

25(OH) Cholecalciferol (Vitamin D₃), an Important Player in the Progression of Neuropathy in Diabetic Case

RUQAYA¹, JAI PARKASH PANJWANI², AMAR LAL DODANI³, TAYYABA KAZMI⁴, AYESHA KHAN⁵

¹Assistant Professor of Physiology, Baqai Medical University, Karachi.

²Associate Professor of Biochemistry, Sir Syed College of Medical Sciences for Girls, Karachi.

³Associate Professor of Physiology, Chandka Medical College Larkana.

⁴Assistant Professor of Anatomy, Baqai Medical University, Karachi.

⁵Senior Registrar Ophthalmology, Peoples University of Medical and Health Sciences for Girls, Nawab Shah.

Correspondence Dr. Ruqaya, Email: amanjai22@yahoo.com

ABSTRACT

Aim: To evaluate the role of vitamin D₃ in nervous conduction studies of subjects suffering with diabetic mellitus.

Methods: For this reason, the nerve conduction velocity was assessed in both healthy and diabetic subjects. Serum level of 25(OH) vitamin D and HbA1c were measured in both groups. 100 participants, 50 healthy and 50 diabetic subjects including both male and female were recruited for the study. The mean ages of male and female in control group were 45±2.54 and 42.34±8.61 years respectively; and the mean ages of males and females of diabetic subjects were 57.5±9.92 and 49.87±8.81 years. In the estimation of vitamin D₃, the mean and standard deviation (SD) in diabetic subjects with neuropathy was significantly low 10.448±2.406ng/ml as compared with diabetic subjects without neuropathy 25.821±4.417ng/ml. p<0.05 was taken as statistical significance value.

Results: Serum level of Vitamin D₃ showed a significant difference between control and diabetic subjects (P < 0.01). Anthropometric measures included age, weight and height, was also recorded for both groups. The study has shown that along with duration of diabetes and elevated level of HbA1c, vitamin D₃ was significantly associated with diabetic neuropathy.

Conclusion: The insufficiency of vitamin D₃ has an adverse effect in the development and progression of diabetic neuropathy. Therefore, Vitamin D supplementation is immediately required to prevent or delay the development of neuropathy in diabetic Case.

Keywords: Nervous conduction velocity, Diabetes mellitus, Neuropathy, HbA1c, Vitamin D₃.

INTRODUCTION

In the Diabetic neuropathy each type of nerve fiber in the body become useless because it effects tangible, autonomic, and engine neurons of fringe sensory system¹. Aseachorgan framework in the body depends on innervations for capacity is therefore subject to pathology¹. Consequently diabetic neuropathy represents various particular disorders due to the effected nerve strands present in every part of the body. In the course of the disorder only few victims reports for the neuropathic pain. The disorder remain undiagnosed until the outcomes of the disorder become extreme¹.

Pervasiveness information for Diabetic Autonomic Neuropathy (DAN) range from 1.6 to 90% contingent upon tests utilized, populaces inspected, sort and phase of sickness. Danger components for the advancement of DAN depends on the duration of diabetes, aging and poor glycemic control for a longer period of time. The comorbidities of diabetes such as dyslipidemia, increased pulse rate may also contribute to DAN. Therefore, regulated glycemic control, lipid balance and circulatory strain control may be important in the remedial action of DAN. Many years of exploration clarifying that the pathophysiology of diabetic neuropathy has been fizzled; in this manner there is a critical need to create a treatment that anticipates or switches its improvement and movement². As of late, various contending or parallel obsessive pathways have started to cross and

supplement one another, lighting up potential pharmacologic targets. These include the formation of advance glycation end products, oxidative species, protein kinase C and the activation of polyol pathway². Previously, a study draws our attention towards the significance of vitamin D in the release of insulin from beta cells of pancreas³. The association of vitamin D and diabetes was found by revelation of the vitamin D receptor in pancreatic tissue; this indicates the influence of this vitamin on the pancreatic tissues and eventually diabetes⁴. Many studies around the world demonstrated the association of vitamin D receptor polymorphism and diabetes type 1^{5,6,7}. The presence of different alleles of vitamin D receptors seems to be responsible for weight gain and impaired glucose hemostasis in patients of diabetes type 2⁸. The gene of vitamin D receptor is consist of 11 exons and is located on chromosome 12q13.11. The polymorphism of this gene is observed at Bsml, Apal, and Taql regions [9]. The polymorphism of vitamin D receptor gene is associated with progression of type 2 diabetes, high blood glucose level in fasting, impaired glucose tolerance and insulin insensitivity [10]. In one study, polymorphism in Apal, Taql, and Bsml regions of vitamin D receptor gene is observed in type 2 diabetic patients. It was also demonstrated that the repetition of "aa" genotype of Apal polymorphism was somewhat higher in Type 2 Diabetic subjects. It is also revealed by the previous study that fasting plasma glucose and glucose intolerance were significantly higher in non-diabetic persons with "aa" genotype contrasted and those with AA genotype. Previously a study signifies the association of polymorphism of vitamin D receptor with insulin resistance¹¹.

Received on 02-04-2019

Accepted on 15-06-2019

MATERIALS AND METHOD

The research is based on cross sectional study. After approval by the ethical committee of Baqai medical university the study is carried out in the Department of Physiology at Baqai Medical College. The diabetic subjects were recruited from the affiliated Baqai Hospitals (Fatima & Baqai institute of diabetology and endocrinology), Karachi and Jinnah Postgraduate Medical Centre, Karachi in the duration of July 2014 till February 2015. 100 participants of both gender were included in the study. Out of this 50 participants were diabetic patients and 50 healthy individuals for comparison were enrolled for this research. The mean ages of diabetic patients inclusive of male and female were 57.61 ± 10 and 49 ± 8.52 . Similarly, the mean ages of healthy individuals inclusive of male and female were 45 ± 2.54 and 42.34 ± 8.61 years. Anthropometric information including name, age, sex, height (centimeter) and weight (kg) was recorded through approved questionnaire for each participant of the study. Nerve conduction velocity along with Vitamin D3 and HbA1c were measured in both control/ diabetic groups.

HbA1c: Blood samples for estimation of HbA1c was collected in Anticoagulants (Potassium EDTA, Ammonium Heparin or Lithium Heparin) tubes. Latex agglutination inhibition assay (Kit Randox Laboratories Limited) was used for its estimation. HbA1c was estimated by agglutination of latex that contain HbA1c specific antibodies of mouse¹². HbA1c in the sample slowed down the speed of agglutination as it competed with the HbA1c agglutinator. The percentage of HbA1c is then calculated using the g/dl HbA1c and Total Haemoglobin values¹³.

Nerve conduction velocity: Nerve conduction velocity is the measure of nerve pathology as it shows the time taken by the electric pulse to travel through the nerve. Nerve conduction velocity is measured by stimulating a nerve that contains both the motor and sensory axons. The excitation of the nerve is done by a brief electrical pulse, this produce enough depolarization for the generation of action potential at the site of stimulation. Meanwhile this induced action potential reach to the distal and proximal directions from the excitation site [14]. After milliseconds this action potential reached to the neuromuscular synapses, produced neuromuscular transmission and eventually generated action potentials along the muscle fibers. The action potential of stimulated muscle fiber was measured by placing surface electrodes on the skin above the muscle and is known as compound muscle action potential¹⁵.

Nerve Conduction Velocity is calculated by dividing Distance with conduction time. Nerve conduction velocity was measured in Motor nerves which include Median nerve, Ulnar Nerve, Tibial Nerve and Peroneal nerve while sensory nerves that included were Radial Nerve, Ulnar Nerve and Sural nerve.

Vitamin D: The 25OH Vitamin D was estimated by ELISA. It is a solid phase Enzyme Linked Immunosorbent Assay and performed on microtiterplates. It is kit based method the kit was manufactured by DI source Immuno Assays S.A, Belgium, Kit KAP1971. [18]. 50 μ l of each Calibrator, Control and test group samples were pipetted into the

appropriate wells [19]. 150 μ l of Incubation Buffer was pipette into all the wells. The absorbance was measured at 450 nm (reference filter 630 nm or 650 nm) within 1 hour²⁰. The concentration of vitamin D was calculated as: $B/BO (\%) = (OD(\text{sample})/OD(\text{Zero Calibrator})) * 100$ Where: OD=Optical Density

Data analysis: SPSS version 22.0 was used for statistical analysis of anthropometric and base line information of the study participants. Normally distributed continuous variables including age, height, weight and BMI for both groups (Control and Test) were presented as mean \pm SD. T-test was used to determine the association between the basic parameters of nerve conduction studies with vitamin D3 and HbA1c levels. Results were considered to be statistically significant at P-value < 0.05 .

RESULTS AND DISCUSSION

The assessment of neuropathies have been rationally done by the nerve conduction studies. As it is an effective method for the diagnosis of neuropathy. In this study nerve conduction velocity (NCV) in right and left side of motor and sensory nerves was assessed using the Power Lab. It is studied previously that decrease of motor nerve conduction velocities (MNCVs) might be an initial indication of neuropathy²², though in some cases reduction in motor nerve conduction velocity alone, is diagnostic for neuropathy²³. This study is focused on the association of nerve conduction velocities (NCV) with Vitamin D3 in subjects with diabetes mellitus. HbA1c level was measured for the conformation of diabetes in known diabetic Case as well as in normal individuals (Table 1)²⁴. HbA1c level was significantly higher in diabetic patients as compared with healthy individuals. The measurement of conduction velocities in motor and sensory nerves are found to be significantly important for the assessment of peripheral neuropathies. In the present study the measurement of motor and sensory nerve conduction was done in normal individuals and diabetic Cases (Table 2 to 5).

In our study a significant reduction in conduction velocity of motor nerve has been found in diabetic Case with neuropathy. Irrespective of the age of diabetic subjects, motor nerve conduction velocity decreases with the duration of diabetes particularly in individuals with uncontrolled diabetes. As expected, a significant decrease in nerve conduction velocities (NCV) were observed in Case compared with those of normal subjects without diabetes²⁸.

The present study has shown that there was a significant difference in serum levels of Vitamin D3 between diabetic subjects and healthy individuals. In the previous research, it was studied that Vitamin D3 deficiency is common among diabetic. The role of Vitamin D3 in diabetic neuropathy is investigated in one study few years ago; according to that research, deficiency of Vitamin D3 was associated with peripheral neuropathy in more than 500 diabetic Case [30]. Our results have shown the positive association of Vitamin D3 level with diabetic neuropathy.

The present study revealed a significant difference in Vitamin D3 levels in diabetic patients with peripheral

neuropathy in comparison with diabetic patients without neuropathy. Since our study had some limitation such as the Cases we recruited were not taking vitamin D3 supplements in the past, there is a need for further investigation to elaborate the role of Vitamin D3 in the progression of neuropathy. Furthermore, diabetic Case with peripheral neuropathy that had Vitamin D3 deficiency must be given supplemental therapy of deficient vitamin and then assessed for the effect of the therapy in the treatment of peripheral neuropathy³³.

Previously, a study based on the significance of vitamin D₃ in the pathogenesis of diabetic neuropathy showed a significant inverse association of these two parameters in diabetic Case (OR 3.4795% CI1.04–11.56). The observation of the previous study revealed

that in the presence of controlled condition in diabetic Case such as levels of HbA1c, LDL, and urinary albumin kept under normal values, there was reduction in nerve conduction velocity due to the deficiency of vitamin D₃²².

It is revealed from the present experimentation that vitamin D₃ deficient diabetic Case had motor or sensory neuropathy or both. No significant differentiation was observed between male and female in regards of reduction in nerve conduction velocity. Recruited Case for our study had full-blown impairment of nerve conduction velocity, although this condition can be controlled or even reverse by regulating blood glucose level and maintaining vitamin D3 level in the normal range³⁴.

Table1: Comparison of RBS, HbA1c and Vitamin D3 in Case and Control.

Parameters	N	Mean	Std. Deviation	P-value
Blood Glucose Control (RBS) mg/dl	50	1.213	12.46	
Case	50	2.315	50.61	<0.01
HbA1c (%) Control	50	5.142	0.406	<0.02
Case	50	6.954	1.09	
Vit.D3 level control ng/ml	50	25.821	4.417	<0.01
Case	50	10.448	2.406	

Table-2: Comparison of Sensory Nerve conduction velocities in right side of case & control.

Nerve	Mean	Std. Deviation	P Value
Median Nerve Control	53.12	4.36	<0.01
Case	49.98	2.14	
Ulnar nerve control	50.7	2.34	<0.01
Case	47.93	1.54	
Sural nerve control	47.6	3.82	<0.01
Case	45.71	3.65	

Table-3: Comparison of Sensory Nerve conduction velocities in left side of case & control.

Nerve	Mean	Std. Deviation	P Value
Median Nerve Control	52.26	3.19	<0.01
Case	49.25	1.59	
Ulnar nerve control	50-41	3.72	<0.01
Case	47.24	1.41	
Sural nerve control	47.3	2.68	<0.01
Case	44.31	3.36	

Table 4: Comparison of Motor Nerve conduction velocities in right side of case & control.

Nerve	Mean	Std. Deviation	P Value
Median Nerve Control	50.21	4.26	<0.01
Case	41.44	4.74	
Ulnar nerve control	52.12	2.82	<0.01
Case	39.64	4.48	
Peroneal nerve control	44.17	3.34	<0.01
Case	44.97	3.21	
Tibial nerve control	47.34	4.19	<0.01
Case	37.64	5.14	

Table-5: Comparison of Motor Nerve conduction velocities in left side of case & control.

Nerve	Mean	Std. Deviation	P Value
Median Nerve Control	48.62	4.59	<0.01
Case	39.81	3.81	
Ulnar nerve control	51.19	2.62	<0.01
Case	37.21	4.72	
Peroneal nerve control	47.98	3.34	<0.01
Case	43.20	3.36	
Tibial nerve control	42.89	4.78	<0.01
Case	35.01	5.75	

CONCLUSION

This study draws our attention towards the essential role of vitamin D₃ in the development and progression of neuropathy in diabetic Case. It can be concluded from this study that Vitamin D₃ supplementation is particularly important for Case with diabetes. As it can provide effective and beneficial outcomes for the prevention and treatment of diabetic neuropathy. Also it is cost effective and an independent factor for treatment of diabetic Case suffering from the neuropathy.

Acknowledgment: This study was conducted at Baqai Fatima Hospital and Jinnah Institute of Post graduate Hospital. We are thankful to Prof. Dr. Sikandar Ali Sheikh for his support and guidance.

REFERENCES

- Duby, J.J., Campbell, R.K., Setter, S.M., & Rasmussen, K.A. "Diabetic neuropathy: an intensive review," *American Journal of Health System Pharmacy*. vol. 61(2), pp. 160-173, 2004.
- Said, G. "Diabetic neuropathy a review. *Nature clinical practice, Neurology*. vol. 3(6), pp. 331-340, 2007.
- Nyomba, B. L., Auwerx, J., Bormans, V., Peeters, T. L., Pelemans, W., Reynaert, J., & De Moor, P. "Pancreatic secretion in man with subclinical vitamin D deficiency," *Diabetologia*. vol. 29(1), pp. 34-38, 1986.
- Hausler, M. R., Whitfield, G. K., Hausler, C. A., Hsieh, J. C., Thompson, P. D., Selznick, S. H., & Jurutka, P. W. "The nuclear vitamin D receptor: biological and molecular regulatory properties revealed," *Journal of Bone and Mineral Research*. vol. 13(3), pp. 325-349, 1998.
- Pociot, F., & McDermott, M. F. "Genetics of type 1 diabetes mellitus," *Genes and immunity*. vol. 3(5), pp. 235-249, 2002.
- Pani, M. A., Knapp, M., Donner, H., Braun, J., Baur, M. P., Usadel, K. H., & Badenhop, K. "Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans," *Diabetes*. vol. 49(3), pp. 504-507, 2000.
- Chang, T.J., Lei, H.H., Yeh, J.I., Chiu, K.C., Lee, K.C., Chen, M. C., & Chuang, L. M. "Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population," *Clinical Endocrinology*. vol. 52(5), pp. 575-580, 2000.
- Cooke, N.E., & Haddad, J.G. "Vitamin-D binding protein (Gc-Globulin)," *Endocrine Reviews*. vol. 10(3), pp. 294-307, 1989.
- Uitterlinden, A. G., Fang, Y., van Meurs, J. B., van Leeuwen, H., & Pols, H. A. "Vitamin D receptor gene polymorphisms in relation to Vitamin D related disease states," *The Journal of Steroid Biochemistry and Molecular Biology*. vol. 89, pp. 187-193, 2004.
- Kahn, S. E., Hull, R. L., & Utzschneider, K. M. "Mechanisms linking obesity to insulin resistance and type 2 diabetes," *Nature*. vol. 444(7121), pp. 840-846, 2006.
- Oh, J. Y., & Barrett-Connor, E. "Association between vitamin D receptor polymorphism and type 2 diabetes or metabolic syndrome in community-dwelling older adults," the Rancho Bernardo Study. *Metabolism*. vol. 51(3), pp. 356-359, 2002.
- Mayer T.K., Freedman Z.R. "Protein glycosylation in diabetes mellitus: A review of laboratory measurements and of their clinical utility," *Clin Chem Acta*. vol. 127, pp. 147 - 184 1983.
- Wolf HU, Lang W., and Zander R, "Alkaline haematin D - 575, a new tool for the determination of haemoglobin as an alternative to the Cyanhaemoglobin method," *Clin Chem Acta*. vol. 136, pp. 83 - 104, 1984.
- Binnie CD., Rowman, AJ and Gutter, TH. "A Manual of Electroencephalographic Technology," Cambridge University, Press, Cambridge 1982.
- Borg, J., Grimby, L. and Hannerz, J. "Axonal conduction velocity and voluntary discharge properties of individual short toe extensor motor units in man," *J. Physiol*. vol. 277, pp. 143-152, 1968.
- Behse, F. and Buchthal, F. "Normal sensory conduction in the nerves of the leg in man," *J. Neural, Neurosurg. Psychiatry*. Vol. 34, pp. 404-414, 1971.
- Bostock, H. and Sears, T.A. "The Inter nodal axon membrane: electrical excitability and continuous conduction in segmental demyelination," *J. Physiol*. vol. 280, pp. 273-301, 1978.
- Dawson-Hughes B., Heaney R.P., Holick M.F., Lips P., Meunier P.J. "Prevalence of Vitamin D insufficiency in an adult normal population," *Osteoporos. Int*. vol. 7, pp. 439-443, 1997.
- Zerwekh J.E. "Blood biomarkers of Vitamin D status," *Am. J. Clin. Nutr*. vol. 87(suppl), pp. 1087S-91S, 2008.
- Bischoff-Ferrari, H.A., Borchers, M., Gudat, F., Durmuller, U., Stahelin, H.B., Dick, W., "Vitamin D receptor expression in human muscle tissue decreases with age," *J. Bone Miner. Res*. Vol. 19, pp. 265-269, 2004b.
- Taha N. M., Vieth R. "The problem of an optimal target level for 25-Hydroxyvitamin D, the test for vitamin D nutritional status," *Clinical Laboratory International*. vol. 34, pp. 28-30. November 2010.
- Shehab D. "Normative DATA of nerve conduction studies in the upper limb in Kuwait, are they different than the western data?," *Med Princ Pract*. vol. 7, pp. 203-208, 1998.
- Shehab D, Moussa MA. "Normal values of lower limb nerve conduction in Kuwait (Part II)," *Med Princ Pract*. vol. 8, pp. 134-137, 1999.
- Riaz, S., Malcangio, M., Miller, M., & Tomlinson, D. R. "A vitamin D₃ derivative (CB1093) induces nerve growth factor and prevents neurotrophic deficits in streptozotocin-diabetic rats," *Diabetologia*. vol. 42(11), pp. 1308-1313, 1999.
- Bostock, H. "Impulse Propagation in experimental neuropathy. In *Peripheral Neuropathy*," 3rd edn. (ed. P.J. Dyck). W.B. Saunders, Philadelphia, P.A., 1993.
- Friedewald WT, Levy RI, Fredrickson DS. "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge," *Clin Chem*. vol. 18, pp. 499-502, 1972.
- Boulton AJ, Malik RA. "Diabetic neuropathy," *Med Clin North Am*. vol. 82, pp. 909-929, 1998.
- Alvarez JA, Ashraf A. "Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis," *Int J Endocrinol*. pp. 351-385, 2010.
- Inomata S, Kadowaki S, Yamatani T, Fukase M, Fujita T. "Effect of 1 alpha (OH)-vitamin D₃ on insulin secretion in diabetes mellitus," *Bone Miner*. Vol. 1, pp. 187-192, 1986.
- Soderstrom, L.H., Johnson, S.P., Diaz, V.A., & Mainous, A.G. "Association between vitamin D and diabetic neuropathy in a nationally representative sample: results from 2001-2004 NHANES," *Diabetic Medicine*. vol. 29(1), pp. 50-55, 2012.
- Fernandes DA, Eyles D, Fe'ron F. "Vitamin D, a neuro-immuno-modulator: implications for neurodegenerative and autoimmune diseases," *Psycho Neuro Endocrinology*. vol. 345, pp. S265-S277, 2009.
- Carlson AN, Kenny AM. "Is Vitamin D Insufficiency Associated with Peripheral Neuropathy," *Endocrinologist*. vol. 17, pp. 319-324, 2007.
- Taylor AV, Wise PH. "Vitamin D replacement in Asians with diabetes may increase insulin resistance," *Postgrad Med J*. vol. 74, pp. 365-366, 1998.
- Reis JP, Michos ED, von Muhlen D, Miller ER. "Differences in vitamin D status as a possible contributor to the racial disparity in peripheral arterial disease," *Am J Clin Nutr*. Vol. 88, pp. 1469-1477, 2008.