Serum Calcium Level in Type 2 Diabetes Mellitus in Khartoum State

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Abstract

The present work is a cross-sectional study aimed to evaluate the serum levels of calcium in patients with type 2 diabetes mellitus, in Khartoum state. This study was done during the period of January to March 2017. The sample size is sixty including both male and female. Calcium and Glucose were measured by Spectrophotometer (Bio-system-BTS310). Data were analyzed using SPSS. The results showed a significant reduction in the mean serum calcium level of diabetic patients (p-value-0.000). However there was an inverse correlation between the serum calcium and (duration, age) of diabetes mellitus, p-value was (0.000, 0.026) R-value (-0.437, -0.287), respectively. This study is effect by age and duration of diabetes in the studied group.

Keywords: Type 2 diabetes mellitus; Diabetic nephropathy; Progranulin

Introduction

Diabetes mellitus is a global epidemic disease that affects more than 150 million people worldwide. It is estimated that a 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 the 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 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 [3,4]

Complications

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

Acute:
• Diabetic ketoacidosis (DKA)
• Hyperglycemic hyperosmolar 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 mg/L. 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 a key role in many important physiological functions 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 myelomas, Increased vitamin D, Thiazide diuretics, Prolonged immobilization [5,6].

**Calcium effect on insulin secretion:** Alterations in calcium flux can have adverse effects on insulin secretion, calcium-dependent process Calcium depletion alone normalized glucose tolerance and insulin secretion in vitamin D-depleted rats In people without diabetes, hypocalcemia 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 type 2 DM exhibit impaired cellular calcium homeostasis including defects in skeletal muscle, adipocytes, and liver [7].

Glucose

It is transported into the beta cell by type 2 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 [8-10].

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 [11,12].

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 depletion 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**

**General objective**
- To estimate serum calcium in Sudanese type 2 diabetes mellitus

**Specific objective**
- To measure blood glucose levels in type 2 diabetes patients
- To measure plasma calcium levels in type 2 diabetes patients
- To correlate between the plasma (calcium and glucose) levels and duration of disease, age, sex

**Materials and Methods**
- 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 type 2 diabetic patients.

Exclusion criteria: Type 2 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 to 4000 rpm, then collected in plain containers and stored at -20 till used for measurements of calcium and glucose.

Calcium-MTB

Calcium in the sample reacts with methylthymolblue in alkaline medium forming a colored complex that can be measured by spectrophotometry.

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

### Contents

**Table 1:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>COD 11527</th>
<th>COD 11507</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Reagent</td>
<td>2 × 50 mL</td>
<td>1 × 250 mL</td>
</tr>
<tr>
<td>B. Reagent</td>
<td>2 × 50 mL</td>
<td>1 × 250 mL</td>
</tr>
<tr>
<td>S. Standard</td>
<td>1 × 5 mL</td>
<td>1 × 5 mL</td>
</tr>
</tbody>
</table>

**Table 2:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium standard(S)</td>
<td>–</td>
<td>10 µL</td>
<td>–</td>
</tr>
<tr>
<td>Sample</td>
<td>–</td>
<td>–</td>
<td>10 µL</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
</tr>
</tbody>
</table>

### Method of glucose reaction

**Glucose oxidase/peroxidase:** Glucose in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry (Table 4) [1].

\[
glucose + \frac{1}{2}O_2 + H_2O \xrightarrow{glucose oxidase} glutonate + H_2O + \text{Quinoneimine} + 4H_2O_2
\]

**Contents:**

**Table 4:**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>COD 11803</th>
<th>COD 11503</th>
<th>COD 11504</th>
<th>COD11538</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. reagent</td>
<td>1 × 50 mL</td>
<td>1 × 200 mL</td>
<td>1 × 500 mL</td>
<td>1 × 1 L</td>
</tr>
<tr>
<td>S. standard</td>
<td>1 × 5 mL</td>
<td>1 × 5 mL</td>
<td>1 × 5 mL</td>
<td>1 × 5 mL</td>
</tr>
</tbody>
</table>

**Calculations**

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

\[
\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} \times \text{Sample dilution factor} = C_{\text{Sample}}
\]

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

### Table 3: Calculations for calcium concentration.

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

### Table 4: Reagents used in the followed protocol.

**Samples Preparation:** 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 prevents glycolysis. Glucose in serum or plasma is stable for 5 days at 2°C to 8°C. Heparin, EDTA, oxalate, and fluoride may be used as anticoagulants.

### Procedure:

- Bring the Reagent to room temperature.

- Pipette into labeled test tubes (Tables 3 and 4).

- Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16°C to 25°C) or for 5 minutes at 37°C.

- Measure the absorbance (A) of the Standard and the Sample at 500 nm against the blank.

- The color is stable for at least 2 hours.

**Citation:** Hus Al, Tahleel B, Hasan AEI, Albagir EH, Mohammad MA, Salah S, Elmahdi SA (2019) Serum Calcium Level in Type 2 Diabetes Mellitus in Khartoum State. Clin Microbiol 8: 332. doi:10.4172/2327-5073.1000331
Table 5: Reagents used in the followed protocol during this research.

Calculations:

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

\[
\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = C_{\text{Sample}}
\]

If the Glucose Standard provided has been used to calibrate (Table 5 and 6).

\[
\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times 100 = \text{mg/dL glucose}
\]

\[
\times 5.55 = \text{mmol/L glucose}
\]

Table 6: Calculations for the glucose standard concentration.

Reference values: Serum and plasma (Table 7) [2].

<table>
<thead>
<tr>
<th>Group</th>
<th>Reference Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn, premature</td>
<td>25-80 mg/dL=1.39-4.44 mmol/L</td>
</tr>
<tr>
<td>Newborn, term</td>
<td>30-90 mg/dL=1.67-5.00 mmol/L</td>
</tr>
<tr>
<td>Children, adult</td>
<td>70-105 mg/dL=3.89-5.83 mmol/L</td>
</tr>
</tbody>
</table>

Table 7: Table explaining the reference value for serum and plasma [2].

Result

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

Figure 2: Represented percentage distribution of gender 52% of patients were males and 48% were females.

Figure 3: Represented correlation between mean concentration levels of calcium among diabetic patients and normal range of calcium.

Figure 4: Shows inverse correlation between duration and calcium level among DM patients.
Figure 5: Shows inverse correlation between age and calcium level among DM patients.

Table 8: Represented characteristics of type 2 DM patients (age, duration, RBG, calcium), respectively.

```
<table>
<thead>
<tr>
<th>Variables</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean ± SD</th>
<th>Mean of ref. value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>20</td>
<td>86</td>
<td>53.9 ± 14.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Duration (60)</td>
<td>1</td>
<td>20</td>
<td>7.5 ± 5.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RBG (60)</td>
<td>98</td>
<td>575</td>
<td>273.4 ± 126.0</td>
<td>170 (140-200)</td>
<td>0</td>
</tr>
<tr>
<td>Calcium(60)</td>
<td>4</td>
<td>10</td>
<td>7.54 ± 1.21</td>
<td>9.45 (8.6-1.3)</td>
<td>0</td>
</tr>
</tbody>
</table>
```

Discussion

This is a cross-sectional study conducted in Khartoum state. Sixty type 2 DM patients were enrolled in both sexes in the period from January 2017 to March 2017. Blood Sample was collected from each individual from both Khartoum Bahri Teaching Hospital and Ibrahim Malik Teaching Hospital. The age range of patients was (20-86 years). In this study, the male’s percentage was 52% while females represent 48% shown in Figure 1. Similar result findings were shown in previous research Snafal, et al. [13].

This cross-sectional studies 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 shown in Table 6 and Figure 2. 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) shown in Figure 3 that agrees to findings in past research study reported in Iraq [14-16]. 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 (Figures 4 and 5) (Table 8).

Conclusion

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

References