

## Estimation of serum calcitonin, phosphate, and calcium in type 2 diabetes mellitus

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### Abstract

**Background and objective:** Type two diabetes is known as insulin-independent diabetes mellitus due to body's inability to respond to insulin and can be managed by lifestyle change and hypoglycemic tablets. This study aimed to compare serum levels of calcitonin, calcium, and phosphate between type two diabetic patients and controls.

**Methods:** The cross-sectional study included 50 patients who have already been diagnosed with type two diabetes mellitus and 50 control subjects without clinical signs of any diseases. The level of serum calcitonin, calcium, and phosphate was estimated for all participants.

**Results:** The mean serum level of calcitonin was  $(2.35 \pm 1.18)$  pg/mL in the case group, while the mean was  $(32.91 \pm 3.88)$  pg/mL in the control group ( $P$  value = 0.001). The mean level of serum phosphate was  $(3.93 \pm 0.08)$  mg/dL and  $(3.73 \pm 0.09)$  mg/dL in the case and control groups, respectively ( $P$  value = 0.117). The mean serum calcium level was  $(9.68 \pm 0.10)$  mg/dL in the case group, and it was about  $(9.67 \pm 0.14)$  mg/dL ( $P$  value = 0.955) in healthy persons. Serum calcitonin was negatively correlated with glycated hemoglobin and serum glucose level.

**Conclusion:** The calcitonin serum level in diabetic patients was significantly lower than in healthy individuals. The level of bone minerals (calcium and phosphate) was not altered in type two diabetic patients when compared with control participants.

**Keywords:** Type two diabetes mellitus; Calcitonin; Calcium; Phosphate.

### Introduction

Type 2 diabetes mellitus (T2DM) is the most common chronic metabolic disorder around the world, is caused by a defect in the secretion of insulin by  $\beta$ -cells in the pancreas and the inability or insulin resistance.<sup>1</sup>

Globally, T2DM is one of the top five non-communicable chronic diseases and is also one of the major causes of morbidity and mortality.<sup>2</sup> Worldwide, around 90% of all patients diagnosed with diabetes mellitus are type two.<sup>3</sup>

Type two diabetes mellitus is caused by the complicated interaction between genetic, environmental, and behavior.<sup>4</sup> Obesity and physical inactivity are the main factors that

cause insulin resistance and then progress to type two diabetes.<sup>5</sup> Loss of weight, increased appetite, frequent urination, thirst, and weakness, are the common symptoms of D2TM.

Calcitonin (thyrocalcitonin) is a type of peptide hormone synthesized and secreted by c cells (parafollicular cells) in the thyroid gland.<sup>6</sup> It has played a significant role in maintaining and regulating calcium-phosphorus balance and metabolism.<sup>7</sup>

Increased concentration of calcium in the circulation stimulates CT secretion, which rapidly decreases calcium concentration by inhibiting osteoclast and elevated excretion of calcium from urine by kidneys.<sup>8,9</sup> It reduced the level of calcium also by

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inhibiting and limiting the absorption of calcium from the intestine to the blood vessel.<sup>10</sup> Indirectly in the bone, CT is promoting the production of bone matrix and mineralization.<sup>11</sup>

Calcium is one of the most important cationic minerals in the human body.<sup>12</sup> Approximately 99% of the calcium in the human body is deposited and stored in the teeth and bone.<sup>13</sup> While about 1% of  $\text{Ca}^{2+}$  is found in the soft tissues, extracellular fluid (ECF), and circulation system.<sup>14</sup>

Calcium has played an essential role in many physiological processes in the body. It is necessary for the building of teeth and bone, metabolic pathways in the cell, contraction process in the muscle, and nerve conduction.<sup>15</sup> Intracellular  $\text{Ca}^{2+}$  plays a critical role in proliferation (survival) and apoptosis (a program of cell death) of cancer cells as well as immune cells.<sup>16,17</sup>

After calcium mineral, phosphate is represented as the second most crucial mineral for the human body.<sup>18</sup> Vitamin D, Parathyroid Hormone (PTH), fibroblast growth factor 23 (FGF-23), and calcitonin have played a significant role in regulating phosphate homeostasis.<sup>19</sup> Phosphate is responsible for several physiological functions in the body such as the mineralization of bone, it is involved in the generation and metabolism of energy through ADP and ATP, acting as a structure in the production of DNA, plasma membrane, and cell signaling, and maintaining acid-base balance.<sup>20,21</sup>

It acts as an integral component in the composition of carbohydrates, fat, and protein.<sup>22</sup> Diabetes mellitus affects the function of calcitonin and parathyroid hormone.<sup>23</sup> In type two diabetes mellitus, the concentration of calcitonin is low.<sup>24</sup> Type two diabetes mellitus can affect electrolyte balances such as calcium, phosphate, magnesium, and potassium.<sup>25</sup> In diabetic patients, abnormal calcium homeostasis is related to impaired glucose metabolism.<sup>26</sup> A diabetic patient has a low intracellular concentration of phosphate and hyperphosphouria because the

intestinal brush border membrane is depolarized for reabsorption of inorganic phosphate by hyperglycemia and defect in homeostatic functions of the kidney.<sup>27</sup>

The aim of the study for estimate serum calcitonin, calcium, and phosphate in type two diabetic patients compared with healthy (control) participants.

## Methods

A cross-sectional study was carried out at Layla Qasim Center for diabetes mellitus in Erbil city and Hemn private laboratory for collecting both cases and controls samples, respectively, from October 2021 to January 2022. The individuals in the study included 100 participants (50 cases and 50 controls). Each group consisted of (32 females and 18 males) and also the age ranged from 40 to 69 in both groups. The case participants have already been diagnosed with type two diabetes mellitus by a specialized physician (endocrinologist).

A specially designed questionnaire was used for the study. The first part of the questionnaire contained questions regarding the demographic data (age, gender, marital status, occupation, and educational level). The second part contained questions to measure the height and weight for calculating Body Mass Index (BMI). The third part was related to lifestyle as smoking status, diet, and exercise questions. The fourth part of the questionnaire composed questions related to the profile of diabetes mellitus as duration, family history, monitoring, and control of T2DM.

## Collection of Specimen

Peripheral blood samples were collected by using standard phlebotomy procedures. Five ml of blood samples were drawn by using a disposable needle and then transferred to two tubes: an EDTA tube for estimation of HbA1c and a gel tube for investigating biochemical parameters (calcitonin, calcium, and phosphate). The gel tube was incubated at room temperature for 15 minutes, then the

serum was separated by centrifugation at 5000 rpm for six minutes. After that, the serum was transferred into Eppendorf tubes and stored at -20°C. The sandwich ELISA and Cobas C 311 were used for measuring the biochemical parameters.

#### Statistical Analysis

Data were analyzed with SPSS (Statistical Package for Social Science) (Version 26). All descriptive data were expressed as mean  $\pm$  standard error of the mean (SEM) for the selected variable. An independent t-test was used for statistical evaluation of numerical data between cases and controls, which included CT, Ca, and P. The probability value ( $P \leq 0.05$ ) statistically means a significant difference, while a  $P$ -value more than 0.05 was considered non-significant difference. In addition, a  $P$ -value of less than 0.001 statistically means a highly significant difference.

## Results

### Demographic characteristics of the participants.

The study included 100 participants of both genders. The case group included those diagnosed with type two diabetes mellitus and the control group included healthy individuals. Each group included 50 participants, it consisted of 32 females and 18 males. The (mean  $\pm$  SE) of age in the case group was (55.06  $\pm$  1.13) years and in the control group was (54.48  $\pm$  1.16) years. The (Mean  $\pm$  SE) BMI was (28.59  $\pm$  0.65) Kg/m<sup>2</sup> and (31.15  $\pm$  0.78) Kg/m<sup>2</sup> in the diabetic and healthy groups, respectively. Most diabetic patients had a family history of T2DM which was about 26 participants and most of the control participants did not have T2DM family history which included 30 individuals. The demographic characteristics of the study are shown in Table 1.

**Table 1** Demographic characteristics of the participants in control and case groups.

Characterization	Case	Control	<i>P</i> -value
<b>Gender No. (%)</b>			1.000*
Males	18 (50%)	18 (50%)	
Females	32 (50%)	32 (50%)	
<b>Age (years) mean <math>\pm</math> SE</b>	55.06 $\pm$ 1.13	54.48 $\pm$ 1.16	0.721**
<b>BMI (Kg/m<sup>2</sup>) mean <math>\pm</math> SE</b>	28.59 $\pm$ 0.65	31.15 $\pm$ 0.78	0.130**
<b>Family history of T2DM No. (%)</b>			0.229*
Yes	26 (52%)	20 (40%)	
No	24 (48%)	30 (60%)	

\*By Chi-square test for qualitative samples.

\*\*By t-test for two independent samples.

**Calcitonin, Calcium, and Phosphate.**

The Mean  $\pm$  Standard Error (SE) of calcitonin was (2.35  $\pm$  1.18) pg/mL in the case participants, while in the control was about (32.91  $\pm$  3.88) pg/mL and the data analysis showed a statistically highly significant difference ( $P \leq 0.001$ ) between the level of serum calcitonin when compared with type two diabetic patients and healthy individuals. The mean  $\pm$  SE value for serum phosphate level in cases was (3.93  $\pm$  0.08) mg/dL, while (3.73  $\pm$  0.09) mg/dL was the mean  $\pm$  SE value for phosphate in controls.  $P$ -value was equal to (0.117) which means there was no statistically significant difference in (mean  $\pm$  SE) of serum phosphate between case and control participants. Regarding, the mean  $\pm$  SE levels of calcium in the serum was (9.68  $\pm$  0.10) mg/dL for diabetic participants and (9.67  $\pm$  0.14) mg/dL for healthy

subjects ( $P = 0.955$ ), and there was no statistically significant difference in the level of serum calcium between the case and control groups (Table 2).

**Effect of gender on the level of serum calcitonin, calcium, and phosphate in diabetic patients.**

The results showed that there was no relationship between gender and the level of serum calcitonin hormone and calcium in case participants. The ( $P \geq 0.05$ ) for calcitonin and calcium statistically considered that there was no significant difference in the mean serum calcium and calcitonin levels between males and females. Regarding, phosphate serum level ( $P \leq 0.05$ ) there was a significant statistical difference in the level of serum phosphate between both genders with type 2 diabetes (see Table 3).

**Table 2** The comparison of the level of serum calcitonin, phosphate, and calcium in diabetic patients with the control group.

Biochemical parameters	Case (N=50)	Control (N=50)	<i>P</i> -value
	Mean $\pm$ SE	Mean $\pm$ SE	
Calcitonin (pg/mL)	2.35 $\pm$ 1.18	32.91 $\pm$ 3.88	0.001*
Phosphate (mg/dL)	3.93 $\pm$ 0.08	3.73 $\pm$ 0.09	0.117*
Calcium (mg/dL)	9.68 $\pm$ 0.10	9.67 $\pm$ 0.14	0.955*

\*By t-test for two independent samples.

**Table 3** Mean  $\pm$  SE of calcitonin, calcium, and phosphate between males and females with type 2 diabetes mellitus.

Biochemical Parameters	Gender		<i>P</i> -value
	Males (Mean $\pm$ SE) N = 18	Females (Mean $\pm$ SE) N = 32	
Calcitonin (pg/mL)	1.82 $\pm$ 1.50	2.63 $\pm$ 1.65	0.747*
Phosphate (mg/dL)	3.71 $\pm$ 0.18	4.06 $\pm$ 0.08	0.044*
Calcium (mg/dL)	9.57 $\pm$ 0.17	9.74 $\pm$ 0.13	0.424*

\*By t-test for two independent samples.

**Correlation coefficient (r) between data variables.**

Correlation of HbA1c with calcitonin, phosphate, and calcium in type two diabetic patients showed that the *P* value was ( $\geq 0.05$ ) for all of them which means that there was no statistically significant correlation of HbA1c with calcitonin, phosphate, and calcium.

The findings showed that a positive (direct) correlation was present between glucose and calcium, and phosphate. Regarding, calcitonin there was a negative (inverse) correlation. As for the HbA1c correlation, the statistical evaluation (*P* value  $\geq 0.05$ ) for all biochemical parameters in the study showed no significant correlation between serum glucose level with calcitonin, phosphate, and calcium.

14 (16.3%) failed to achieve CR, 13 (15.1%) died during or before induction chemotherapy and 9 (10.5%) of them were lost to follow up.

The association between remission status post induction chemotherapy and the phenotype pattern expression is shown in Table 7.

In comparison between the two groups (CR and No-CR) in relation to the type of aberrant CD marker, no significant association was noticed as most of them were associated with higher frequency of CR except CD56 and CD64, both were associated with No-CR when expressed but the significance level was not reached.

**Table 4** Correlation between HbA1c and calcitonin, phosphate, and calcium represented by correlation coefficient (r) and *P*-value.

Parameters	HbA1c	
	r	<i>P</i> value
Calcitonin (pg/mL)	-0.005	0.976
Calcium (mg/dL)	0.034	0.815
Phosphate (mg/dL)	0.220	0.126

**Table 5** Correlation between serum glucose level with calcitonin, phosphate, and calcium represented by correlation coefficient (r) and *P*-value.

Parameters	Serum glucose level	
	r	<i>P</i> value
Calcitonin (pg/mL)	-0.169	0.298
Calcium (mg/dL)	0.176	0.222
Phosphate (mg/dL)	0.002	0.989

## Discussion

Throughout the world, Type 2 Diabetes Mellitus (T2DM) is a metabolic disease characterized by a defect in the secretion of insulin from the pancreas and reduces the sensitivity of insulin (insulin resistance).<sup>28</sup>

In the present cross-sectional study, we compared the serum calcitonin hormone (CT) level and minerals such as calcium and phosphate in type two diabetic patients with healthy individuals. Calcitonin (CT) is a peptide hormone produced and released by the thyroid gland from c-cells involved in increasing the calcium in the blood.<sup>8</sup>

We demonstrated that the level of (CT) was significantly lower in type two diabetic patients when compared with healthy individuals. The mechanism by which T2DM affects the level of CT is unclear. The current findings is in disagreement with the results of the previous study, which showed that the level of serum calcitonin was significantly higher in type one and type two diabetes mellitus than in healthy individuals, it also described that calcium causes an elevated level of calcitonin.<sup>29</sup> However, a recent study showed that the level of calcitonin significantly decreased in type two diabetic patients with complications.<sup>30</sup>

Calcium (Ca) is one of the most crucial cationic minerals, and for building the body's skeletal structure it is one of the most crucial minerals.<sup>15</sup> The results showed that there was no significant difference in the mean level of calcium between case and control participants. This is in agreement with the results of the previous study, which statistically ( $P$  value  $\geq 0.05$ ) found that there was no significant difference in the mean serum calcium level between patients and non-diabetic subjects.<sup>31</sup> Phosphate has played an essential role in the body. It is involved in several metabolic processes with Ca stored in the bone.<sup>21</sup> In the present study, the level of serum phosphate was not altered between diabetes and non-diabetes individuals.

In 2014, Revathi and Amaldas reported a study about clinical study of serum phosphate and magnesium in type II diabetes mellitus, the analyzed data showed that the serum phosphate level was significantly lower in diabetic patients than the level in control subjects. It is in disagreement with the present cross-sectional study.<sup>32</sup>

The data analysis in this study demonstrated that gender did not have an impact on altering the level of serum calcitonin and calcium in patients with type two diabetes mellitus. There was no significant difference in both levels between males and females ( $P$ -value  $\geq 0.05$ ). Regarding calcitonin, no study indicated the effect of gender on serum calcitonin levels in diabetic individuals. Regarding calcium, the result is in accordance with a previous study that has shown no significant statistical difference in the mean serum calcium levels between females and males.<sup>33</sup>

The present study demonstrated that gender has a significant effect on the mean serum phosphate level when comparing males with females ( $P$  value  $\leq 0.05$ ) as there was a significant difference in the mean serum phosphate level in comparing both genders.

A previous study showed that there was no significant difference in the mean phosphate level in serum between males and females with type two diabetes.<sup>33</sup> Furthermore, the statistical analysis showed that the correlation of HbA1c with calcium and phosphate was a positive (direct) correlation, as  $P$  value was ( $\geq 0.05$ ) indicating no significant correlation between HbA1c and phosphate as well as with calcium levels in serum of T2DM patients.

The previous studies have shown negative (inverse) correlation between HbA1c with phosphate and calcium serum concentrations in type two diabetic patients, and this is in disagreement with the correlation result of the current study.<sup>27,34</sup>

As for HbA1c correlation, the glucose correlation indicated a positive (direct) correlation was present between glucose with phosphate and calcium. At  $P$  value  $\geq 0.05$  there was no statistically significant correlation present between serum glucose level with phosphate and calcium. This result is in agreement with the previous study that demonstrated the positive (direct) correlation of phosphate with serum glucose level.<sup>35</sup>

Regarding the correlation between calcium and serum level of glucose, the prior study reported a negative (inverse) correlation between calcium and glucose in type two diabetic individuals.<sup>36</sup>

This study found a negative (inverse) correlation between calcitonin and HbA1c as well as with glucose ( $P$  value  $\geq 0.05$ ). There were no available previous studies that reported the correlation of calcitonin in type 2 DM.

### Conclusion

The level of serum calcitonin is negatively correlated with the serum level of glucose and glycated hemoglobin. The level of calcitonin was reduced in case participants. The level of phosphate and calcium were not affected by hyperglycemia and insulin resistance in type two diabetic patients and a positive correlation was detected between glycated hemoglobin and glucose with phosphate and calcium.

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### Competing interests

The authors declare that they have no competing interests.

### References

- Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of type 2 diabetes mellitus. *Int J Mol Sci.* 2020; 21(17):6275. <https://doi.org/10.3390/ijms21176275>
- Abdullah N, Attia J, Oldmeadow C, Scott RJ, Holliday EG. The architecture of risk for type 2 diabetes: understanding Asia in the context of global findings. *Int J Endocrinol.* 2014. <https://doi.org/10.1155/2014/593982>
- Lal BS. Diabetes: causes, symptoms and treatments. *Public health environment and social issues in India.* 2016;1.
- Serbis A, Giapros V, Kotanidou EP, Galli-Tsinopoulou A, Siomou E. Diagnosis, treatment and prevention of type 2 diabetes mellitus in children and adolescents. *World J Diabetes.* 2021; 12(4):344. <https://doi.org/10.4239/wjd.v12.i4.344>
- Punthakee Z, Goldenberg R, Katz P. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Can J Diabetes.* 2018; 42:S10–5. <https://doi.org/10.1016/j.cjcd.2017.10.003>
- Danila R, Livadariu R, Branisteanu D. Calcitonin revisited in 2020. *Acta Endocrinol (Buchar).* 2019; 15(4):544. <https://doi.org/10.4183/aeb.2019.544>
- Rigoldi F, Metrangolo P, Redaelli A, Gautieri A. Nanostructure and stability of calcitonin amyloids. *J Biol Chem.* 2017; 292(18):7348–57. <https://doi.org/10.1074/jbc.m116.770271>
- Hamdy RC, Daley DN. Oral calcitonin. *Int J Womens Health.* 2012; 4:471. <https://doi.org/10.2147%2FIJWH.S24776>
- Hsiao CY, Chen TH, Chu TH, Ting YN, Tsai PJ, Shyu JF. Calcitonin induces bone formation by increasing expression of Wnt10b in osteoclasts in ovariectomy-induced osteoporotic rats. *Front Endocrinol.* 2020; 613. <https://doi.org/10.3389/fendo.2020.00613>
- Wongdee K, Rodrat M, Teerapornpantakit J, Krishnamra N, Charoenphandhu N. Factors inhibiting intestinal calcium absorption: hormones and luminal factors that prevent excessive calcium uptake. *J Physiol Sci.* 2019; 69(5):683–96. <https://doi.org/10.1007/s12576-019-00688-3>
- Gosink J, AG E. Parathyroid hormone, calcitonin and vitamin D testing in calcium and bone metabolic disorders. *Medlab magazine* 2015; 2:26–8.
- Pu F, Chen N, Xue S. Calcium intake, calcium homeostasis and health. *Food Sci Hum Wellness.* 2016; 5(1):8–16. <http://doi.org/10.1016/j.fshw.2016.01.001>
- Zhou Y, Xue S, Yang JJ. Calciomics: integrative studies of Ca<sup>2+</sup>-binding proteins and their interactomes in biological systems. *Metallomics.* 2013; 5(1):29–42. <https://doi.org/10.1039%2Fc2mt20009k>
- Veldurthy V, Wei R, Oz L, Dhawan P, Jeon YH, Christakos S. Vitamin D, calcium homeostasis and aging. *Bone Res.* 2016; 4(1):1–7. <https://doi.org/10.1038/boneres.2016.41>
- Raskh S. The importance and role of calcium on the growth and development of children and its complications. *Int J Res Appl Sci Biotechnol.* 2020; 7(6):162–7. <https://doi.org/10.31033/ijrasb.7.6.24>
- Schwarz EC, Qu B, Hoth M. Calcium, cancer and killing: the role of calcium in killing cancer cells by

- cytotoxic T lymphocytes and natural killer cells. *Biochim Biophys Acta*. 2013; 1833(7):1603–11. <https://doi.org/10.1016/j.bbamcr.2012.11.016>
17. Varghese E, Samuel SM, Sadiq Z, Kubatka P, Liskova A, Benacka J, et al. Anti-cancer agents in proliferation and cell death: the calcium connection. *Int J Mol Sci*. 2019; 20(12):3017. <https://doi.org/10.3390/ijms20123017>
18. Raina R, Garg G, Sethi SK, Schreiber MJ, Simon JF, Thomas GJ. Phosphorus metabolism. *J Nephrol Ther*. 2012; 1:S4. <https://doi.org/10.4172/2161-0959.S3-008>
19. Shobeiri N, Adams MA, Holden RM. Phosphate: an old bone molecule but new cardiovascular risk factor. *Br J Clin Pharmacol*. 2014 ; 77(1):39–54. <https://doi.org/10.1111%2Fbcp.12117>
20. Penido MG, Alon US. Phosphate homeostasis and its role in bone health. *Pediatr Nephrol*. 2012; 27(11):2039–48. <https://doi.org/10.1007/s00467-012-2175-z>
21. Dobenecker B, Kienzle E, Siedler S. The Source Matters—Effects of High Phosphate Intake from Eight Different Sources in Dogs. *Animals*. 2021; 11(12):3456. <https://doi.org/10.3390%2Fani11123456>
22. Gupta R, Laxman S. Cycles, sources, and sinks: Conceptualizing how phosphate balance modulates carbon flux using yeast metabolic networks. *Elife*. 2021; 10:e63341. <https://doi.org/10.7554/elife.63341>
23. Wongdee K, Krishnamra N, Charoenphandhu N. Derangement of calcium metabolism in diabetes mellitus: negative outcome from the synergy between impaired bone turnover and intestinal calcium absorption. *J Physiol Sci*. 2017; 67(1):71–81. <https://doi.org/10.1007/s12576-016-0487-7>
24. Hegedüs L, Moses AC, Zdravkovic M, Le Thi T, Daniels GH. GLP-1 and calcitonin concentration in humans: lack of evidence of calcitonin release from sequential screening in over 5000 subjects with type 2 diabetes or nondiabetic obese subjects treated with the human GLP-1 analog, liraglutide. *J Clin Endocrinol Metab*. 2011; 96(3):853–60. <https://doi.org/10.1210/jc.2010-2318>
25. Ahn C, Kang JH, Jeung EB. Calcium homeostasis in diabetes mellitus. *J Vet Sci*. 2017; 18(3):261–6. <https://doi.org/10.4142/jvs.2017.18.3.261>
26. Zhu J, Xun P, Bae JC, Kim JH, Kim DJ, Yang K, et al. Circulating calcium levels and the risk of type 2 diabetes: a systematic review and meta-analysis. *Br J Nutr*. 2019; 122(4):376–87. <https://doi.org/10.1017%2F0007114519001430>
27. Bora GK, Rajkakati R, Kakati S, Yadav S. Serum Inorganic Phosphate Concentration and Glycated Haemoglobin Percent in Type 2 Diabetes Mellitus-A Hospital Based Study. 2016; 6(10):96–104.
28. Roden M, Shulman GI. The integrative biology of type 2 diabetes. *Nature*. 2019; 576(7785):51–60. <https://doi.org/10.1038/s41586-019-1797-8>
29. Blasiak M, Kuska J, Kokot F, Woch W. Serum levels of calcitonin, parathyroid hormone and 25-hydroxycholecalciferol in patients with diabetes mellitus. *Endokrynol Pol*. 1989; 40(5):241–50.
30. Al-Attaby AK, Al-Lami MQ. Effects of duration and complications of type 2 diabetes mellitus on diabetic related parameters, adipocytokines and calcium regulating hormones. *Iraqi J Med Sci*. 2019:2335–61. <https://doi.org/10.24996/ijms.2019.60.11.5>
31. Hamad NA, Eltayeb LB, Brair SL, Hussein K. A clinical study of serum calcium, phosphorus, and alkaline phosphates level in type II Diabetes mellitus among Sudanese population in Khartoum State, 2012. *Neelain Med J*. 2013; 3:42–50.
32. Revathi R, Amaldas J. A clinical study of serum phosphate and magnesium in type II diabetes mellitus. *Int J Med Res Health Sci*. 2014; 3(4):808–12. <https://doi.org/10.5958/2319-5886.2014.00005.8>
33. Yousif AA, Ahmed SO. Estimation of Serum Calcium and Phosphorus Levels in Sudanese Patients with Type 2 Diabetes Mellitus (Doctoral dissertation, Sudan University of Science and Technology). 2014.
34. Hassan SA, Elsheikh WA, Rahman N, ElBagir NM. Serum calcium levels in correlation with glycated hemoglobin in type 2 diabetic sudanese patients. *Advances Diabetes Metab*. 2016; 4(4):59–6. <https://doi.org/10.13189/ADM.2016.040401>
35. Raikou VD, Kyriaki D, Gavriil S. Importance of serum phosphate in elderly patients with diabetes mellitus. *World J Diabetes*. 2020; 11(10):416. <https://doi.org/10.4239%2Fwjid.v11.i10.416>
36. Kanchana N, Saikumar P. Serum calcium levels in type 2 diabetes mellitus. *J Dent Med Sci*. 2014; 13(8):01–3. <https://doi.org/10.9790/0853-13820103>