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Association of serum vitamin d level with diabetic polyneuropathy in rural population of central India

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***Abstract*---**To evaluate association of serum Vitamin D level and development of polyneuropathy. This was a case -control study and the study was conducted on an outpatient basis. Fifty-five subjects were recruited from the Medicine OPD of UPUMS Saifai. All subjects had type-2 diabetes (male and female, 40-60 years old). The Michigan Neuropathy Screening Instrument (15-point questionnaire) was used to identify patients with neuropathy (score > 7/15). 20 patients did not have neuropathy and were designated the control group. 35 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 (16. 41 ng/ml with the SD of: 2.026) in comparison to the controls (38.76 ng/ml with the SD of 5.483). However, there was no correlation between the vitamin D level and the

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detailed quantitative neuropathy score in the study group. There is an association between serum 25- hydroxyl vitamin D level and diabetic polyneuropathy. We did not find significant association between the level of 25-hydroxyvitamin D and the severity of diabetic neuropathy , this association may be producible with a larger sample size in a longitudinal study.

***Keywords*---**serum vitamin D, diabetic polyneuropathy, neuropathy, 25-hydroxyvitamin D.

Introduction

Neuropathies are characterized by a progressive loss of nerve fiber function. A widely accepted definition of diabetic peripheral neuropathy is "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after exclusion of other causes."[1] Neuropathies are the most common complication of diabetes mellitus (DM), affecting up to 50% of patients with type 1 and type 2 DM. In type 1 diabetes mellitus distal polyneuropathy typically becomes symptomatic after many years of chronic prolonged hyperglycemia. Conversely, patients with type 2 diabetes mellitus may present with distal polyneuropathy after only a few years of known poor glycemic control; sometimes, these patients already have neuropathy at the time of diagnosis. The factors leading to the development of diabetic neuropathy are not understood completely, and multiple hypotheses have been advanced[2]. It is generally accepted to be a multifactorial process[3]. Development of symptoms depends on many factors, such as total hyperglycemic exposure[4] and other risk factors such as elevated lipids, blood pressure, smoking, increased height, and high exposure to other potentially neurotoxic agents[5] such as ethanol. Genetic factors may also play a role[6]. Important contributing biochemical mechanisms in the development of the more common symmetrical forms of diabetic polyneuropathy likely include the polyol pathway, advanced glycation end products, and oxidative stress.

Polyol pathway

Hyperglycemia causes increased levels of intracellular glucose in nerves, leading to saturation of the normal glycolytic pathway. Extra glucose is shunted into the polyol pathway and converted to sorbitol and fructose by the enzymes aldose reductase and sorbitol dehydrogenase. [7] Accumulation of sorbitol and fructose lead to reduced nerve myoinositol, decreased membrane Na^+/K^+ -ATPase activity, impaired axonal transport, and structural breakdown of nerves, causing abnormal action potential propagation. This is the rationale for the use of aldose reductase inhibitors to improve nerve conduction. [8]

Advanced glycation end products

The nonenzymatic reaction of excess glucose with proteins, nucleotides, and lipids results in advanced glycation end products (AGEs) that may have a role in disrupting neuronal integrity and repair mechanisms through interference with nerve cell metabolism and axonal transport. [9]

Oxidative stress

The increased production of free radicals in diabetes may be detrimental via several mechanisms that are not fully understood. These include direct damage to blood vessels leading to nerve ischemia and facilitation of AGE reactions. Despite the incomplete understanding of these processes, use of the antioxidant alpha-lipoic acid may hold promise for improving neuropathic symptoms.[10]

Related contributing factors

Problems that are a consequence of or co-contributors to these disturbed biochemical processes include altered gene expression with altered cellular phenotypes, changes in cell physiology relating to endoskeletal structure or cellular transport, reduction in neurotrophins, and nerve ischemia.[11] Clinical trials of the best-studied neurotrophin, human recombinant nerve growth factor, were disappointing. With future refinements, however, pharmacologic intervention targeting one or more of these mechanisms may prove successful. In the case of focal or asymmetrical diabetic neuropathy syndromes, vascular injury or autoimmunity may play more important roles.[12] Vitamin D has been experimentally linked to the regulation of neurotrophin levels and neuronal Ca^{2+}homeostasis, both of which may provide a neuroprotective effect [12]. The influence of vitamin D on nerve function is supported in an animal model of diabetic rats with deficiencies in nerve growth factor synthesis; treatment of these rats with vitamin D increased nerve growth factor production and prevented neurotrophic deficit[13]. The data in humans regarding vitamin D insufficiency and diabetic neuropathy are limited. A recently reported prospective study of 51 patients with Type 2 diabetes and associated chronic, painful neuropathy found that conservative vitamin D supplementation for 3 months resulted in a nearly 50% decrease in pain scores[14]. This study will evaluate the association between vitamin D insufficiency and peripheral neuropathy and neuropathic pain in a representative sample of people with diabetes.

Material and Methods

We recruited men and women, 40 to 60 years old, with type-2 diabetes as defined by American Diabetes Association (ADA) criteria.

Exclusion criteria

- Patients with diseases other than diabetes known to be associated with peripheral neuropathy
- Patients with 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, HbA1caverage 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. All subjects gave their witnessed informed consent before entering the study, which was approved by local investigational research board in our centre. All the samples were collected in 4 week period (JUNE 2021). Patients with neuropathy were subsequently evaluated in detail by a neurologist, using the Michigan DiabeticNeuropathy Scoring System. The total MDNS score comes to 46 points[15] and the score >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. 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. Each of these scores was analyzed against vitamin D independently and as a compost of both scores added together ("Total neuropathy score") versus 25- hydroxyvitamin D level alone. 25-hydroxyvitamin D concentrations in serum were measured in all subjects using the LC-TMS methodology[16].

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 Wilcox on-Mann-Whitney test. Other continuous variables were compared using a t-test or Wilcox 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 polyneuropathy and controls are reported in Table 1.Age, prevalence of male and female, HbA1c within the last 3 months, prior medical treatments for diabetic neuropathy and retinopathy were comparable among patients with diabetic polyneuropathy and controls.

Table 1

Variables	Cases (with neuropathy)	Control (without Neuropathy)
No. of Patients	35	20
M/F	20/15	12/8
HbA1C	9.19±1.93	7.13±0.51
GFR	≥60	≥60
Retinopathy	2/33	0/20
Medication	7/28	0/20

Table 2

	Vit D Level (ng/ml) Cases	Vit D Level(ng/ml)Control	P-Value
Male	15.69±1.132 (20males)	38.80±5.372(12males)	0.0001
Female	14.68±1.648 (15females)	38.78±6.131(8females)	0.0001
Total	16.41±2.026	38.76±5.483	0.0001

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 neuropathy group (Table 2) as was expected based on prior studies[17,18]. However in our study the 25-hydroxyvitamin D level in male and female controls were identical. 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.

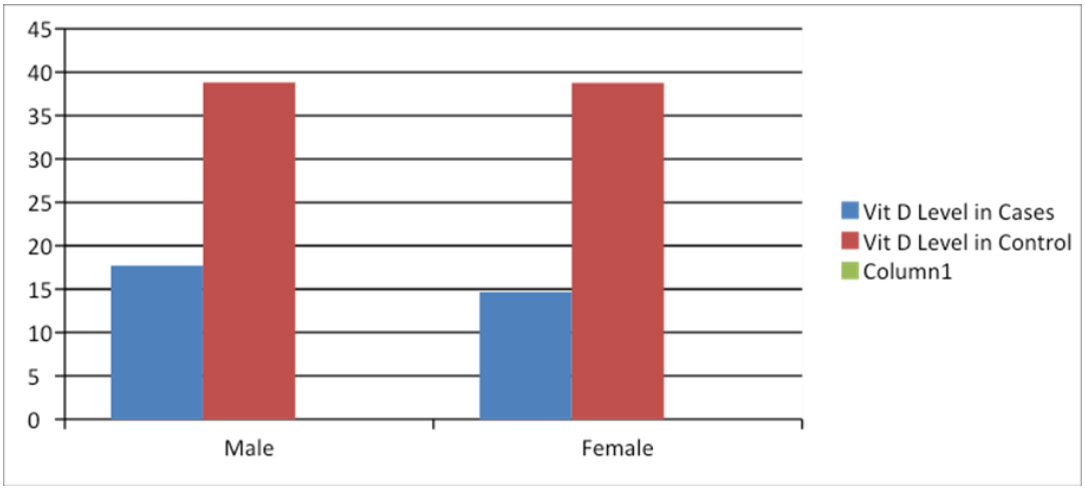

Figure1. 25 (OH) Vitamin D Level in Cases and Control Groups

Discussion

The present study shows that diabetic patients with neuropathy and poor glycemic controll have lower 25-hydroxyvitamin D levels than patients without neuropathy and good glycemic control, identified by a screening questionnaire instrument and HbA1c (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. It has been reported that 25-hydroxyvitamin D level is lower in diabetic women in over all ethnicities. 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. Some animal models have shown evidence that 25-hydroxyvitamin D can potentiate nerve regeneration[19], also there is a role for vitamin D in the development of the peripheral and central nervous system in human[20]. Other human studies have shown 25-hydroxyvitamin D can potentiate nerve growth factor production in human epidermal keratinocytes[21], or can alleviate neuropathic pain in diabetic patients[22]. 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 OPD in which it can be expected that the population be enriched for those with complications. Therefore we cannot eliminate a possible unintentional bias.

Second, 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, 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.

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