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Literature Review

Calcium Level in Diabetes Type Two Patients in Sudan

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Abstract

The current study is a cross-sectional study aimed at assessing calcium level in patients with type 2 diabetes in Khartoum State, conducted between January and March 2017. The sample size is 60, including both males and females, and calcium and glucose were analyzed using the spectrophotometer. The results showed a decrease in blood calcium levels for diabetics (probability value 0.000) we found that there was an inverse relationship between calcium level and (age and period of diabetes) value (0.000, 0.026) and its values (-0.437, -0.287) respectively the summary of this study that it is affected by age and period of diabetes.

Keywords: Calcium; Type 2 Diabetes; Glucose

Introduction

Diabetes mellitus is a global epidemic disease that affects more than 150 million people worldwide. It is estimated that global number of adults suffering from all forms of diabetes will reach 439 million in 2030; most of them type 2 diabetes mellitus cases. Diabetes mellitus is a major cause of morbidity and mortality. [1]. The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy, nephropathy, and neuropathy [2]. Diabetes mellitus is known to have its effect on almost all body systems causing various structural and biochemical changes in tissues. From this study it is hypothesized that alteration in calcium flux may adversely affect the insulin secretion as it is a calcium dependent process.

Over 99% of total body calcium is found in bones and teeth, where it functions as a key structural metabolism, serving as a signal for vital physiological processes, including vascular contraction, blood clotting, muscle contraction and nerve transmission. Inadequate intakes of Calcium have been associated with increased risk of osteoporosis, nephrolithiasis, insulin resistance and obesity. Most of these disorders have treatments but no cures. Calcium is unique among nutrients (WHO 2006).

Literature Review

Diabetes mellitus is actually a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action.

Classification of diabetes mellitus

- Type 1 diabetes is characterized by inappropriate hyperglycemia primarily a result of pancreatic islet cell destruction and a tendency to ketoacidosis.
- Type 2 diabetes mellitus is characterized by hyperglycemia as a result of an individual's resistance to insulin with an insulin secretory defect.
- Gestational has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy.
- Other specific types of diabetes are associated with certain conditions (secondary), including genetic defects of cell function or insulin action, pancreatic disease, diseases of endocrine origin, drug- or chemical-induced insulin receptor abnormalities, and certain genetic syndromes type 2 constitutes the majority of the diabetes cases. Most patients in this type are obese or have an increased percentage of body fat distribution in the abdominal region [4].

Complications

Include microvascular problems such as nephropathy, neuropathy and retinopathy. Increased heart disease is also found in patients with diabetes.

Acute and chronic complications

Acute:

- Diabetic ketoacidosis (DKA)
- Hyperglycemic hyperosmolar is Syndrome (HHS)
- Hypoglycemia
- Metformin associated lactic acidosis, MALT.

Chronic:

- Nephropathy
- Retinopathy
- Neuropathy
- Macrovascular diseases (CHD, peripheral vascular disease, stroke).

Calcium

Calcium is the fifth most common element in the body and the most prevalent cation the skeleton contains 99% of the body calcium predominantly as extracellular crystals of unknown structure with a composition approaching that of hydroxyapatite.

Biochemistry and physiology

In blood virtually of the calcium is in the plasma which has a mean normal calcium concentration of approximately 9.5 mgL. Calcium exists in three physiochemical states in plasma with approximately 50% free 40% bound to plasma proteins primarily albumin and 10% complexed with small anions.

The free calcium fraction is the biologically active form Its concentration in plasma is tightly regulated by the calcium regulating hormone PTH and vitamin D.

Intercellular calcium has key role in many important physiological function including muscle contraction, hormone secretion, glycogen metabolism, and cell division.

Causes of hypocalcemia

Primary hypoparathyroidism glandular aplasia, destruction, or removal Hypomagnesemia Hypermagnesemia Hypoalbuminemia (total calcium only, ionized not affected by) chronic liver disease, nephrotic syndrome, malnutrition Acute pancreatitis, Vitamin D deficiency, Renal disease, Rhabdomyolysis, Pseudohypoparathyroidism.

Causes of hypercalcemia

Primary hyperparathyroidism adenoma or glandular hyperplasia hyperthyroidism benign familial hypocalciuria malignancy.

Multiple myeloma, increased vitamin D, thiazide diuretics, prolonged immobilization [5].

Calcium effect on insulin secretion

Alterations in calcium flux can have adverse effects on insulin secretion, a calcium-dependent process Calcium repletion alone normalized glucose tolerance and insulin secretion in vitamin D-depleted rats in people without diabetes, hypocalcaemia is associated with impairment of insulin release.

Calcium effect on insulin action

Calcium is essential for insulin-mediated intracellular processes in insulin responsive tissues such as skeletal muscle and adipose tissue with a very narrow range of Ca^{2+} needed for optimal insulin-mediated functions Changes in Ca^{2+} in primary insulin target tissues contributes to alterations in insulin action.

Impairment of insulin receptor phosphorylation, a calcium dependent process leading to impaired insulin signal transduction and decreased glucose transporter activity Changes in Ca^{2+} modulate adipocyte metabolism, which may promote triglyceride accumulation via increased de novo lipogenesis and inability to suppress insulin-mediated lipolysis leading to fat accumulation Patients with type2 DM exhibit impaired cellular calcium homeostasis including defects in skeletal muscle, adipocytes, and liver [6].

Glucose

Glucose is transported into the beta cell by type2 glucose transporters (GLUT2). Once inside, the first step in glucose metabolism is the phosphorylation of glucose to produce glucose-6-phosphate. This step is catalyzed by hexokinase; it is the rate limiting step in glycolysis, and it effectively traps glucose inside the cell. As glucose metabolism proceeds, ATP is produced in the mitochondria. The increase in the ATP: ADP ratio closes ATP-gated potassium channels in the beta cell membrane. Positively charged potassium ions (K^+) are now prevented from leaving the beta cell. The rise in positive charge inside the beta cell causes depolarization. Voltage-gated calcium channels open allowing calcium ions (Ca^{2+}) to flood into the cell. The increase in intracellular calcium concentration triggers the secretion of insulin via exocytosis [7].

The type 2 ryanodine receptor (RyR2) is a Ca^{2+} release channel on the endoplasmic reticulum (ER) of several types of cells, including cardiomyocytes and pancreatic β cells. In cardiomyocytes, RyR2-dependent Ca^{2+} release is critical for excitation-contraction coupling; however, a functional role for RyR2 in β cell insulin secretion and diabetes mellitus remains controversial [8].

Rationale

Calcium ion plays an important role in glycemic Control by affecting the biosynthesis and release of insulin from the beta cell of the pancreas. Calcium repletion alone normalized glucose tolerance and insulin secretion. In people without diabetes, hypocalcemia is associated with impairment of insulin release so the estimation of calcium is important to monitor insulin secretion.

Objectives of the Study

General objective: To estimate serum calcium in Sudanese type2 diabetes mellitus.

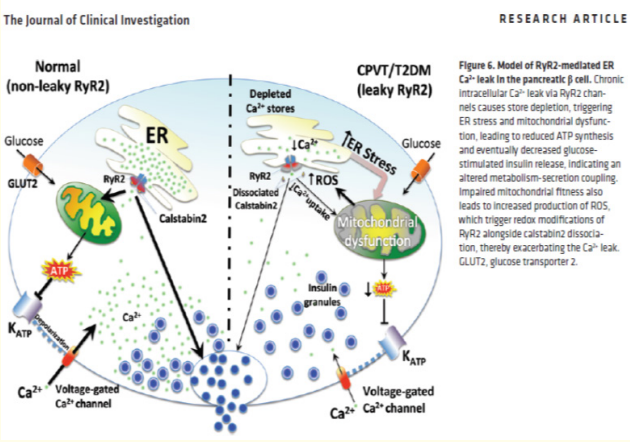

Figure: Model of RyR2-mediated ER Ca2+ leak in the pancreatic β cell. Chronic intracellular Ca2+ leak via RyR2 channels causes store depletion, triggering ER stress and mitochondrial dysfunction, leading to reduced ATP synthesis and eventually decreased glucose-stimulated insulin release, indicating an altered metabolism-secretion coupling. Impaired mitochondrial fitness also leads to increased production of ROS, which trigger redox modifications of RyR2 alongside calstabin2 dissociation, thereby exacerbating the Ca2+ leak. GLUT2 glucose transporter 2.

Specific objective:

1. To measure blood glucose levels in type 2 diabetes patients.
2. To measure plasma calcium levels in type 2 diabetes patients.
3. To correlate between the plasma (calcium and glucose) levels and duration of disease, age, sex.

Materials and Methods

Materials

Study design: Cross-sectional study design.

Study area: The study was carried in Khartoum state.

Study duration: The study was conducted in the period from January - March 2017.

Inclusion criteria: All Male and female type2 Diabetic patients.

Exclusion criteria: Type2 diabetes mellitus with (renal disease, thyroid disease, bone disease).

Sample collection: About 4 ml of blood was collected from each patient in heparinized and fluoride oxalate containers for measurement of calcium and glucose respectively. The plasma was obtained after centrifugation at 3000 - 4000 rpm, then collected in plain containers and stored at -20°C till used for measurements of calcium and glucose.

Methods

Calcium-MTB

Principle of the method

Calcium in the sample reacts with methyl thymol blue in alkaline medium forming a coloured complex tha`t can be measured by spectrophotometry.

Hydroxyquinoline is included in the reagent to avoid magnesium interference 1, 2.

Contents

	COD 11527	COD 11507
A. Reagent	2 x 50 mL	1 x 250 mL
B. Reagent	2 x 50 mL	1 x 250 mL
S. Standard	1 x 5 mL	1 x 5 mL

Table A

Composition

A. Reagent: Potassium cyanide 7.7 mmol/L, ethanolamine 1.5 mol/L.

B. Reagent: Methyl thymol blue 0.1 mmol/L, hydrochloric acid 10 mmol/L, hydroxyquinoline 17 mmol/L.

S. Calcium/Magnesium Standard: Calcium 10 mg/dL (2.5 mmol/L), magnesium 2 mg/dL.

Aqueous primary standard.

Storage: Store at 15 - 30ºC.

Working reagent: Mix equal volumes of Reagent A and Reagent B (Note 1). Mix gently. Stable for 2 days at 2 - 8ºC.

Procedure

1. Pipette into labelled test tubes: (Notes 1, 2).
2. Mix thoroughly and let stand the tubes for 2 minutes at room temperature.
3. Read the absorbance (A) of the Standard and the Sample at 610 nm against the Blank. The colour is stable for at least 1 hour.

Calculations: The calculation concentration in the sample is calculated using the following general formula:

$$\frac{A\ sample}{A\ standard} \text{ x C Standard x Sample dilution factor = C Sample.}$$

	Blank	Standard	Sample
Calcium Standard (S)	–	10 µL	–
Sample	–	–	10 µL
Working Reagent	1.0 mL	1.0 mL	1. 0 mL

Table B

If the Calcium Standard provided has been used to calibrate (Note 3).

Serum and plasma	Urine
x 10 = mg/dL calcium	x 20 = mg/dL calcium
x 2.5 = mmol/L calcium	x5=mmol/L calcium

Table C

Reference values

Serum and plasma: 8.6 - 10.3 mg/dL = 2.15 - 2.58 mmol/L.

Method of glucose

Glucose oxidase/peroxidase

Principle of the method: Glucose in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry [1].

$$\text{Glucose} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{Guconate} + H_2O$$

$$2\,H_2O_2 + 4\text{ - Amino antipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O.$$

Contents

	COD 11803	COD 11503	COD 11504	COD 11538
A. Reagent	1 x 50 mL	1 x 200 Ml	1 x 500 mL	1 x 1L
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 Ml	1 x 5 mL

Table D

Composition

A. Reagent: Phosphate 100 mmol/L, phenol 5 mmol/L, glucose oxidase > 10 U/mL, peroxidase > 1 U/mL, 4-aminoantipyrine 0.4 mmol/L, pH 7.5.

S. Glucose/Urea/Creatinine Standard: Glucose 100 mg/dL (5.55 mmol/L), urea 50 mg/dL, creatinine 2 mg/dL. Aqueous primary standard.

Storage: Store at 2-8ºC.

Reagent preparation: Reagent and Standard are provided ready to use.

Samples

Serum or plasma collected by standard procedures. Serum or plasma must be separated from the red cells promptly to prevent glycolysis. The addition of sodium fluoride to the blood sample prevent glycolysis. Glucose in serum or plasma is stable for 5 days at 2 - 8ºC. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

Procedure

1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes: (Note 1).
3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16 - 25ºC) or for 5 minutes at 37ºC.
4. Measure the absorbance (A) of the Standard and the Sample at 500 nm against the Blank.

	Blank	Standard	Sample
Glucose Standard (S)	_	10 µL	_
Sample	_	_	10 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

Table E

The colour is stable for at least 2 hours.

Calculations: The glucose concentration in the sample is calculated using the following general formula:

$$\frac{A\ sample}{A\ standard} \text{ x CStandard= CSample}$$

If the Glucose Standard provided has been used to calibrate (Note 2).

	x 100 = mg/dL glucose
	x 5.55 = mmol/L glucose

Table F

Reference values

Serum and plasma [2]

Newborn, premature	25 - 80 mg/dL = 1.39 - 4.44 mmol/L
Newborn, term	30 - 90 mg/dL = 1.67 - 5.00 mmol/L
Children, adult	70 - 105 mg/dL = 3.89 - 5.83 mmol/L

Table G

Results

This was cross sectional study which conducted in total of 60 type 2 diabetic mellitus patients in Khartoum state.

Figure 1 represented percentage distribution of gender 52% of patients were males and 48% were females.

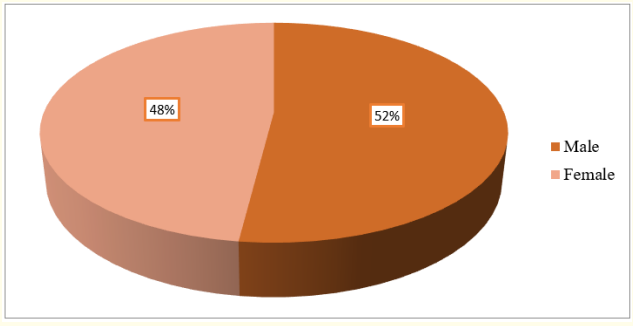

Figure 1: Percentage distribution of gender.

Table 1 represented Characteristics of type 2 DM patients (age, duration, RBG, calcium), respectively.

Variables	Minimum	Maximum	Mean ± SD	Mean of ref. value	p.value
Age (Years)	20.00	86.00	53.9 ± 14.6	-	-
Duration (60)	1.00	20.00	7.5 ± 5.5	-	-
RBG (60)	98.00	575.00	273.4 ± 126.0	170 (140 - 200)	0.000
Calcium (60)	4.00	10.00	7.54 ± 1.21	9.45 (8.6 - 1.3)	0.000

Table 1: General demographic statistics of diabetic patients.

Figure 2 represented correlation between mean concentration levels of calcium among diabetic patients and normal range of calcium.

Correlation between mean levels of calcium among diabetic patient and (duration, age) when calculated showed in both figures as follows.

Figure 3 shows inverse correlation between duration and calcium level among DM patients.

Figure 4 shows inverse correlation between age and calcium level among DM patients.

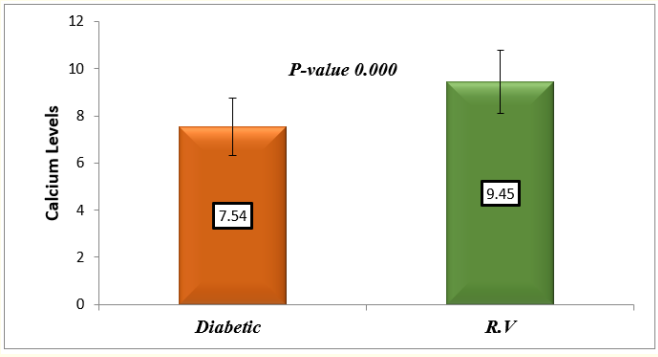

Figure 2: Mean concentration level of calcium among diabetic patients.

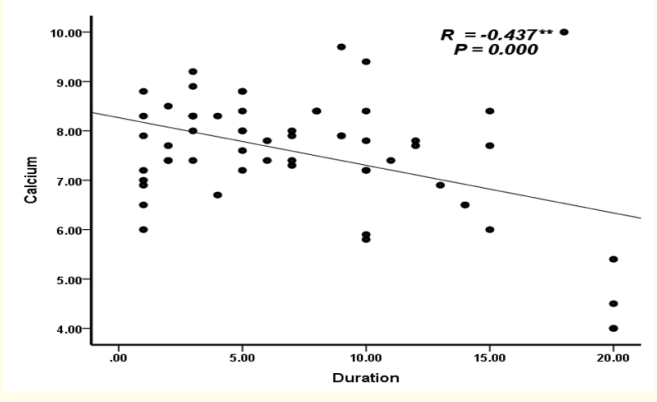

Figure 3: Correlation between duration of diabetic and calcium levels.

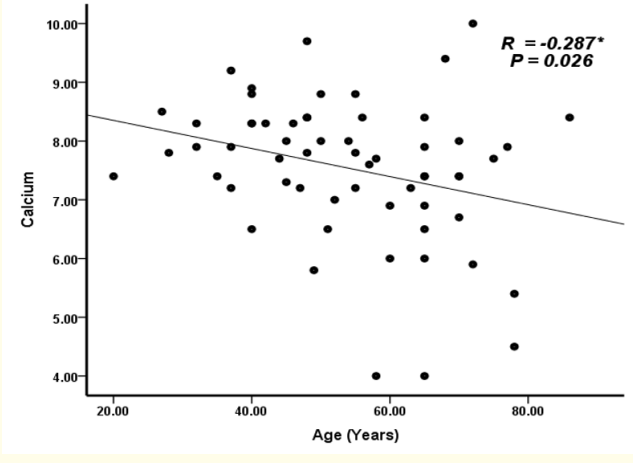

Figure 4: Correlation between age of diabetic patient and calcium levels.

Discussion

This is a cross-sectional study conducted in Khartoum state. Sixty type 2 DM patients were enrolled in both sex in the period from January 2017 - March 2017. Blood Sample was collected from each individuals from both Khartoum bahri Teaching Hospital and Ibrahim Malik Teaching Hospital. The age range of patients was (20 - 86 years). In this study the males percentage was 52% while females represent 48% show in figure 1. Similar result findings was shown in previous research Snafal., *et al* [9].

This cross sectional study of DM type 2 characteristic age-duration-random were shown on the mean of RBG was 273.40 ± 126.08 and mean of duration was 7.53 ± 5.52 and mean of age was 53.92 ± 14.64 and mean of calcium was 7.54 ± 1.21 show in table 1. The mean level of calcium in DM patients was 7.54 mg/dl when compared with mean calcium among healthy individuals level 9.45 mg/dl. Revealed a significant decreased among DM patients, p-value (0.000) show in figure 2 that agrees to findings in past research study reported by Shatha R., *et al.* in Iraq [10]. Pearson's correlation study when applied between calcium and (duration, age) of DM patients, revealed an inverse correlation P-value (0.000, 0.026) R-value (0.437, 0.287), respectively (Figure 3 and 4).

Conclusion

Calcium level was reduced among diabetic patients, as well as correlation study revealed inverse correlation between calcium (duration, age).

Recommendation

- Regular checkup calcium for diabetic patient.
- Calcium supplementation should inter to treat management and help in better glycemic control for patients.
- For further study take large sample size.

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