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Research Article

Assessment of HbA1c %, Serum Calcium, Magnesium Levels in Egyptian (Cairo gov.) Diabetic Type 2 Patients

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Abstract

Introduction: Patients with type 2 diabetes have been shown to have an elevated prevalence of hypo- calcemia/ hypomagnesemia.

Aim: To evaluate the correlation of serum calcium and magnesium with controlled and uncontrolled type 2 diabetes mellitus.

Subjects and methods: This case-control study was conducted on 160 adult type 2 diabetic patients (males and females) and 80 matched normal people. Glycosylated hemoglobin (HbA1c), calcium (Ca) and magnesium (Mg) were measured in all subjects.

Results: Patients with uncontrolled type 2 diabetes have higher risk of being hypocalcemia and/ or hypomagnesemia.

Conclusion: Monitoring Ca and Mg levels and managing diabetes effectively are crucial to prevent complications associated with Ca and Mg imbalances.

Introduction

Diabetes mellitus (DM) is one of most prevalent non-communicable diseases and it represents a major endemic problem all over the world. T2DM has been recognized as a leading cause of morbidity and mortality [1]. Hyperglycemia is the main feature of diabetes mellitus due to absolute (type1) or relative deficiency and/or impaired insulin leading to impaired metabolism of macro and micro nutrients [2].

Trace elements as magnesium and calcium are a cofactor for various enzymes in carbohydrate metabolism [3]. Calcium (Ca) an essential mineral play role in bone and tooth mineralization; cellular signaling transduction and insulin secretion and glucose homeostasis [4-6]. Abnormal Ca homeostasis could potentially be involved with defects in insulin action and disorders in glucose homeostasis, contributing to Type 2 DM development [4,7,8].

Diabetic patients, especially poorly controlled type 2 diabetes [having higher glycosylated hemoglobin (HbA1c)] might be at higher risk of hypocalcemia and/ or hypomagnesimia (low serum Ca, Mg levels) compared to those with controlled diabetes [4-6]. Hyypocalcemia may be due to impaired intestinal calcium absorption (reduction in calcium uptake), increased urinary excretion, and changes in bone metabolism. Hypomagnesemia (low magnesium) can also contribute to hypocalcemia in diabetics and might have some effect in the development of diabetic complications with other risk factors [4-6].

Magnesium (Mg) has many roles as regulating calcium levels, preventing the onset of diabetes and cardiovascular diseases, antioxidant functions [9]. Low level of serum magnesium has been correlated with the presence of oxidative stress and magnesium supplements might cause a reduction in Reactive Oxygen Species (ROS) [9,10]. Magnesium may

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influence the regulation of insulin signaling, phosphorylation of the insulin receptor kinase, and insulin's function in cellular glucose uptake [11,12].

Material and methods

This is a case-control study

Sample size calculation: A power analysis was used to determine our sample size based for assessing the associations between serum calcium, magnesium, and their Ca/Mg ratio. A confidence level of 95% was selected for the sample size calculation, with a population proportion of 90%, and a significance level of 0.05. Following these parameters, the analysis indicated that a minimum of 139 participants was necessary to achieve reliable results.

A total of one hundred and sixty (160: 40 male, 40 female uncontrolled diabetic; and 40 male, 40 female controlled diabetic) and 80 apparently, healthy individuals as controls matched in age and sex were enrolled in this study. There are sufficient safeguards to protect participants' confidentiality and privacy. The study protocol conformed to the Helsinki Declaration, the ethical norm of the World Medical Association for human testing. Definition of uncontrolled or controlled glycemia were done according to American Diabetes Association (ADA) 2021 [14] based on HbA1c level where patients with HbA1c < 7.0 considered as patients with good glycemic control while patients with HbA1c > 7.0 considered as patients with poor uncontrolled glycemic control. A written consent from every patient was taken.

Inclusion criteria: Egyptian patients with type 2`DM were included; Age range: 40-55 years; Taking oral drugs. Absence of additional disease diagnosis (metabolic or liver diseases, Type 1 DM, hyperparathyroidism, cancer, etc.), Duration of diabetes > 5 years.

Exclusion criteria: Patients with hypertension, renal disease and heart disease were excluded. Patients taking insulin injection, Pregnant and/or lactating women were excluded. Patients taking mineral as Ca, Mg supplements.

Blood sample and analysis: Fasting (6-8 hr) blood samples was withdrawn from all participant during 2024 year. The estimation of the parameters was carried out within 4-6 hrs. The samples were analyzed for serum calcium and magnesium by using appropriate kits. Glucose was determined using accorging to Barham and Trinder 1972 [15]. HbA1c was determined using Chemica Diagnostica kits accorging to Little and Rohlfing 2013 [16]. Ca was measured using BIOLABO Kits, France, according to Tietz 1999 (Calcium Arseno III Method) [17]; Mg was measured using Atlas Medical kits, Germany, according to Burtis, et al. 1999; and Tietz, et al. 1995 [18,19]. Unicauum spectrophotometer was used [16].

Quality controls: The precision and accuracy of all methods used in this study were checked using suitable control sera kits (Human multisera normal/upnormal using linear kits, spain).

Statistical analysis: Data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The following tests were used: one-way analysis of variance (ANOVA), followed by post hoc Turkey's test, and Pearson's correlation coefficient (r) test. *p* value of ≤ 0.05 was considered significant.

Results and discussion

Table 1 and Figures 1,2 represents the effect of type 2 DM on serum Ca, Mg levels and the HbA1c% in one hundred and sixty (40 male, 40 female uncontrolled diabetic; and 40 male, 40 female controlled diabetic) Egyptian patients compared to their matched control.

From the analysis of the data in the present work (Table 1, Figures 1,2), it was found that patients with uncontrolled diabetes mellitus showed an increased ratio of HbA1c and decreased levels of serum calcium, magnesium in contrast to non-diabetic subjects and normal healthy subjects (Table 1, Figures 1,2). In the present study, the mean HbA1c level in uncontrolled diabetic patients was (9.06 ± 0.42, 8.89 ± 0.40; 8.97 ± 0.42 %) respectively and it was significantly different when compared with respective groups of normal healthy subjects or controlled diabetic groups. Also, the mean serum calcium level in uncontrolled diabetic patients was (7.54 ±

Table 1: Comparison of the laboratory data between normal subjects and diabetics type 2 with poor and good glycemic control.

		Glucose mg/100 ml	HbA1c%	Mg mg/100 ml	Ca mg/100 ml	Ca/Mg
M C	Mean ± SD	88.38 ± 4.57^{c}	6.20 ± 0.21^{e}	2.42 ± 0.19^{b}	9.38 ± 0.52^{a}	3.89 ± 0.35^{d}
F C	Mean ± SD	86.34 ± 6.36^{c}	6.02 ± 0.29^{f}	2.47 ± 0.11^{a}	9.17 ± 0.56^{c}	3.73 ± 0.31^{e,}
M+F C	Mean ± SD	87.36 ± 5.60^{c}	6.11 ± 0.27^{e,f}	2.44 ± 0.16^{a,b}	9.27 ± 0.55^{b}	3.81 ± 0.34^{d,e}
M UCD	Mean ± SD	256.31 ± 24.30^{a}	9.06 ± 0.42^{a}	1.53 ± 0.10^{d}	7.54 ± 0.46^{f}	4.94 ± 0.39^{a}
F UCD	Mean ± SD	260.86 ± 25.51^{a}	8.89 ± 0.40^{b}	1.54 ± 0.11^{d}	7.49 ± 0.36^{f}	4.87 ± 0.37^{a}
M+F UCD	Mean ± SD	258.58 ± 24.86^{a}	8.97 ± 0.42^{b}	1.54 ± 0.11^{d}	7.51 ± 0.41^{f}	4.90 ± 0.38^{a}
M D	Mean ± SD	123.41 ± 8.54^{b}	6.53 ± 0.24^{c}	1.96 ± 0.10^{c}	9.00 ± 0.29^{d}	4.61 ± 0.21^{b}
F D	Mean ± SD	121.22 ± 7.65^{b}	6.35 ± 0.21^{d}	1.98 ± 0.08^{c}	8.86 ± 0.36^{e}	4.49 ± 0.22^{c}
M+F D	Mean ± SD	122.31 ± 8.13^{b}	6.44 ± 0.24^{d}	1.97 ± 0.09^{c}	8.93 ± 0.33^{e}	4.55 ± 0.22^{b,c}

MC: male control; FC: female control; M+F C: both male and female control (total); M UCD: male uncontrolled diabetic; FC: female uncontrolled diabetic; M+F C: both male and female uncontrolled diabetic; M CD: male controlled diabetic; FC: female controlled diabetic; M+F C: both male and female controlled diabetic;
Columns containing different upper lower letters are significant at $p < 0.001$

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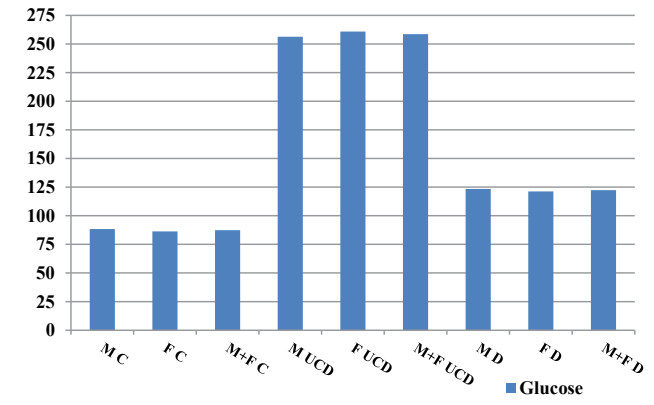

Figure 1: Comparison of the laboratory data between normal subjects and diabetics type 2 with poor and good glycemic control for glucose level (mg/dl).

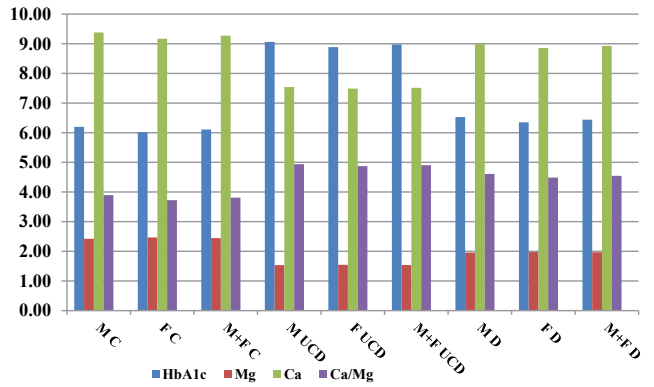

Figure 2: Comparison of the laboratory data between normal subjects and diabetics type 2 with poor and good glycemic control for HbA1c%, Ca, Mg (mg/dl) and Ca/Mg ratio.

0.46, 7.49 ± 0.36; 7.51 ± 0.41 mg/dl) for male, female, total male and female(total) groups respectively and it was significantly different when compared with respective groups of normal healthy subjects or controlled diabetic groups. The same was for Mg level. The mean serum Mg level in uncontrolled diabetic patients was (1.53 ± 0.10, 1.54 ± 0.11; 1.54 ± 0.11 mg/dl) for male, female, total male and female (total) groups respectively and it was significantly different when compared with respective groups of normal healthy subjects or controlled diabetic groups. Also controlled diabetic groups reveal significant decrease in serum Mg, Ca when compared with normal healthy groups but percent decrease of controlled groups (around 19, 3.8 %) is less than uncontrolled groups (around 37.0, 20.0 %) from normal healthy groups (Table 2).

Our results agree with Puri 2013 [20]; and Khalil 2016 [21]; Vijayalakshmi, et al. 2016 [22]; Safaa, et al. 2016 [23]; and Marshnil, et al. 2018 [24] whom found a significant reduction in the mean serum calcium levels of their DM participants compared to non-DM subjects/controls.

The role of calcium in the etio-pathogenesis of diabetes is multifactorial. Calcium is important for insulin mediated intracellular processes. Calcium is necessary for insulin receptor phosphorylation and proper signal transduction and thus optimal GLUT-4 transporter activity [4,7]. Relation between diabetes mellitus and Ca are inconsistent and has diverse contradictory results. Our results for serum Ca level in uncontrolled diabetic patients disagree with Suh, et al. 2017 [25]; Becerra-Tomás, et al. 2014 [4]; Rooney et al. 2016 [5]; Lorenzo, et al. 2014 [26]; Yamaguchi, et al. 2011 [27] where they stated that Ca concentration may be directly associated with the risk of developing Type 2 DM; It also disagree with Kim, et al. 2014 [28]; and Zaccardi, et al. 2015 [7] where they reported the null correlation between circulating Ca and Type 2 DM. These diverse contradictory results might be due to several factors as different method for Ca determination and multiple negative feedbacks are involved in circulating Ca homeostasis regulation. Shalileh, et al. 2010 [29]; and Mitri, et al. 2011 [30] reported that Ca supplementation has no significant effect on insulin resistance in obese adults, on insulin secretion or sensitivity and on glycaemia in adults at risk of Type 2 DM.

The decrease in serum calcium levels may be attributed to a number of factors as urinary loss of Ca due to hyperglycemia, poor excretion of phosphorus leading to accumulation of phosphorus in blood then P binds with ionized blood Ca leading to hypocalcemia [31]. A reduction in PHT secretion as a result of hypomagnesemia may lead to disruption in calcium homeostasis i.e. hypocalcemia [31].

Our results agree with Misirlioğlu, et al. 2020 [32,33] where they found that HbA1c levels were significantly higher in the hypomagnesemic group (poor controlled) than in the normomagnesemic group. Our results came in consistency with Khanna, et al. 2020 [34]; k Bhoi B, mukeSh 2020 who found that serum magnesium levels in uncontrolled diabetics were significantly lower than controlled diabetics or normal controls. The average serum magnesium levels were 1.53, 1.72, 1.89 mg/dl for cases (uncontrolled, controlled diabetics and normal controls, respectively). Wahid, et al. 2017 [35]; Winzer, et al. 2019 [36] and found that hypomagnesemia was more frequent in people with type 2 diabetes,

Results represented in Table 1, Figures 1,2 show that low serum Mg levels and high Ca/Mg ratio are associated with an increased risk of poor glycemic control in patients with Type 2 DM. Rosanoff and Rising 2010 [37] stated that it is better to evaluate Ca/Mg ratio than Ca, Mg alone. Shah, et al. 2021 [38] stated that high Ca/Mg ratio is associated with an increased risk of Type 2 DM in the population,while Huang, et al. 2014 found that Ca/Mg ratio between 2.0 and 2.5 and adequate Mg intake in diabetic elderly patients are important for reducing the risk of cardiovascular disease.

Table 2: Percent % decrease from normal healthy control groups.

% decrease from normal	Mg	Ca
M UCD	-36.72	-19.6
F UCD	-37.44	-18.35
M+F UCD	-37.08	-18.98
M D	-19.24	-4.08
F D	-19.91	-3.43
M+F D	-19.58	-3.76

M UCD: male uncontrolled diabetic; FC: female uncontrolled diabetic; M+F C: both male and female uncontrolled diabetic; M CD: male controlled diabetic; FC: female controlled diabetic; M+F C: both male and female controlled diabetic;

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Our results agree with Kamile and Ali 2023 [39] where they found that Ca/Mg ratio is significantly higher in poor uncontrolled type 2 diabetic patients.

Results of Table 3 reveal a significant negative correlation between serum calcium and HbA1c in participants with Type 2 DM (r = -0.42, -0.42, -0.40; $p \leq 0.001$), for male, female, total male and female uncontrolled diabetic groups was found suggesting that low calcium levels had a deleterious effect on long-term glycemic control. Cellular calcium signaling is regulated by the Vitamin D precursor 1, $25(OH)_2D_3$ which drive secretion in several cell types as pancreatic secretory cells [31]. Also calcium signaling is linked with the regulation of apoptosis in Type 2 DM [37]. Our result (Table 3) are in agreement with Safaa, et al. 2016 [23]; and Marshnil &Mythili 2018 [24]; and Harriet, et al. 2020 [41] where they found a significant negative correlation between Ca and HbA1c and disagree with Yamaguchi, et al. 2011 [27] where they found a positive correlation between serum calcium and fasting plasma glucose in men ($p \leq 0.5$) but not in women without explanation. It is known that fasting glucose does not reflect control of glycemia (giving glucose level at time o measurement) opposite to HbA1c which reflect the average glycemia over a 3-month period.

Conclusion

Regular monitoring of serum calcium and magnesium levels is important for individuals with diabetes, especially those with poor blood sugar control. Early detection and management of Ca and Mg imbalances can help prevent complications associated with deficiencies or disruptions in Ca and Mg homeostasis.

Author contributions

All authors have accepted and shared responsibility for the entire content of this manuscript and approved its submission.

Informed consent

Informed consent was obtained from all individuals included in this study.

Table 3: Pearson Correlation between HbA1c level and Serum Magnesium and Calcium level in normal control and Type 2 DM (controlled and uncontrolled groups).

		Mg		Ca	
	n	r	P	r	P
M C	40	0.09	NS	0.18	NS
F C	40	0.13		0.13	
M+F C	80	0.04		0.1	
M UCD	40	-0.42	S	-0.42	S
			$p < 0.0001$		$p < 0.0001$
F UCD	40	-0.42		-0.42	
M+F UCD	80	-0.41		-0.4	
M D	40	0.11	NS	-0.07	NS
F D	40	-0.02		-0.13	
M+F D	80	0.01		-0.01	

MC: male control; FC: female control; M+F C: both male and female control (total); M UCD: male uncontrolled diabetic; FC: female uncontrolled diabetic; M+F C: both male and female uncontrolled diabetic; M CD: male controlled diabetic; FC: female controlled diabetic; M+F C: both male and female controlled diabetic; S: significant; NS: non-significant.

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