Association of Diabetic Neuropathy and Low Serum Calcium Levels in Diabetic Patients

Suresh Kumar P, T. Ramakrishna, E. Sreekumaran

Abstract— Disturbances in Calcium (Ca2+) and phosphorus metabolism were observed in uncomplicated type 1 Diabetes Mellitus and type 2 Diabetes Mellitus patients. Hormones like PTH, calcitonin etc are involved in it. There is a growing body of evidence that sensory neuropathy in diabetes is associated with abnormal calcium signaling in dorsal root ganglion neurons. Abnormal calcium signaling in diabetes has pathologic significance as elevation of calcium influx and cytosolic calcium release has been implicated in other neurodegenerative conditions characterized by neuronal dysfunction and death. Serum Ca2+ levels were assessed and found that 53.1 % males and 46.9 % females, whose calcium was analyzed, are having low serum Ca2+ values. Mean serum calcium values of hyper-insulinemic patients was 8.06 mg%. Males had lower values in both hyper insulinemic (7.94 mg% for male vs 8.26 mg% for female) as well as low insulin groups (8.2 mg% for male vs 8.8 mg% for female). It is essential that we have to look beyond blood sugar and lipids, and that it may be important to assess serum calcium and other relevant micro and macro nutrients levels and to correct any deficiency by supplementing the same.

Index Terms— Calcium, Diabetes Mellitus, Hypocalcemia, Hyper-Insulinemia, Neuropathy.

1 Introduction

Disturbances in calcium and phosphorus metabolism were observed in uncomplicated type 1 diabetes and type 2 diabetes patients.[1],[2] But the physiological hormone control involving PTH (Parathyroid hormone) and Calcitonin were found to be preserved. There are studies that the mean values of serum Ca2+ were identical in control as well as diabetic patients, whereas the PTH values were found to be lower in diabetic patients though not statistically significant.[3] But Migdalis et al. reported lower levels of Ca2+ vs. control and non-neuropathic and lower Mg2+ vs. control, despite similar PTH levels.[4] Eventhough these results are mutually contradictory, they found that 'Ca2+-Mg2+-ATPase' pump is an important regulator of intracellular calcium concentration. This diabetic neuropathy study group had found significantly lower levels of ATPase, compared to controls and compared to diabetic patients without neuropathy. They also had lower levels of Ca2+ and Mg2+ vs. control and non-neuropathic vs. control, despite similar PTH levels. There is a growing body of evidence that sensory neuropathy in diabetes is associated with abnormal calcium signaling in dorsal root ganglion (DRG) neurons. Abnormal calcium signaling in diabetes has pathologic significance as elevation of calcium influx and cytosolic calcium release has been implicated in other neurodegenerative conditions characterized by neuronal dysfunction and death. The calcium "set point" hypothesis suggests that modest changes in cytosolic calcium ([Ca2+]i) over prolonged periods may be injurious to the cell.[5],[6] It was noted that neuronal resting intracellular Ca2+ abnormalities increased progressively with the duration of diabetes.[2] It was not only the function of neurons but, that of other organelles in the body like neutrophils that are also abnormal due to improper functioning of Ca2+ in T2DM. In type 2diabetes impaired neutrophil function leads to increased bacterial infection and cardiovascular disease. Intriguingly, increased intracellular Ca2+ is associated with platelet hyper function in diabetes.[7] It was also found that altered Ca2+ transients of vascular smooth muscle cells to vasoconstrictors may contribute to altered regulation of blood flow in diabetes.[8] However, to the best of our knowledge surprisingly no study seems to have been undertaken to assess calcium levels in diabetics in India.

2 Materials and Methods

160 diabetic patients were selected for the study. Blood sample is collected in plain tube without tourniquet application. Samples were allowed to clot, centrifuged and the serum separated from the samples is assayed for Calcium levels using Arsenazo III method in the Beckman AU 480 analyzer. A calcium value of 8.5 mg/dL is considered as the lower cut of normal calcium level. Samples were collected from all the participants after getting informed consent. The values were compared using chi square test (Medcalc 12.0) and $p<=0.05$ is considered significant.

3 Results

The study polulation includes 160 diabetic subjects (53.1 % male and 46.9 % female). The results of the study are given in Table.1 and Figure 1. Mean serum calcium values of hyper-insulinemic patients was 8.06 mg%. Males had lower values in both hyperinsulinemic (7.94 mg% for male vs 8.26 mg% for female) as well as low insulin groups (8.2 mg% for male vs 8.8 mg% for female). Males from both

- *Suresh Kumar P is currently the Medical Director of Dr. Suresh's DiabCare India, East West Cross Road, Nadakkavu, Kozhikkode, Kerala, India-673011. Email: srshkmrp@gmail.com, Phone: +91 495 4012123*
- *T. Ramakrishna is currently Emeritus professor, Dept of life Sciences, Calicut University*
- *E. Sreekumaran is currently Reader, Physiology, Dept. of Life Science, Calicut University*

groups had lower calcium levels compared to females. There is a statistically significant decrese in calcium levels in the total population (p=0.035), but was not significant in the male and female groups.

Table 1. Calcium distribution of the study population (n=160)

Category	Male (n=85)		Female(n=75)		Total(n=160)	
	n(%)	p	n(%)	p	n(%)	p
Normal Calcium	40(47.1%)	0.189	30(40%)	0.140	70(43.8%)	0.035
Below Normal Ca^{2+}	45(52.9%)	NS	45(60%)	NS	90(56.3%)	SIG

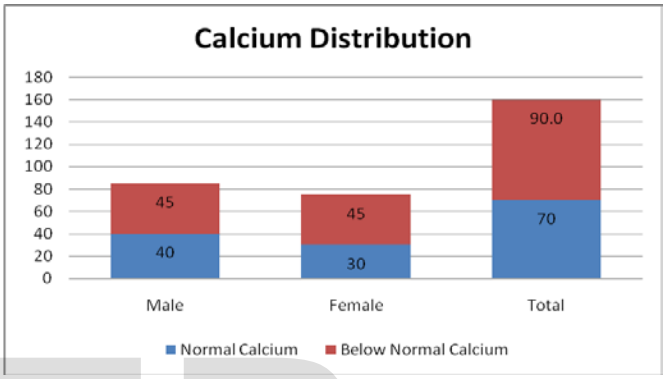
NS: Not significant, SIG: Significant. A calcium value of 8.5 is used as the lower cut of normal Calcium levels.

4 Discussion

Disturbances in Ca2+ and phosphorus metabolism were observed in uncomplicated type 1 diabetes and type 2diabetes patients.[2] But the physiological hormone control involving PTH (Parathyroid hormone) and calcitonin were found to be preserved. Advani et al., in 2004 reported that the mean values of serum Ca2+ were identical in control as well as diabetic patients, whereas the PTH values were found to be lower in diabetic patients though not statistically significant. But Migdalis et al. had lower levels of Ca2+ vs. control and non-neuropathic and lower Mg2+ vs. control, despite similar PTH levels.[4] These results are mutually contradictory and we found that our diabetic patients had a lower mean serum Ca2+ levels (8.06 mg%). Mean values were lower in males (7.94 mg% vs 8.26 mg% for female). Lower calcium levels in males were observed among non-hyperinsulinemic group as well. This indicates that hypocalcemia is a sequel of insulin resistance, because males had more insulin resistance in the study population, both by the anthropometric criteria as well as the biochemical parameters of fasting insulin levels. It is possible that the altered Ca2+ metabolism and its lower serum levels may be indicative of a severer degree of insulin resistance in diabetics as it was shown that the Calcium and Mg2+ abnormalities were related to the degree of insulin resistance.

There is a growing body of evidence that sensory neuropathy in diabetes is associated with abnormal calcium signaling in dorsal root ganglion (DRG) neurons. Abnormal calcium signaling in diabetes has pathologic significance as elevation of calcium influx and cytosolic calcium release has been implicated in other neurodegenerative conditions characterized by neuronal dysfunction and death. Impaired regulation of calcium channels by G proteins is an important mechanism contributing to an enhanced calcium influx in diabetes. Various pathological lesions observed in animal and human models of diabetes includes segmental demyelination; atrophy; loss of myelinated and unmyelinated fibers; Wallerian degeneration; segmental and paranodal demyelination; and blunted nerve fiber regeneration. [9],[10] Elevation of calcium currents observed in peripheral and spinal sensory neurons in rodent models of diabetes results in increased cytosolic calcium release from internal stores and impaired calcium resequestration.[11],[12] The latter causing prolonged cytosolic calcium elevation has been implicated in the pathogenesis of neuronal injury in a variety of neurodegenerative disorders. The calcium "set point" hypothesis[13] suggests that modest changes in cytosolic calcium ([Ca2+]i) over prolonged periods may be injurious to the cell. It was noted that neuronal resting intracellular Ca2+ abnormalities increased progressively with the duration of diabetes.[2] This is also in accordance with our results correlating the duration of diabetes with the degree of hypocalcaemia. Diabetic neurons release more Ca2+ from cytosolic pools and demonstrate impaired ability to resequester Ca2+, compared to non-diabetic controls.

Figure 1. Distribution of Calcium Levels



Normal Clacium levels are above or equal to 8.5 mg/dL and Below normal calcium levels are below 8.5 mg/dL

It was not only the function of neurons but, that of other organelles in the body like neutrophils that are also abnormal due to improper functioning of Ca2+ in T2DM. In type 2diabetes impaired neutrophil function leads to increased bacterial infection and cardiovascular disease. Many neutrophil functions depend on Ca2+ signaling, which involves release of Ca2+ from intracellular stores and subsequently translocation of stores via the cytoskeleton to the plasma membrane, causing store mediated Ca2+ entry (SMCE) into the cell. Abnormal Ca2+ signaling is likely to be important in the pathogenesis of diabetic complications.

The cause of hypocalcaemia detected in the present study, need further evaluation. It could either be due to intracellular deficiency of Mg2+ or probably due to the abnormal functioning of one of the least attended counter-regulatory hormones called PTH (parathormone) secondary to insulin resistance. This aspect needs more focused research to look at the cause for hypocalcaemia in type 2 diabetics. It was reported that unlike in the case of Mg2+, Ca2+ and PO4 does not show increased renal wasting in Diabetes.

Dietary deficiency alone is unlikely reason for low Ca2+ levels, which will usually be compensated through mobilization from bone. The deficiency or abnormal functioning of Calcium and related minerals can lead to various neurological and musculoskeletal symptoms which can affect the

normal life of the patients and can be easily and effectively treated with ordinary calcium supplements in combination with Vit.D3 (and magnesium) available in the market.

Now it is evident that in the management of diabetes, we have to look beyond blood sugar and lipid levels alone. It may be important to assess serum calcium and other relevant micro and macro nutrient levels and to correct any deficiency by supplementation. Serum Ca2+ levels will give us an indirect estimate of intracellular Mg2+ also. Information on all these aspects is necessary to completely understand and manage diabetes.

5 Summary And Conclusion

Calcium metabolism, one of the least attended areas in the management of diabetes, was abnormal in this study population. The lower mean serum calcium levels among the diabetics, predominantly among male diabetics and especially in the poorly controlled group, shows that we should spread out our attention to the areas of mineral metabolism also, apart from concentrating merely on blood sugar and lipids in the management of diabetic pateients. It is possible that the altered calcium metabolism and its lower serum levels in diabetics may be indicative of a more severe degree of insulin resistance. This is one clinical problem that if detected sufficiently early, can be corrected easily with medication.

Having known that calcium metabolism is closely related to the survival and functioning of nervous tissue, especially ganglions in the dorsal horns, it is obvious that its deficiency may have a bearing on the severity of the manifestations of neuropathic signs and symptoms. So, it is suggested that all diabetics should be investigated for their mineral status like calcium and magnesium levels. Nearly half of the patients in this study had sub-normal levels of calcium. Supplementing this deficiency may delay the apoptosis of the neurons and thus primarily and or secondarily prevent neuro-degeneration in diabetes.

6 Referecne

[1] Pittas, Anastassios G., Joseph Lau, Frank B. Hu, and Bess Dawson-Hughes. "The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis." The Journal of Clinical Endocrinology & Metabolism 2007; 92(6):2017-2029.

[2] Pyörälä, Eeva. Interaction in dietary counselling of diabetic children and adolescents. University of Helsinki, 2000.

[3] Advani, A., S. M. Marshall, and T. H. Thomas. "Impaired neutrophil store-mediated calcium entry in Type 2 diabetes." European journal of clinical investigation 2004; 34(1): 43-49.

[4] Migdalis, I. N., K. Xenos, K. Chairopoulos, N. Varvarigos, E. Leontiades, and K. Karmaniolas. "Ca2+ Mg2 ATPase activity and ionized calcium in Type 2 diabetic patients with neuropathy." Diabetes research and clinical practice 2000; 49(2): 113-118.

[5] De Waard M, Liu H, Walker D, Scott VES, Gurnett CA, Campbell KP Direct binding of G-protein βγ complex to voltage-dependent calcium channels. Nature 1997; 385:446–450

[6] Ikeda SR Double-pulse calcium channel current facilitation in adult rat sympathetic neurons. J Physiol (Lond) 1991;439:181–214.

[7] Ishii, H., F. Umeda, T. Hashimoto, and H. Nawata. "Increased intracellular calcium mobilization in platelets from patients with type 2 (non-insulin-dependent) diabetes mellitus." Diabetologia 1991; 34(5): 332-336.

[8] Guerrero-Hernandez, Agustin, and Alexei Verkhratsky. "Calcium signalling in diabetes." Cell calcium 2014; 56(5): 297-301.

[9] Bean, Bruce P. "Classes of calcium channels in vertebrate cells." Annual Review of Physiology 1989; 51(1): 367-384.

[10] Doupnik, C. A., and R. Y. K. Pun. "G-protein activation mediates prepulse facilitation of Ca2+ channel currents in bovine chromaffin cells." The Journal of membrane biology 1994; 140(1): 47-56.

[11] Gispen, Willem Hendrik, and Geert-Jan Biessels. "Cognition and synaptic plasticity in diabetes mellitus." Trends in neurosciences 2000;23(11): 542-549.

[12] Cens, Thierry, Sophie Restituito, Alice Vallentin, and Pierre Charnet. "Promotion and inhibition of L-type Ca2+ channel facilitation by distinct domains of the β subunit." Journal of Biological Chemistry 1998;273(29): 18308-18315.

[13] De Waard, Michel, Julien Hering, Norbert Weiss, and Anne Feltz. "How do G proteins directly control neuronal Ca2+ channel function?." Trends in pharmacological sciences 2005; 26(8): 427-436.