Original Article

Low Serum Calcium Levels Occur in Nigerian Adults with Type 2 Diabetes and Correlates Negatively with Their Glycosylated Hemoglobin Levels: A Case-control Study

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Abstract

Background: The prevalence of diabetes mellitus (DM) has increased globally making it a major public health concern. Serum calcium levels, together with other minerals, play an important role in the regulation of plasma glucose. The aim of this study was to determine the serum calcium levels in individuals with Type 2 DM (T2DM) as well as its relationship with their clinical characteristics and glycemic control. **Materials and Methods:** This was a cross-sectional study carried out at the University of Nigeria Teaching Hospital, Enugu State. A total of 300 participants were recruited, comprising 150 participants with DM and 150 age- and gender-matched normal participants as control. A questionnaire was administered to obtain clinical characteristics. Serum calcium and glycosylated hemoglobin (HbA1c) were measured. Mean ± standard deviation was calculated, and an independent *t*-test was used to determine the mean difference. Pearson's correlation was done to establish the correlation between serum calcium and HbA1c. Data were analyzed with SPSS version 23. **Results:** This study showed that the mean serum calcium level for the T2DM participants was significantly reduced (2.16 ± 0.17) when compared to that of the controls (2.21 ± 0.17) ($P = 0.02$). Furthermore, individuals with T2DM showed a significant negative correlation between serum calcium levels and HbA1c-a long-term measure of glycemic control ($r = 0.273$; $P = 0.001$). **Conclusion:** Participants with T2DM were found to have a lower mean level of serum calcium. In addition, T2DM participants who had poor glycemic control were more likely to have hypocalcemia.

Keywords: Calcium, correlation, glycosylated hemoglobin, type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia from relative insulin deficiency, resistance, or both.[1] The estimated global prevalence of DM among adults aged 20–79 years in 2019 was reported by the International Diabetes Foundation to be 9.3%, affecting 463.0 million adults, and this figure is projected to increase to 578.4 million by 2030 and eventually, to 700.2 million adults, in 2045.[2] A recent systematic review of DM prevalence in Nigeria (2018), revealed a nation-wide prevalence of 5.7%, though with marked regional variations in prevalence.[3] Type 2 DM (T2DM) is the most common type of DM and results from a combination of insulin resistance and less severe insulin deficiency.[1] T2DM is also the most commonly seen diabetes presentation in older adults, though in recent times, it has been noted to be increasing in incidence among children, adolescents, and younger adults due to poor diet, obesity, and sedentary lifestyle.

DM as a complex chronic disease, requires multifactorial risk-reduction strategies beyond glycemic control.[4] The long-term metabolic derangement in DM leads to the affectation of different organs and systems in the body including the kidneys, nerves, eyes, and cardiovascular system. Insulin is the key hormone required for the storage and controlled release of chemical energy in form of glucose from food in the body[1]

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and hence, is the primary determinant of glycemic control. The landmark United Kingdom Prospective Diabetes Study showed that patients with tight control of glycaemia had fewer chronic complications;[5] therefore, adequate glycemic control should be the primary aim of treatment. Short-term glycemic control of patients can be determined by self-monitoring of blood glucose using glucose meters.[6] However, the glycosylated hemoglobin (HbA1c) levels, reflects the average glycemia over approximately 3 months and has a strong predictive value for DM complications.[5,6]

Calcium is a protean intracellular messenger involved in many biological processes.[7] More than 99% of the calcium present in the human body is in the bone, the remaining being found mostly in the blood and extracellular fluid.[8] The biologically active form of calcium is the unbound (ionized) form and this ionized calcium needs to be maintained within a narrow range, because of the critical role it plays in a wide range of cellular functions.[8] Calcium is mainly regulated by parathyroid hormone (PTH) and Vitamin D and to a lesser extent, by calcitonin.[9]

Insulin secretion is a complex physiologic process which is highly calcium-dependent, and occurs following a Vitamin D-mediated surge in intracellular calcium. This surge then triggers activation of calcium-dependent phospholipid protein kinase, which through intermediary pathways, leads to fusion of insulin-containing granules to the cell membrane of the pancreatic islets beta cells, with subsequent release of insulin-rich granule contents.[1] In situations where calcium levels are too low or too high, the pancreatic islets and insulin target cells then fail to respond appropriately to the physiologic stimulus.[8]

Reduced serum calcium in patients with T2DM is attributed to excessive urinary calcium loss due to chronic hyperglycemia. PTH secretion is then stimulated in response to increased urinary calcium loss, to help maintain serum calcium levels.[10]

The aim of this study was to determine the serum calcium levels in participants with T2DM and to determine any relationship between serum calcium levels, clinical features of DM patients and their HBA1c levels.

Materials and Methods

This was a cross-sectional study carried out at the once weekly diabetes clinic of the University of Nigeria Teaching Hospital (UNTH) Enugu State Nigeria, over a 7 months period, from January to July 2017.

Study population

One hundred and fifty sequentially presenting and consenting adults were recruited through systematic random sampling from the diabetes clinic. One hundred and fifty age- and gender-matched nondiabetic participants were recruited as a comparator group.

Patients with renal disease and other endocrine diseases were excluded from the study.

Ethical Clearance was obtained from the Health Research and Ethics Committee of UNTH. Details of the study were explained to the participants and written informed consent obtained for each participant. Data were collected with the aid of a structured questionnaire and additional clinical data were obtained from the medical records to aid in determining participants' eligibility.

The procedure was clearly explained to all the study participants. For all the participants, 3 ml of blood was collected into a plain bottle and allowed to clot for 3 min, then centrifuged for 10 min. The serum was separated and used for the estimation of serum calcium using the colorimetric method. Three milliliters of blood was collected into an ethylenediaminetetraacetic acid bottle for HbA1c estimation for the participants with DM. The blood samples were stored at a temperature of 2°C–8°C and analyzed within 24 h using an automated HbA1c machine(In2itTM (I) system). Fasting blood glucose for the controls was done to exclude DM, and was estimated with the aid of Accu-Check Active® glucometers and test strips which uses the hexokinase method for glucose estimation, with results comparable to blood glucose concentrations in venous plasma as recommended by the International Federation of Clinical Chemistry and Laboratory Medicine.

Data collected from this study were analyzed using the SPSS statistical package version 22 (Chicago, IL, USA). Mean and standard deviation was used for the continuous variables. Chi-square test was used to compare the differences in the demographic characteristics of the study population. Independent *t*-test was used to compare the mean difference of serum calcium between the DM participants and healthy controls. Characteristics of patients with and without hypocalcemia were also compared, using Chi-square for categorical variables and Student's *t*-test for continuous variables. Pearson's correlation coefficient was used to correlate serum calcium level with HbA1c in participants with T2DM. In all, critical $P < 0.05$ was regarded as significant, and conclusions were drawn based on this level of significance.

Results

The mean ages of both the T2DM participants and controls were comparable at 61.54 ± 8.70 years and 61.21 ± 9.08 years, respectively ($P = 0.91$). The gender distribution of both the DM participants and control were also similar. The DM group had 61 males and 89 females, while the control group had 65 males and 85 females. Other demographic characteristics are shown in Table 1.

As shown in Table 2, the mean serum calcium level in the DM participants was significantly lower when compared to nondiabetes control group ($P = 0.02$).

Clinical characteristics of the subjects with diabetes mellitus

A total of 94 (64%) patients had DM for >5 years. Treatment was done with one or more oral medication in 133 patients,

whereas 17 (11.3%) were on both insulin and oral drugs. There was no significant difference between the calcium levels of males (2.18 ± 0.16) and females (2.19 ± 0.18) with diabetes, $P = 0.13$. Other clinical characteristics of the T2DM participants with and without hypocalcemia are illustrated in Table 3.

The mean HbA1c for all the participants with T2DM was 7.09% ± 2.05% and 79 participants (52.7%) had good glycemic control using a cut-off HbA1c level of <7%. The T2DM participants who had hypocalcemia had a mean HbA1c of 7.49 ± 1.98, while those with normal calcium levels had a mean HbA1c of 6.90 ± 1.98. Subsequently, there was a significant negative correlation between serum calcium and HBA1c in participants with T2DM ($P = 0.001$), as shown in Table 4 and illustrated in Figure 1.

Discussion

The aim of the present study was to determine the baseline serum level of calcium in Nigerian adult participants with T2DM compared to controls in addition to correlating serum calcium levels with clinical characteristics and their HbA1c.

Table 1: Sociodemographic characteristics of the study subjects

Variable	DM subjects, *n* (%)	Control, *n* (%)	Chi square	*P*
Mean age (years)	61.54±8.70	61.21±9.08	0.01**	0.91
Age group(years)				
40-49	10 (6.7)	13 (8.7)	0.55	0.97
50-59	53 (35.3)	53 (35.3)		
60-69	62 (41.3)	58 (38.7)		
>70	25 (16.6)	26 (17.3)		
Gender				
Male	61 (40.7)	65 (43.3)	0.22	0.64
Female	89 (59.3)	85 (56.7)		
Marital status				
Married	132 (88.0)	139 (92.7)	6.14	0.11
Divorced	0 (0.0)	2 (1.3)		
Widowed	16 (10.7)	9 (6.0)		
Never married	2 (1.3)	0 (0.0)		
Occupation				
Trader	52 (34.7)	41 (27.3)	7.22	0.21
Farmer	28 (18.7)	32 (21.3)		
Professional	16 (10.7)	31 (20.7)		
Artisans	35 (23.3)	28 (18.7)		
Housewife	6 (4.0)	5 (3.3)		
Retired	13 (8.7)	13 (8.7)		

**t-test. DM: Diabetes mellitus

Table 2: Comparison of mean serum calcium levels in subjects with diabetes mellitus and controls

	DM subjects	Controls	*P*
Mean serum calcium	2.16±0.17	2.21±0.17	0.02*

*Significant values. DM: Diabetes mellitus

The age and gender of the DM participants and controls in our study were comparable, with no significant difference between the two groups.

The prevalence of T2DM has traditionally been known to be higher in older adults. However, increasing prevalence is now being noticed in young people. Indeed, the American diabetes association, in 2012, projected an increase in the diagnosis of T2DM in those less 20 years by up to 49%.[11] Luckily, this worrisome trend is yet to be seen in our environment as all the participants with T2DM were more than 40 years of age, with the majority of them being between 50 and 69 years.

Low-socioeconomic status has also been associated with T2DM due to various reasons;[12,13] therefore, it was not surprising that participants with T2DM had fewer professionals and more artisans when compared to the controls.

Table 3: Comparison of clinical characteristics of type-2 diabetes mellitus patients with and without hypocalcemia

Variable	Hypocalcemia, *n* (%)	Normal, *n* (%)	Chi square	*P*
Age (years)				
<65	33	65	0.130	0.718
≥65	16	36		
Gender				
Male	18	43	0.466	0.495
Female	31	58		
History of smoking				
Yes	1	8	2.023	0.155
No	48	93		
History of alcohol use				
Yes	15	30	0.013	0.909
No	34	71		
History of hypertension				
Yes	37	83	0.917	0.338
No	12	18		
Duration of illness (years)				
≥5	31	63	0.11	0.916
<5	18	38		
Glycemic control (%)				
HbA1c ≥7	26	45	0.958	0.328
HbA1c <7	23	56		

HbA1c: Glycosylated hemoglobin

Table 4: Correlation between serum calcium levels and glycosylated hemoglobin in diabetes group

Serum calcium	HBA1c
Pearson coefficient	−0.273
P	0.001

HbA1c: Glycosylated hemoglobin

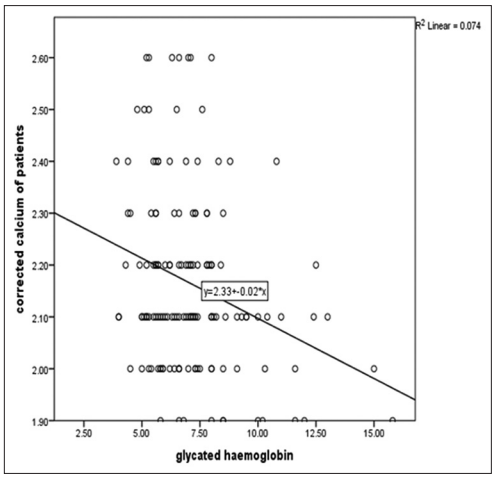

Figure 1: Scatter diagram showing a negative correlation between serum calcium and glycosylated hemoglobin in Type 2 diabetes mellitus subjects ($P = 0.001$)

There were more patients with long duration of diabetes (>5 years). This may have contributed to the relatively poor glycemic control in the DM subjects, as a longer duration of diabetes may result in declining beta-cell function and attendant poor glycemic control.

The results from this study showed that serum calcium levels were significantly reduced in participants with T2DM compared to normal subjects (2.16 ± 0.17 vs. 2.21 ± 0.17, respectively, $P = 0.02$). This decrease in serum calcium levels may be attributed to a number of factors. First, there is a heightened urinary loss of calcium as a result of hyperglycemia, which has been described by Sultan *et al*.[14] and this urinary calcium loss has been found to be proportional to the degree of glycosuria. In addition, volume depletion, which is common among T2DM subjects, may induce varying degrees of renal impairment, resulting in poor excretion of phosphorus and ultimately, high phosphate levels. The high levels of phosphorous then accumulate and avidly binds ionized calcium in the blood, resulting in hypocalcemia.[15] Furthermore, overtime, there is a gradual reduction in the secretion of PTH, (an effect mediated by hypomagnesemia – commonly seen in patients with diabetes), which may subsequently contribute to the disruption in calcium homeostasis, with resultant hypocalcemia.[15] This finding resonates with reports by several researchers elsewhere, who also found a significant reduction in the mean serum calcium levels of their DM participants compared to non-DM subjects/controls[16-18] but differs from that reported by Yousif and Ahmed[19] who found no mean difference in the level of serum calcium between individuals with diabetes and normoglycemic participant. They attributed their finding to the relative availability of dietary calcium sources and partial control of hyperglycemia in their participants with DM, as studies have suggested that calcium optimization lessens the symptoms of DM and may even reduce the risk of developing T2DM in adults.[15,20] Arun *et al*.[21] also did not find any difference in serum calcium levels between T2DM participants with hyperglycemia and those with normoglycemia. However, it must be stressed that all their participants had T2DM already and were then classified using HbA1c cutoff level of 6.5%. Another study by Abubaker and Mohammed[22] in Sudan did not show any significant difference between mean serum calcium level in participants with DM and control.

From our results, only a fraction above half (52.7%) of the T2DM participants had good long-term glycemic control, as evidenced by an HbA1c <7%.[23] The T2DM participants who had hypocalcemia, had a mean HbA1c of 7.49 ± 1.98 while those with normal calcium levels had a mean HbA1c of 6.90 ± 1.98. These figures eventually revealed a negative correlation between serum calcium and HbA1c in participants with T2DM ($r = -0.273$, $P \leq 0.01$), suggesting that low calcium levels had a deleterious effect on long-term glycemic control. This is not surprising, as cellular calcium signaling, strongly regulated by the Vitamin D precursor 1, $25(OH)_2D_3$, has been shown to drive cellular responses and secretion in several cell types, including pancreatic secretory cells.[15] In addition, sustained calcium signaling is linked with the regulation of apoptosis in T2DM and other diseases.[24,25] Although various studies report conflicting results in the relationship between serum calcium levels and blood glucose levels, a relatively large prospective study suggests that a daily calcium intake of >1200 mg in combination with appropriate amounts of Vitamin D, was associated with a 33% risk reduction of T2DM, compared with a daily intake of <600 mg of calcium.[20] This result is in agreement with some earlier reports elsewhere.[16,17,26] In contrast, Yamaguchi *et al*.[27] showed a positive correlation between serum calcium and fasting plasma glucose in men ($P \leq 0.5$) but not in women. It is not clear why their finding was only positive for men. HbA1c is known to reflect the average glycemia over a 3-month period, and fasting plasma glucose only gives the current glycemic level at the time of measurement. The fact that Yamaguchi *et al*.[27] used fasting plasma glucose in their study may also account for the difference found in their result because fasting plasma glucose does not represent effectively, long-term control of glycemia.

In the study, the presence of hypocalcemia was not significantly related to older age, longer duration of diabetes, or the presence of hypertension in the participants with diabetes. This suggests that the effect of hypocalcemia on HbA1c levels found in this study is largely due to the hypocalcemia *per se* and unrelated to these other factors.

Conclusion

This study found that in participants with T2DM, serum calcium levels were lower compared to participants without diabetes. There was also a significant negative correlation between serum calcium and HbA1c. Screening for serum calcium levels may need to be done more frequently, especially in those with persistent or recalcitrant hyperglycemia, with a view to correcting the low calcium level, if present, as this may ultimately translate to better glycemic control.

The cross-sectional nature of the study limits our significant findings to mere associations as causality cannot be claimed from our results. In addition, point of care glucometers and HbA1c testing machines were used to assess fasting blood sugar in the controls and long-term glycemic control in participants with diabetes, respectively, using finger-prick blood samples. However, results obtained from them have been shown to have a fairly good correlation to results obtained in the laboratories, using venous plasma.[28,29]

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Conflicts of interest

There are no conflicts of interest.

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