., 2007; Scragg et al., 2004; Pittas et al., 2007).

However in our study the 25-hydroxyvitamin D level in male and female controls were identical. The mean 25-hydroxyvitamin D level was not optimal in both groups (Table 2).

Serum vitamin D level was lower among the patient with proven diabetic neuropathy (Figure 1). We did not find any association between level of serum vitamin D with the severity of diabetic neuropathy (Figure 2).

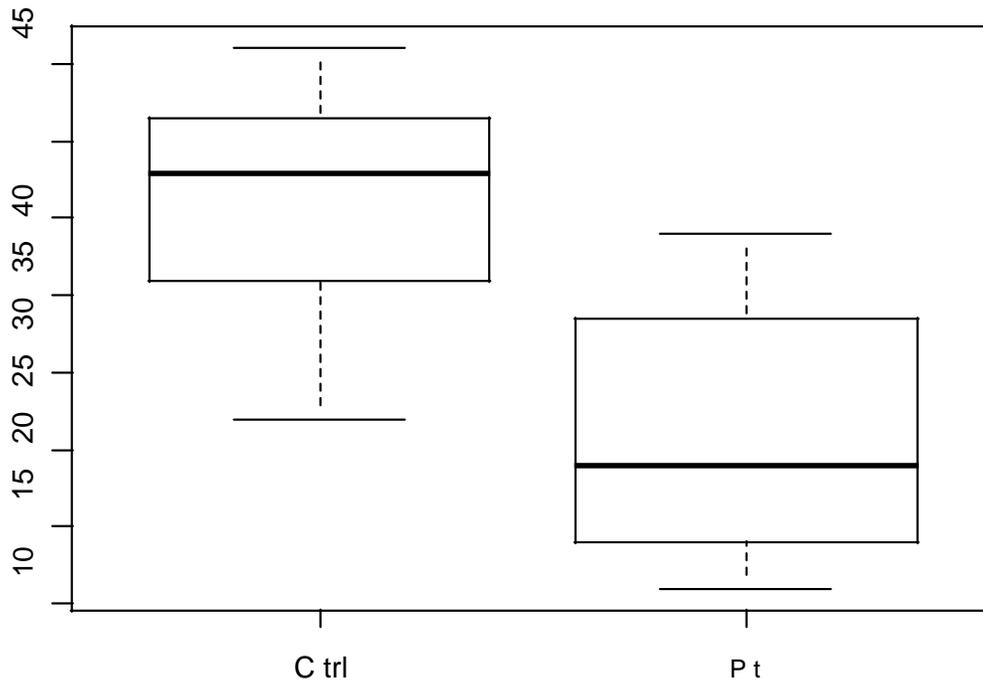
## DISCUSSION

The present study shows that diabetic patients with neuropathy have lower 25-hydroxyvitamin D levels than patients without neuropathy identified by a screening questionnaire instrument (Figure 1). This is an

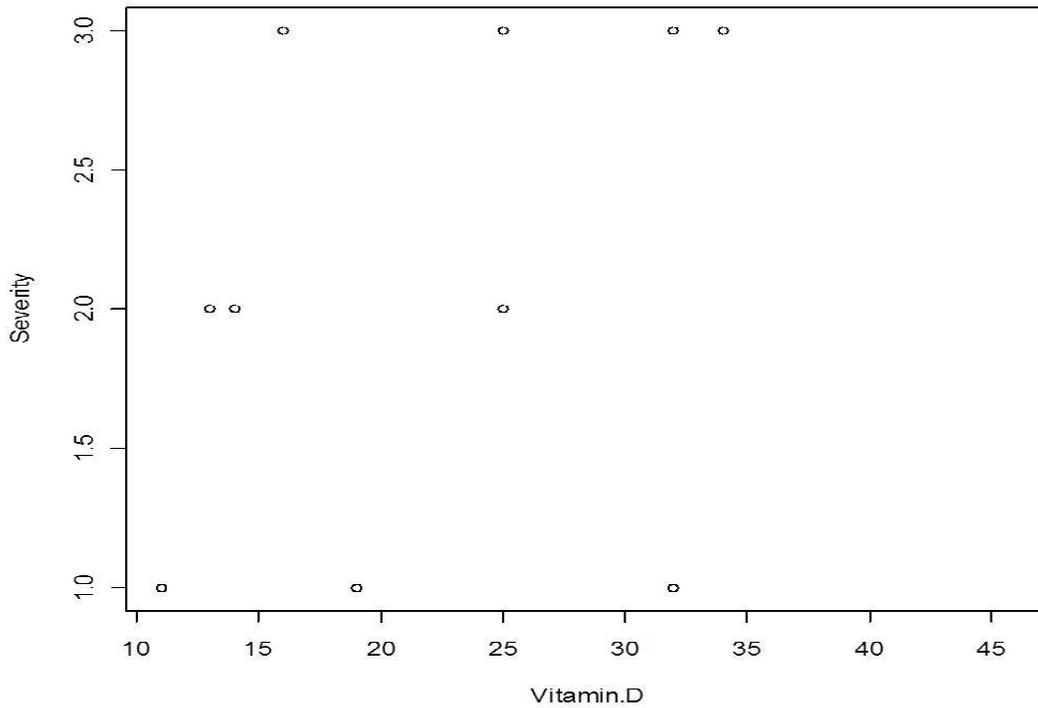
association and causality cannot be inferred. The severity of neuropathy, as assessed by a comprehensive scoring tool, was not correlated with the actually 25-hydroxyvitamin D level (Figure 2). It has been reported that 25-hydroxyvitamin D level is lower in diabetic women in overall ethnicities (Mattila et al., 2007; Scragg et al., 2004; Pittas et al., 2007). Female patients with diabetic neuropathy had lower vitamin D levels than male patients with diabetic neuropathy. However, the vitamin D levels were similar in both male and female in the control group. The gender differences in the vitamin D level are controversial and the reasons for that are not clear.

Patients with diabetes have a lower 25-hydroxyvitamin D level than the general population and there are some pathophysiologic observations that might link lower levels of 25-hydroxyvitamin D with neuropathic injury.

There has been demonstrated in some epidemiological association studies that diabetic patients have lower 25-hydroxyvitamin D level (Mattila et al., 2007; Scragg et al., 2004; Pittas et al., 2007). Some animal models have shown evidence that 25-hydroxyvitamin D can potentiate nerve regeneration Chabas et al., (2008), also there is a role for vitamin D in the development of the peripheral and central nervous system in human (Garcion et al., 2002; Eyles et al., 2004). Other human studies have shown 25-hydroxyvitamin D can potentiate nerve growth factor production in human epidermal keratinocytes (Fukuoka et al., 2001), or can alleviate neuropathic pain in diabetic patients (Lee et al., 2008; Valensi et al., 2005).



**Figure 1.** Comparison of overall serum 25-hydroxyvitamin D levels in naturopathic patients (n=11) versus control (n=11) patients.



**Figure 2.** Association of vitamin D and severity of diabetic neuropathy (Total Neuropathy Score) in patients with diabetic neuropathy was assessed using a Pearson correlation with permutation test calculated p-value ( $p = 0.7$ ).

There are several limitations to this study. First, the patients studied were not a random sample of all patients with and without neuropathy; they were recruited sequentially from a diabetes clinic in which it can be expected that the population be enriched for those with complications. Therefore we cannot eliminate a possible unintentional bias. Secondly, this study was conducted in a northeastern medical center, and 25-hydroxyvitamin D levels in the northeastern US are frequently low, especially in the winter months when this study was conducted. These data may not be as dramatic in regions with a lesser prevalence of 25-hydroxyvitamin D deficiency. Thirdly, 25-hydroxyvitamin D concentrations fluctuate in an individual and we used a single vitamin D concentration measurement, not an average over time that might be more reflective the risk of pathophysiologic process. However, our data suggests that the ambient 25-hydroxyvitamin D concentration is not tightly linked to the severity of neuropathy, suggesting that any influence of 25-hydroxyvitamin D on nerve function would be over a long term.

In summary, we believe that this observation is suggestive of an inverse association of 25-hydroxyvitamin D and the presence of neuropathy. We did not find significant association between the level of 25-hydroxyvitamin D and the severity of diabetic neuropathy (Figure 2), this association may be producible with a larger sample size in a longitudinal study. Further studies in a more representative sample would be needed to verify this observation and suggest a pathophysiologic link.

## REFERENCES

- American Diabetes Association. Total Prevalence of Diabetes & Pre-diabetes. Available at <http://www.diabetes.org/diabetes-statistics/prevalence.jsp>
- Chabas JF, Alluin O, Rao G, Garcia S, Lavaut MN, Risso JJ, Lagre R, Magalon G, Khrestchatsky M, Marqueste T, Decherchi P, Feron F (2008). Vitamin D2 potentiates axon regeneration, *J. Neurotrauma*, 25 (10): 1247-1256.
- Eyles DW, Smith S, Robert K, Martin H, John JM (2004). Distribution of the Vitamin D receptor and 1 $\alpha$ -hydroxylase in human brain. *J. Chem. Neuroanatomy*, 29 (5): 21-30.
- Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA (1994). A practical two-step, Quantitative Clinical and Electrophysiological Assessment for the Diagnosis and Staging of Diabetic Neuropathy. *Diabetes care*. 17(11): 1281-9.
- Fukuoka M, Sakurai K, Ohta T, Kioki M, Katayama L (2001), Tacalcitol, an Active Vitamin D3, Induces Nerve Growth Factor Production in Human Epidermal Keratinocytes. *Skin Pharmacol. Appl. Skin Physiol.*, 14(4): 226-233.
- Garcion E, Wion-Barbot P, Montero-Menei CN, Berger F, Didier W (2002). New clues about vitamin D functions in the nervous system, *Trends in Endocrinology and Metabolism*, pp. 1043-2760.
- Lee P, Chen R (2008), Vitamin D as an analgesic for patients with type-2 diabetes and neuropathic pain, *Arch. Int. Med.*, 168(7): 771-772.
- Lensmeyer GL, Wiebe DA, Binkley N, Drezner MK (2006). HPLC Method for 25-Hydroxyvitamin D Measurement: Comparison with Contemporary Assays. *Clin. Chem.*, 52: 61120-1126.
- Mattila C, Kenkt P, Mannisto S, Rissanen H, Laaksonen MA, Montonen J, Reunanen J (2007). Serum 25-Hydroxyvitamin D Concentration and Subsequent Risk of type 2 Diabetes. *Diabetes Care*, 30(10): 2569-2570.
- Pittas AG, Lau J, Hu FB, Dawson-Hughes B (2007). The Role of Vitamin D and Calcium in Type 2 Diabetes. A Systematic Review and Meta-Analysis. *J. Clin. Endocrinol. Metab.*, 92(6): 2017-2029.
- Scragg R, Sower SM, Bell C (2004). Serum 25-Hydroxyvitamin D, diabetes and ethnicity in the third national health and nutrition examination survey, *Epidemiology, health services, psychosocial research. Diabetes Care*, 27(12): 2813-2818.
- Valensi P, Le Devehat C, Richard JL, Farez C, Khodabandehlou T, Rosenbloom RA, Le Fante C (2005). A multicenter, double-blind, safety study of QR-333 for the treatment of symptomatic diabetic peripheral neuropathy. A preliminary report. *J. Diabetes and its Complications*. 19 (5): 247-253.