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Diabetes Mellitus and serum calcium levels- A case control study

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ABSTRACT

Introduction

Diabetes mellitus (DM) is a worldwide metabolic disease characterized by chronic hyperglycemia, which results from insufficient insulin secretion and/or insulin resistance in
target tissues or both . Chronic hyperglycemia leads to macrovascular and microvascular complications in various organs such as heart , brain , kidney , eyes etc. It not only disturbs the normal functioning of vital organs but also impairs calcium metabolism by disrupting the functions of calcium regulating organs namely intestine , bone and kidney.

Calcium is an important element for several biological processes, such as cell division and growth, blood coagulation, cardiovascular homeostasis, hormone responses, neural electrical activity, and bone formation. The present article thus focuses on the underlying mechanisms
of the negative effects of DM on calcium homeostasis as well as their long-term consequences.

AIMS AND OBJECTIVES

a) To measure the level of Serum Calcium in patient with Type-2 Diabetes mellitus
b) To measure the level of Serum Calcium in controls.
c) To compare the levels of serum calcium in both groups.

MATERIALS AND METHODOLOGY

This is a case control study .The study was conducted for a duration 2 year .The study was carried out on 70 cases of clinically diagnosed type 2 diabetes mellitus in the age group 35-55 years in KIMS Koppal. Seventy age and sex matched healthy subjects were taken as controls . Informed consent was taken from all the subjects. Ethical clearance was obtained from Institute's ethical clearance committee. Diabetes Mellitus was diagnosed as per the WHO diagnostic criteria[14].

STATISTICAL TEST

Data is expressed in terms of mean ± SD. Chi- square test was applied to estimate the difference between the two groups of population. Unpaired 't'-test was used to study the changes in serum calcium levels between the study groups. p value <0.05 was considered statistically significant.

Results : Our study showed a significantly decreased levels of serum calcium in cases compared to controls. ($p<0.05$)

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Conclusion: It is clear that DM, hyperglycemia and associated complications (microvascular damage) interfere with normal functions of the three calcium-regulating organs, namely the intestine, bone, and kidney. Hence maintaining the serum calcium levels within normal range and also supplementing with calcium supplements may prevent long term bone effects in DM.

Keywords : Calcium, Diabetes Mellitus, Fasting blood sugar , post prandial blood sugar, hyperglycemia

Introduction

The incidence OF type 2 diabetes mellitus (type 2 DM) is increasing at an alarming rate both nationally and worldwide, with more than 1 million new cases per year diagnosed in the United States alone[1,2]. Diabetes is the fifth leading cause of death in the United States, and it is also a major cause of significant morbidity. Although our current methods of treating type 2 DM and its complications have improved, prevention of the disease is preferable. Indeed, epidemiological data suggest that nine of 10 cases of type 2 DM could be attributed to habits and forms of modifiable behavior[3,4] .

Potentially modifiable environmental risk factors for type 2 DM have been identified, the major one being obesity. Although weight loss (achieved by any means) has been shown to be successful in delaying type 2 DM, it is difficult to achieve and maintain long term. Therefore, identification of environmental and easily modified risk factors is urgently needed to prevent development of type 2 DM in the 41 million Americans who are at risk of the disease[5].

Calcium is an element that plays an important role not only in skeletal mineralization but also in a wide range of biological functions[6] . In recent decades, insulin resistance and secretion have been shown to depend on calcium homeostasis. The secretion of insulin in response to an elevated concentration of plasma glucose is a Ca2+-dependent process. Alterations in insulin secretion have also been involved with disorders in blood glucose homeostasis[7], and increasing cytosolic calcium has been associated with an increase in the expression of GLUT4 transporters in the myocyte, which, in turn, increases the insulin-stimulated glucose transport activity in these cells[8] . Because both defects in insulin secretion and insulin action are related to type 2 diabetes[9,10] , it is expected that abnormal calcium homeostasis could play an important role in the development of type 2 diabetes.

We conducted this study in our area to see for the changes in serum calcium levels in patients with type 2 diabetes mellitus in our locality .

Findings from epidemiological studies are inconsistent. Some observational studies have indicated that increased serum Ca concentration may be directly associated with the risk of developing T2DM[11-13] , whereas others reported the null correlation between circulating Ca and prevalent T2DM[14] .

Also, it has been observed that elevated serum Ca levels were related to insulin resistance[15], reduced insulin sensitivity and impaired glucose tolerance[15] , but not a decrease in insulin secretion[16] . These contradictory results may be partially attributable to diverse Ca assessments, for example, total serum Ca and albumin-adjusted serum Ca, which are different biomarkers of Ca status[17]. Because approximate 40 % of Ca in the serum is bound to albumin[18] , it is vital to know the serum albumin level when evaluating the total serum Ca.

Moreover, circulating Ca homeostasis is exquisitely regulated by multiple negative feedback loops which involve several integrated hormonal responses and target organs[18] . Thus, each of these components may mediate the possible Ca-related risk of diabetes. Interestingly, two double-blind, placebo-controlled, randomised clinical trials reported non-significant effects of Ca supplementation on insulin resistance in obese adults , as well as insulin secretion, insulin sensitivity and glycaemia in adults at risk of T2DM(26) . Of note, these studies have relatively short follow-up periods, insufficient numbers of participants and heterogeneous characteristics between populations[19,20].

Objectives

The objectives of this study are :

a) To measure the level of Serum Calcium in patient with Type-2 Diabetes mellitus

b) To measure the level of Serum Calcium in controls.

c) To compare the levels of serum calcium in both groups.

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Materials and methods:

This is a case control study .The study duration is 2 years. The study was carried out on 70 cases of clinically diagnosed type 2 diabetes mellitus in the age group 40-60 years , attending out patient department (OPD) of department of medicine , Koppal Institute of Medical Sciences (KIMS) ,Koppal. Seventy age and sex matched healthy subjects were taken as controls . Ethical clearance was obtained from Institute's ethical clearance committee. Informed consent was taken from both cases and controls after explaining the procedure. Diabetes Mellitus was diagnosed as per the WHO diagnostic criteria[21].

Ethical clearance was obtained from Institute's ethical clearance committee. Informed consent was taken from both cases and controls after explaining the procedure.

Exclusion criteria:

- Diabetes Mellitus with complications- diabetic nephropathy
- Chronic kidney diseases, Malabsorptive disorders
- Patients with osteoporosis, bone diseases,
- Thyroid and parathyroid disorders
- Patients on steroids therapy
- Pregnant and lactating women

Biochemical analysis: A sample of 3 ml venous blood is collected in both fasting and post prandial state under aseptic precautions . It is allowed to clot and serum was separated by centrifugation.

The following parameters are studied.

1 FBS and PPBS –Glucose oxidase peroxidase method[22,23] .(kits supplied by Erba Diagnostics) . The parameters were read using semi auto analyser (STAT FAX 3300).
2 HbA1c was estimated by Nycocard reader II[24].
3 Serum Calcium-Arsenazo 3 method, end point assay

Statistical analysis :Data was expressed in terms of mean ± SD. Chi- square test was applied to estimate the difference between the two groups of population. Unpaired 't'-test was used to study the changes in serum magnesium and zinc in between cases and controls. Pearson correlation was performed to establish the relationship between study variables. p value <0.05 was considered statistically significant.

RESULTS :

This was a comparative case control study conducted on 70 cases of type 2 DM (n=70) and 70 age and sex matched healthy controls(n=70). Serum calcium was estimated, analyzed and correlated with HbA1c, FBS and PPBS. The results were expressed as mean ± standard deviation.

The mean age (in years) of cases was 45.5±10.7 years and that of controls was 48 ±10.4 years and was not significant. **Table 1** shows comparision of serum calcium , FBS, PPBS and HbA1c levels in both groups and was statistically significant($p<0.05$). The mean serum levels (mg/dL) in cases was 8.1±0.3, and in controls was 8.9±0.7 and was highly significant ($p <0.0001$)**.**

Table 2 shows the mean age of cases and controls .

Figure 1 shows the correlation between glycosylated hemoglobin and serum calcium levels

DISCUSSION:

We conducted a case control study on total 140 subjects.We observed a relative decrease in serum calcium levels in cases as compared t controls. Several lines of evidence have suggested that DM in both humans and rodents profoundly affects renal calcium handling, such as decreasing 1,25(OH)2D3 synthesis, as a consequence of prolonged hyperglycemia and dysregulation of calciotropic hormone metabolism[25-28].

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Tubular calcium reabsorption in STZ-induced diabetic rats was markedly reduced, which could be reversed by insulin administration[29]. A decrease in insulin production and release in T1DM or insulin resistance in T2DM is believed to contribute to a reduction in renal calcium (and magnesium) reabsorption. A study in isolated perfused mouse thick ascending limb showed that insulin significantly increased the transepithelial potential difference—a driving force for passive calcium reabsorption—as well as the transepithelial calcium and magnesium transport[30]. Therefore, this driving force for calcium reabsorption is probably reduced under diabetic condition, thereby culminating in increased calcium excretion. On the other hand, amino acids that can induce insulin secretion are able to induce tubular calcium reabsorption[31]. Nevertheless, in parathyroidectomized female rats subjected to intravenous arginine infusion, the arginine-induced insulin secretion was associated with urinary calcium excretion[32].

This discrepancy can be explained by an increase in glomerular filtration rate secondary to amino acid loading, which leads to renal calcium excretion. Indeed, the stimuli or pattern of insulin secretion may also determine the final outcome of renal calcium handling.

In addition, renal calcium loss in DM can result from dysregulation of the calciotropic hormones, such as PTH and 1,25(OH)2D3. It has been reported that the PTH-induced stimulation of 1,25(OH)2D3 production was impaired in diabetic condition, whereas insulin treatment successfully rescued 1,25(OH)2D3 production. Another evidence comes from the study of hyperinsulinemic ZDF rats (a T2DM model), in which a reduction in the renal expression of megalin and disabled (Dab)-2 proteins can cause an impaired receptor-mediated endocytosis of vitamin D-binding protein-bound 25(OH)D3, leading to urinary loss of 25(OH)D3 and a decrease in circulating 25(OH)D3 level[33]. It is of interest that the bone-derived phosphaturic hormone FGF-23 probably contributes to changes in renal calcium handling during diabetic condition. FGF-23 has recently been demonstrated to be a negative regulator of intestinal calcium absorption[34,35].

CONCLUSION:

Conclusion: It is clear that DM, hyperglycemia and associated complications(microvascular damage) interfere with normal functions of the three calcium-regulating organs, namely the intestine, bone, and kidney.

DM as well as elevated glucose concentration diminish calcium absorption, presumably by down regulating nuclear vitamin D receptor expression and inducing oxidative stress.

Hence maintaining the serum calcium levels within normal range and also supplementing with calcium supplements may prevent long term bone effects in DM.

Future study: Further studies on these parameters on large scale is required to establish diagnosic and prognostic role of these parameters in Diabetes mellitus.

Limitations of the study :Small number of the sample

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Conflict of interest : None declared

References

1) Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, Marks JS 2003 Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA 289:76 –79.
2) Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC 2001 Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 345:790 –97.
3) Benjamin SM, Valdez R, Geiss LS, Rolka DB, Narayan KM 2003 Estimated number of adults with prediabetes in the US in 2000: opportunities for prevention. Diabetes Care 26:645– 49 .
4) Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, DawsonHughes B 2006 Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 84:18 –28.

ISSN:0975-3583,0976-2833 VOL14,ISSUE06,2023

5) Holick MF 2006 High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 81:353–73 .
6) Peacock M. Calcium metabolism in health and disease. Clin J Am Soc Nephrol 2010;5 (Suppl. 1):S23–S30.
7) Mears D. Regulation of insulin secretion in islets of Langerhans by Ca(2+)channels. J Membr Biol 2004;200:57–66 .
8) Ojuka EO, Jones TE, Nolte LA, et al. Regulation of GLUT4 biogenesis in muscle: evidence for involvement of AMPK and Ca(2+). Am J Physiol Endocrinol Metab 2002;282:E1008–E1013.
9) American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33(Suppl. 1):S62–S69.
10) Levy J, Stern Z, Gutman A, Naparstek Y, Gavin JR 3rd, Avioli LV. Plasma calcium and phosphate levels in an adult noninsulin-dependent diabetic population. Calcif Tissue Int 1986;39:316–18.
11) Mears D (2004) Regulation of insulin secretion in islets of Langerhans by Ca(2+) channels. J Membr Biol 200, 57–66.
12) Wollheim CB & Sharp GW (1981) Regulation of insulin release by calcium. Physiol Rev 61, 914–73.
13) Ojuka EO, Jones TE, Nolte LA, et al. (2002) Regulation of GLUT4 biogenesis in muscle: evidence for involvement of AMPK and Ca(2+). Am J Physiol Endocrinol Metab 282, E1008–E1013.
14) Begum N, Leitner W, Reusch JE, et al. (1993) GLUT-4 phosphorylation and its intrinsic activity. Mechanism of Ca(2+)-induced inhibition of insulin-stimulated glucose transport. J Biol Chem 268, 3352–56.
15) American Diabetes Association (2010) Diagnosis and classification of diabetes mellitus. Diabetes Care 33, S62–S69.
16) Procopio M, Magro G, Cesario F, et al. (2002) The oral glucose tolerance test reveals a high frequency of both impaired glucose tolerance and undiagnosed type 2 diabetes mellitus in primary hyperparathyroidism. Diabet Med 19, 958–61.
17) Lorenzo C, Hanley AJ, Rewers MJ, et al. (2014) Calcium and phosphate concentrations and future development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetologia 57, 1366–74.
18) Khan AH & Pessin JE (2002) Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. Diabetologia 45, 1475–83.
19) Kim MK, Kim G, Jang EH, et al. (2010) Altered calcium homeostasis is correlated with the presence of metabolic syndrome and diabetes in middle-aged and elderly Korean subjects: the Chungju Metabolic Disease Cohort study (CMC study). Atherosclerosis 212, 674–681.
20) Yamaguchi T, Kanazawa I, Takaoka S, et al. (2011) Serum calcium is positively correlated with fasting plasma glucose and insulin resistance, independent of parathyroid hormone, in male patients with type 2 diabetes mellitus. Metabolism 60, 1334–133.
21) Alvin C Powers. Diabetes Mellitus, In: Harrisons Principles of Internal Medicine . 18th Ed. Mc Graw Hill, p2968-69.
22) Lott JA, Turner K. Evaluation of Trinder's Glucose Oxidase Method for Measuring Glucose in Serum and Urine. Clin.Chem 1975: 21(12):1754-60 .
23) Burtis A, Ashwood R, Bruns E, editors. Estimation methods for glucose. Teitz textbook of clinical chemistry and molecular diagnostics, 5th edition. New Delhi: 2012. p 720-21.
24) Burtis A, Ashwood R, Bruns E, editors. Glycated hemoglobin. Teitz textbook of clinical chemistry and molecular diagnostics, 5th edition. New Delhi: 2012. p 1441-46.
25) Raskin P, Stevenson MR, Barilla DE, Pak CY (1978) The hypercalciuria of diabetes mellitus: its amelioration with insulin. Clin Endocrinol (Oxf) 9:329–335.
26) Anwana AB, Garland HO (1990) Renal calcium and magnesium handling in experimental diabetes mellitus in the rat. Acta Endocrinol (Copenh) 122:479–486.
27) Garland HO, Forshaw AG, Sibley CP (1997) Dietary essential fatty acid supplementation, urinary calcium excretion andreproductive performance in the diabetic pregnant rat. J Endocrinol 153:357–363 .
28) Huang CQ, Ma GZ, Tao MD, Ma XL, Liu QX, Feng J (2009) The relationship among renal injury, changed activity of renal 1-a hydroxylase and bone loss in elderly rats with insulin resistance or type 2 diabetes mellitus. J Endocrinol Invest 32:196–201 .
29) Singh HJ, Garland HO (1989) A comparison of the effects of oral and intravenous glucose administration on renal calcium excretion in the rat. Q J Exp Physiol 74:531–540.
30) Birdsey TJ, Husain SM, Garland HO, Sibley CP (1995) The effect of diabetes mellitus on urinary calcium excretion in pregnant rats and their offspring. J Endocrinol 145:11–18.

ISSN:0975-3583,0976-2833 VOL14,ISSUE06,2023

31) Verhaeghe J, Bouillon R, Nyomba BL, Lissens W, Van Assche FA (1986) Vitamin D and bone mineral homeostasis during pregnancy in the diabetic BB rat. Endocrinology 118:1019–1025
32) Kashef S, Karamizadeh Z (2002) Hypercalciuria, hyperphosphaturia and growth retardation in children with diabetes mellitus. Iran J Med Sci 27:11–14.
33) Lee CT, Lien YH, Lai LW, Chen JB, Lin CR, Chen HC (2006) Increased renal calcium and magnesium transporter abundance in streptozotocin-induced diabetes mellitus. Kidney Int 69:1786–91 .
34) Mandon B, Siga E, Chabardes D, Firsov D, Roinel N, De Rouffignac C (1993) Insulin stimulates Na, Cl-, Ca2, and Mg2 transports in TAL of mouse nephron: cross-potentiation with AVP. Am J Physiol 265:F361–F369
35) Gollaher CJ, Wood RJ, Holl M, Allen LH (1984) A comparison of amino acid-induced hypercalciuria in sham-operated and parathyroidectomized rats. J Nutr 114:622–626.

TABLES AND FIGURES

Table 1 shows comparision of serum calcium , FBS, PPBS and HbA1c levels in both groups

Characteristics	Groups	Mean ±SD	T	P
FBS (mg/dL)	Cases	204.5±54.4	11.1	0.001*
	Controls	90.1±12.3		
PPBS(mg/dL)	Cases	310.3±62.6	17.2	0.001*
	Controls	110.9±8.6		
HbA1c(%)	Cases	7.9±0.6	15.8	0.001*
	Controls	5.4±0.5		
Serum calcium (mg/dl)	Cases	7.1±0.2	18.5	0.001
	Controls	9.2±0.5		

Table 2 shows the mean age of cases and controls .

	cases	controls
Mean age in years	45yrs	46yrs

Figure 1 shows the correlation between glycosylated hemoglobin and serum calcium levels