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Minerals and Glucose Among Patients With Vitamin (D) Deficiency

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Abstract

According to previous studies, vitamin D deficiency might increase the risk of mineral deficiency, and type 2 diabetes mellitus. The aim of this study was to evaluate the level of calcium, phosphorus, calcium to phosphorus ratio, magnesium, and fasting blood glucose in blood serum of patients with vitamin D deficiency. This case-control study comprised 71 patients, 30 subjects sufficient in vitamin D classified as control group (Mean value of vitamin D was 30.67. ± 5.94 ng/dl), and 41 patients suffering from vitamin D deficiency, and insufficiency chosen as the case group (Mean value of vitamin D was 9.79 ± 3.12 ng/dl) .There was lower significant difference in the level of calcium (Ca), calcium–to-phosphorus ratio (Ca/P), and magnesium (Mg) in cases compared to control, but there was higher significant difference in the level of phosphorus (P), and fasting blood glucose (FBG) in cases compared to controls. Serum vitamin D levels showed significantly positive correlations with Ca, and Ca/P ratio, and negative correlation with P. However, vitamin D demonstrated insignificant positive correlation with Mg, and insignificant negative correlation with FBG. The study concluded that, vitamin D deficiency associated with diabetes risk, in addition to mineral deficiency, which associated with various disorders, such as skeletal deformities, osteoporosis, Osteomalacia, cardiovascular diseases, and metabolic syndrome.

Keywords: Vitamin D deficiency, calcium, phosphorus,magnesium, and fasting blood glucose.

Partly due to differences in 25(OH)D measurement techniques and the variability of vitamin D levels in the human body, there is a lack of consensus on the cut-off points that denote different vitamin D status categories. The conservatively used cut-off points in the last decade are, vitamin D deficiency, 25(OH)D < 25 nmol/L (< 10 ng/ml), which implicates that, below this level, adverse effects like rickets and osteomalacia are observed in children , and adults; vitamin D insufficiency, 25(OH)D of 25-50nmol/L (10-20ng/ml) between this range increased bone resorption and an elevated risk for secondary hyperparathyroidism are seen; and adequate status, 25(OH)D > 50nmol/L (> 20ng/ml) [1].

Phosphorus and calcium are interrelated because hormones, such as vitamin D and parathyroid hormone (PTH), regulate the metabolism of both minerals. In addition, phosphorus and calcium make up hydroxyapatite, the main structural component in bones and tooth enamel [2]. The combination of high phosphorus intakes with low calcium intakes increases serum PTH levels, but the evidence is mixed on whether the increased hormone levels decrease bone mineral density [3].

The major biological function of vitamin D is to maintain normal blood levels of calcium and phosphorus [4]. Vitamin D aids in the absorption of calcium, helping to form and maintain strong bones. Recent research also suggests that sufficient intake of this vitamin may provide protection from and decrease an individual's risk of developing osteoporosis, hypertension, cancer, and several autoimmune diseases [4].

The total amount of dietary calcium intake affects the level of absorption. On the other hand, excess in the available phosphate may reduce the passive shift of calcium by forming insoluble salts in the intestinal lumen. Thus, the relative ratio of calcium and phosphorus intakes seems to be another factor to regulate intestinal calcium absorption. Epidemiologically, a reduced intake ratio of calcium to phosphorus is associated with a reduction in bone mineral density (BMD) in healthy perimenopausal women [5, 6]. It has an important role in bone mineralization, as it increases the absorption of Ca in the small intestine, promotes osteoblastic activity and maintains serum concentration of Ca , and P in the normal range [7].

The adequate balance of magnesium and vitamin D is essential for maintaining the physiologic functions of various organs. Vitamin D helps regulate calcium and phosphate balance to maintain healthy bone functions [8]. Skeletal muscles, heart, teeth, bones, and many other organs require magnesium to sustain their physiologic functions. Furthermore, magnesium is needed to activate vitamin D. Abnormal levels in either of these nutrients can lead to serious organ dysfunctions [9].

In a study published in 2011, it was stated that lowvitamin D levels increased prediabetes risk, and in healthy adolescent males, the insulin levels decreased as the vitamin D level increased [10]. The type 2 diabetes risk was lower for those whose 25(OH)D levels were higher [11]. On the other hand, in a study carried out in postmenopausal women, it hasbeen reported that low vitamin D levels were not associatedwith diabetes risk [12] . On the other hand , Studies conducted on adolescents, revealed that the low level of vitamin D is obviously accompanied by abdominal obesity, elevated hypertension, high fasting blood sugar (FBS), and metabolic syndrome [13]. The main objective of this study is to assess the serum level of Vitamin D. The specific objectives of this present research study are to demonstrate the impact of low levels of vitamin D on Ca, P, Ca/ P ratio, Mg, and FBG.'

Materials and Method

Subjects study

A total of seventy-one patients who applied to the outpatient clinics of Al -zaytouna specialized hospital. Their ages were between 18 to 85 years and they were coming from different parts of Sudan. Al -zaytouna specialized hospital located in Khartoum State, Khartoum is the largest city of the country it had an estimated total population of approximately 6 million people according to the national population census in 2009. The subjects were divided into two main groups, group one (41 patients) represented the case group (Mean value of vitamin D was 9.79 ± 3.12 ng/dl). The second group (30 patients) was chosen as controls (Mean value of vitamin D was 30.67.79 ± 5.94 ng/dl).

Study Design

This is a case-control study with sequential recruitment of study participants with deficient and insufficient vitamin D, diagnosed by physicians depending on laboratory investigation, and those without the disease (control). The laboratory tests for the biochemical parameters were conducted to assess vitamin D deficiency, serum calcium, serum phosphorus, and serum magnesium.

Data Collection

Samples (4 ml) were collected from the participants by venipuncture into a dry tube (4 ml) using a 5 ml nontoxic, pyrogen-free, sterilized disposable syringes (Cathy Yougo®, France). Biochemical parameters were assessed in clotted samples .The blood samples were then centrifuged (BECKMAN Instruments Inc., USA) at 2500 rpm for 5 minutes, and serum was transferred into separate Eppendorf tubes using sterile micropipettes and stored in the freezer at −35°C for subsequent batch analysis of serum vitamin D, calcium, phosphorus, magnesium, and fasting blood glucose.

Ethical Consideration

Ethical clearance was obtained from the Faculty of postgraduate, university of Al-Neelain. Administrative clearance was obtained from the Director of Al -zaytouna Specialized Hospital in

Khartoum, the capital city of Sudan. Furthermore, authorization was obtained from the chief of the laboratory.

Methods

Measurement of Vitamin D

Vitamin D was measured according to the a competitive chemiluminescence immunoassay (CLIA) technique method described by [14].

Measurement of Ca

Calcium was measured according to the method based on the reaction of calcium with5-nitro-5'-methyl-BAPTA (NM-BAPTA) under alkaline conditions to form a complex described by [15, 16].

Measurement of P

Phosphorus was measured according to the method, phosphomolybdate uv using ammonium molybdate in sulfuric acid described by [17].

Measurement of Mg

Magnesium was measured according to the method based on the reaction of magnesium with xylidyl blue in an alkaline solution containing ethylene glycol tetraacetic acid (EGTA) .This method described by[18].

Measurement of glucose level

Glucose was measured according to enzymatic method with hexokinase described by [19].

Statistical analysis

Data were analyzed using the statistical software package IBM SPSS statistics for windows, version 16 (SPSS Inc., USA). The normality of the continuous data was evaluated using the Kolmogorov-Smirnov test. Data were expressed as percentages, mean ±standard deviation of the mean (SDM). Student's t test was used to assess the difference between group means of parametric variables. The relationship between variables was analyzed utilizing Pearson's or Spearman's correlation coefficient. Statistical significance was designated as $p \leq 0.05$.

Results

Patients suffering from insufficient and deficient vitamin D

Seventy-one adults who participated in this study are classified into groups according to the vitamin D levels: group one was deficient, and insufficient in vitamin D (case group) represented 57.75% (n=41), group two was healthy (control) represented 42.25% (n=30).

Table (1) shows that the mean value of calcium (mg/dl) was (9.27± 0.26) in control group, whereas, (8.66 ± 0.54) in case group. There were lower significant differences between the deficient group compared with the control group.

In addition, the mean value of phosphorus level (mg/dl) was (3.72± 0.51) in healthy patients, and (5.00± 0.94) in the patients deficient vitamin D (case group). There were higher significant differences between the deficient group compared with the control group.

Mean value of calcium to phosphorus ratio was (2.55± 0.47) in control, and (1.81± 0.45) in cases. There were lower significant differences between the deficient group compared with the control group.

The mean value of magnesium level (mg/dl) was (2.06± 0.13) in control group, and (1.96± 0.24) in patients with vitamin D deficiency. There were lower significant differences between the deficient group compared with the control group.

Mean value of fasting blood glucose level (mg/dl) was (101.77± 14.28) in healthy patients, or control, and (119.51± 36.29) in patients with vitamin D deficiency. There were higher significant differences between the case group and control.

The association between serum vitamin D concentration and, Ca, P, Ca/P ratio, Mg, and FBG.

Table (2) indicates that vitamin D deficiency level shows significant positive correlation with Ca (r = 0. 0.589, and P = 0.000), and with Ca/P ratio (r = 0.662, and P = 0.000). In contrast, table (2) revealed significant negative correlation between vitamin D and phosphorus (r = -0.644, and P = 0.000). Table (2), also shows insignificant positive correlation with Mg (r = 0.186, and P = 0.126), and negative with FBG (r = -0.229, and P = 0. 055).

Table (1): Levels of calcium (Ca), phosphorus (P), Ca/P ratio, magnesium (Mg), and fasting blood glucose (FBG) of the study population.

Measurement (mg/dL)	Control group (n=30)	Case group (n=41)	t-test value	P- value
Calcium	9.27± 0.26	8.66 ± 0.54	-5.63	0.000
Phosphorus	3.72± 0.51	5.00± 0.94	6.81	0.000
Ca: P ratio	2.55± 0.47	1.81± 0.45	-6.79	0.000
Magnesium	2.06± 0.13	1.96± 0.24	-2.10	0.039
FBG	101.77± 14.28	119.51. ± 36.29	2.54	0.014

* The mean difference is significant at the 0.05 level. Data are expressed as Mean± SD.

Table (2): Serum vitamin D level in relation to calcium (Ca), phosphorus (P), Ca/P ratio, magnesium (Mg), and fasting blood glucose (FBG**)** levels of the study population.

Parameters	**Correlation Coefficients (r)**	**P-value**
Ca (mg/dl)	0.589	0.000
P (mg/dl)	-0.644	0.000
Ca/P ratio	0.662	0.000
Mg (mg/dl)	0.186	0.126
FBG (mg/dl)	-0.229	0.055
P value < 0.05 is considered significant		

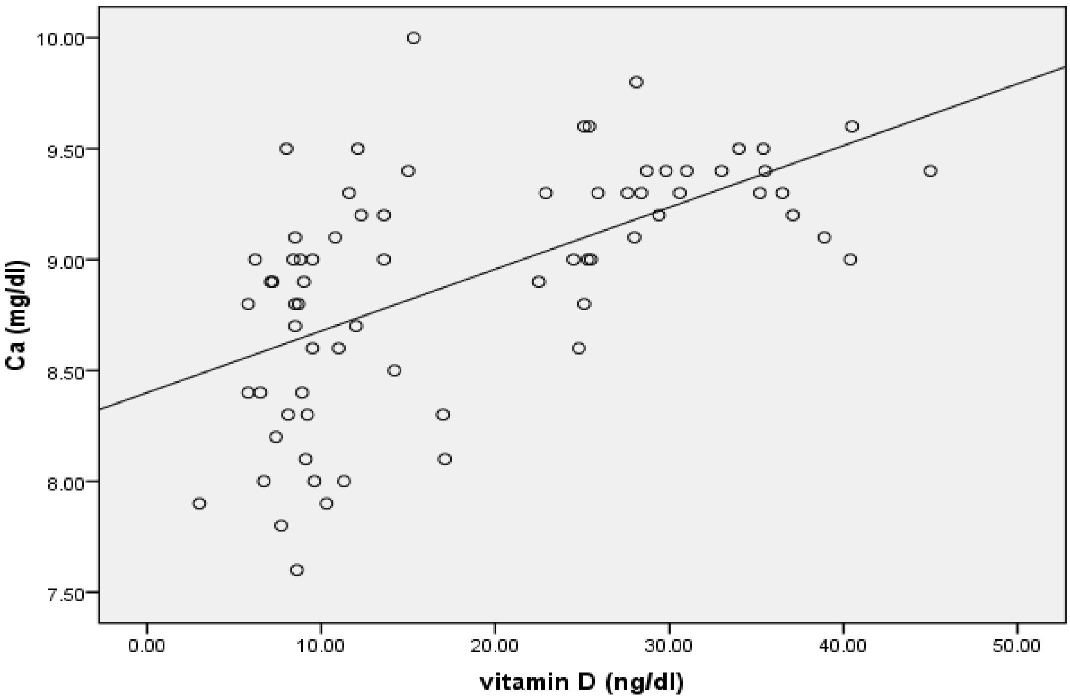

Figure (1). Serum vitamin D level in relation to serum calcium

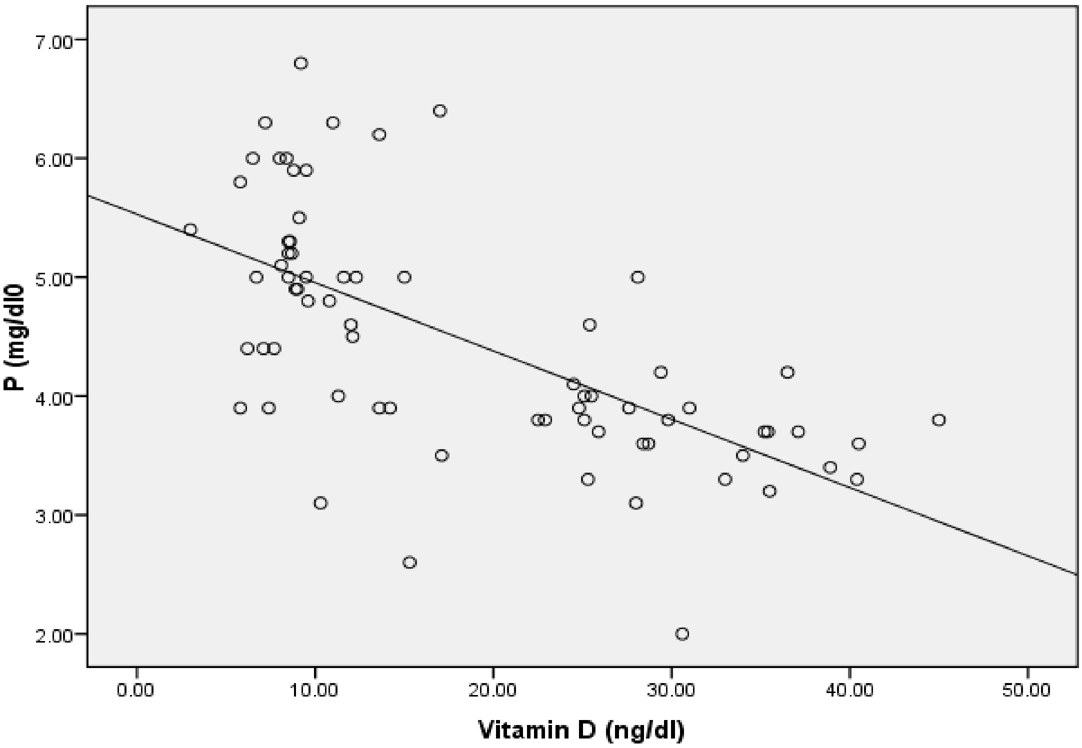

Figure (2). Serum vitamin D level in relation to serum phosphorus

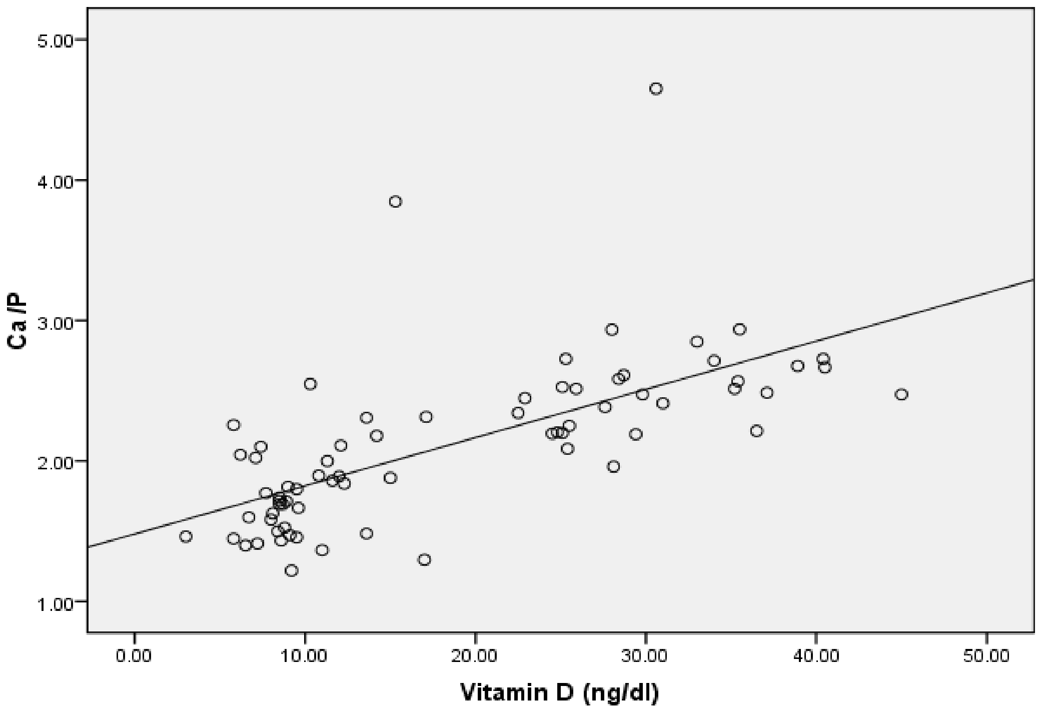

Figure (3). Serum vitamin D level in relation to Ca/P ratio

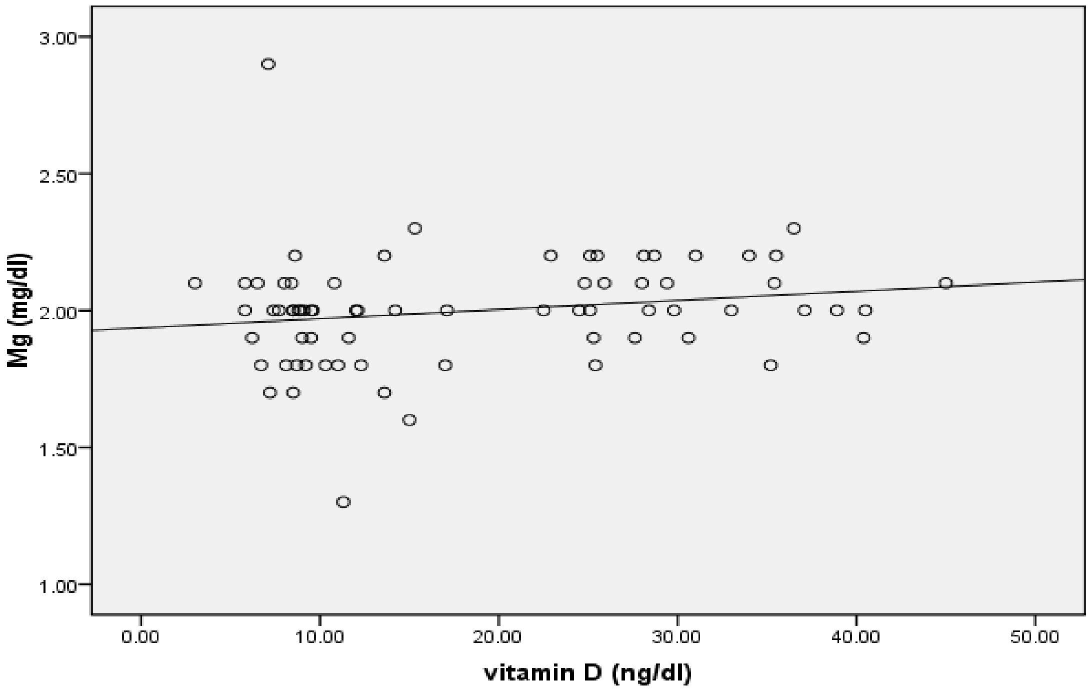

Figure (4), Serum vitamin D level in relation to serum magnesium

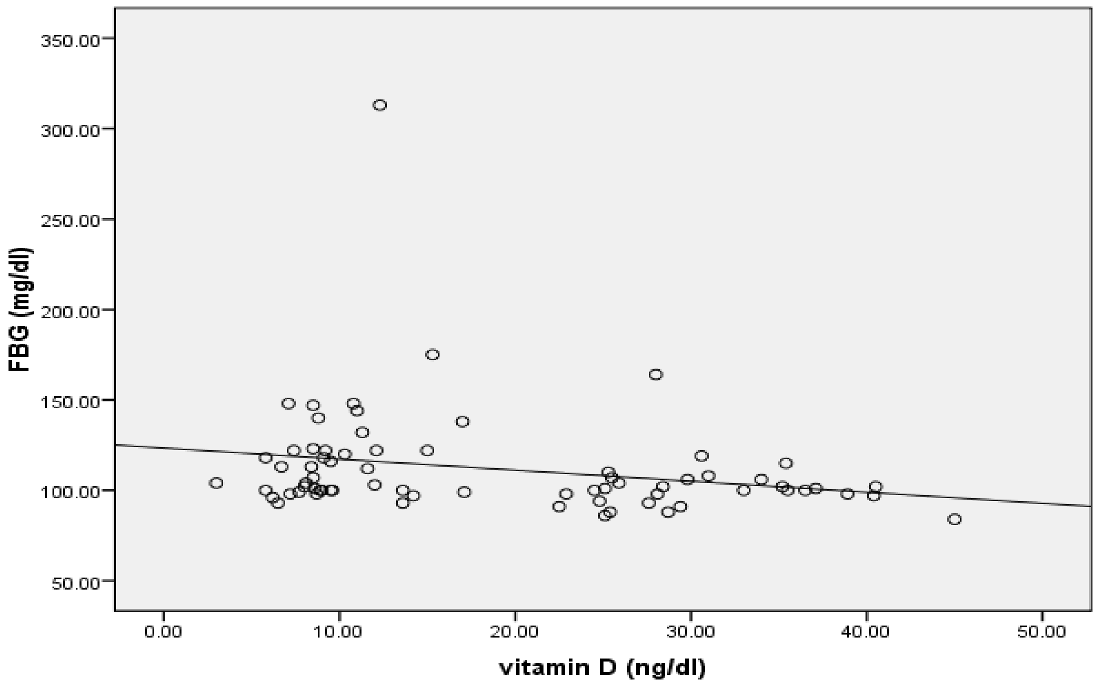

Figure (5). Serum vitamin D level in relation to serum fasting blood glucose

Discussion

Vitamin D deficiency has a significant effect on health, including low bone mineral density, increased risk of fractures and falls [20]. In addition, adequate blood vitamin D levels and intake of vitamin D may decrease the risk of cancer, type I diabetes, multiple sclerosis ,and rheumatoid arthritis [21].

As indicated in the present results, serum calcium was significantly decreased in the case groups compared to controls (P=0.000) . Similar results were obtained by [22] Calcium is needed for our heart, muscles, and nerves to function properly and for blood to clot. Inadequate calcium significantly contributes to the development of osteoporosis and Osteomalacia. Vitamin D and calcium are both required for the maintenance of musculoskeletal health, among others [23].

As shown in Table (1), high level of phosphorus is observed with low level of vitamin D in the deficient vitamin D patients (P= 0.000). This result explains that Serum phosphate level is regulated by intestinal phosphate absorption, renal phosphate handling, and equilibrium of extracellular phosphate with that in bone or intracellular fluid. Several hormone such as parathyroid hormone, 1, 25-dihydroxyvitamin D and fibroblast growth factor 23 (FGF23) are regulate the serum phosphorus by modulating intestinal phosphorus absorption, renal phosphorus reabsorption and/or bone metabolism and also dietary phosphorus rapidly enhances renal phosphorus excretion [24].

The results of the Ca/P ratio showed that, there were lower significant differences in the cases compared to the control group according to vitamin D status (P=0.000). These results indicate that, the Ca/P ratio in the case groups was lower, because calcium levels in the case groups decreased whereas, the phosphorus levels in the case group increased. The major biologic function of vitamin D in humans is to maintain serum calcium and phosphorus concentrations within the normal range by enhancing the efficiency of the small intestine to absorb these minerals from the diet [25].

The results revealed that, serum magnesium was significantly decreased in cases compared to the control group (P= 0.039). Carpenter et al reported that ,lower levels of serum magnesium are associated with hypovitaminosis D [26]. . Magnesium assists in the activation of vitamin D, which helps regulate calcium and phosphate homeostasis to influence the growth and maintenance of bones. All of the enzymes that metabolize vitamin D seem to require magnesium, which acts as a cofactor in the enzymatic reactions in the liver and kidneys. Deficiency in either of these nutrients is reported to be associated with various disorders, such as skeletal deformities, cardiovascular diseases, and metabolic syndrome. It is therefore essential to ensure that the recommended amount of magnesium is consumed to obtain the optimal benefits of vitamin D[27].

Data presented in this study showed serum fasting blood glucose was significantly increased in cases compared to the controls group (P=0.014) . Such finding coincides with that results obtained by [28]. A study by Hyppönen, et al., published in the November 2001 issue of "Lancet," showed that supplementation of vitamin D in infants reduced the incidence of type I diabetes [29].

Vitamin D may have a beneficial effect on insulin action either directly, by stimulating the expression of insulin receptors and thereby enhancing insulin responsiveness for glucose transport [30], or indirectly via its role in regulating extracellular calcium and ensuring normal calcium influx through cell membranes and an adequate intracellular cytosolic calcium pool because calcium is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscles and adipose tissues [31].

Table (2), and figure (1) showed a significantly, positive correlation between the serum levels of calcium and vitamin D level (P =0.000). This finding is in agreement with the results reported by [32]. In addition, table (2), and figure (3) also showed a significantly, positive correlation between serum levels of Ca/P ratio, and vitamin D levels (P =0.000). These findings are agreed with the results obtained by [5]. The results in table (2), and figure (2) indicated a significantly, negative correlation between the serum levels of phosphorus and vitamin D level (P = 0.000). This finding coincides with that obtained by [33]. On the other hand, table (2) and figure (4) showed insignificantly positive correlation between the serum levels of magnesium, and vitamin D (P =0.126).This findings agreed with the results reported by [34]. Data presented in table (2),and figure (5) showed insignificant negative correlation between serum fasting blood glucose and vitamin D level (P =0.055). These findings are agreed with the results obtained by [35].

Conclusion

Serum Ca , ratio of Ca/P, and Mg was significantly lower in cases compared to controls according to vitamin D status whereas, serum P, and FBG was higher in cases compared to control. Serum Vitamin D levels showed significant positive correlations with Ca and ratio of Ca/P, and negative correlation with P. The study concluded that, vitamin D deficiency associated with diabetes risk, in addition to mineral deficiency, which associated with various disorders, such as skeletal deformities, osteoporosis ,osteomalacia, hyperphosphatemia, cardiovascular diseases, and metabolic syndrome.

Recommendations

Follow up a particular diet and taking vitamin D and calcium-rich food is recommended.

- Supplementation of vitamin D in patients with hypovitaminosis could be useful in management and treatment strategy of the vitamin D deficiency risks.
- Regular visits to clinics to take early steps for avoid and manage vitamin D deficiency complications risk.

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References

1. Nowson, CA& Margerison, C. Vitamin D intake and vitamin D status of Australians. Med J. (**2002**) ; 177(3), 149-152.
2. Trautvetter, U.,Ditscheid, B., Jahreis, G., Glei, M. Habitual intakes, food sources and excretions of phosphorus and calcium in three German study collectives. Nutrients. (**2018**);10.
3. Anderson, J.L., May, H.T., Horne, B.D., Bair, T.L., Hall, N.L., Carlquist, J.F., et al . Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general health care population. The American journal of cardiology. (**2010**). ;106(7):963-968.
4. Barclay, L. Management of vitamin D deficiency reviewed. (**2009**); 4:208 – 210.
5. Brot, C., Jorgensen, N., Madsen, OR., Jensen, L.B., Sorensen, O.H. Relationships between bone mineral density, serum vitamin D metabolites and calcium: Phosphorus intake in healthy perimenopausal women. J Intern Med. (**1999**). ; 245: 509– 516.
6. Lukert, B.P., Carey, M., Tieman, S., Goodnight, L., Helm, M., Hassanein, R., Stevenson, C., Stoskopf, M., Doolan, L . Influence of nutritional factors on calcium-regulating hormone and bone loss. Calcif Tissue Int. (**1987**) ; 40: 119– 125.
7. Hollick, M.F.Vitamin D deficiency D. N. Engl. J. Med. (**2007**) ;357:266–281. [PMID: 17634462 DOI: 10.1056/NEJMra070553].
8. Dusso, A.S .Update on the biologic role of the vitamin D endocrine system. Curr Vasc Pharmacol (**2014**). ;12(2):272-277.
9. Vacek, J.L., Vanga, S.R., Good, M., Lai, S.M., Lakkireddy, D., Howard, P.A. Vitamin d deficiency and supplementation and relation to cardiovascular health. Am J Cardiol. (**2012**) ;109:359–63.
10. Gupta, A.K., Brashear, M.M., Johnson ,W.D. Prediabetes and Prehypertension in healthy adults are associated with low vitamin D levels. Diabetes Care. (**2011**) 34(3):658–660.
11. Pittas, A.G., Sun, Q., Manson, J.E., DawsonHughes, B., Hu, F.B. Plasma 25-hydroxyvitamin D concentration and risk of incident type 2 diabetes in women. Diabetes Care. (2**010**);33(9): 2021-2023.

12. Robinson, J.G., Manson, J.E., Larson, J., Liu, S., Song, Y., et al. Lack of association between 25(OH)D levels and incident type 2 diabetes in older women. Diabetes Care. (**2010**); 34 (3) : 628-634.
13. Vacek, J.L., Vanga, S.R., Good, M., Lai, S.M., Lakkireddy, D., Howard, P.A. Vitamin d deficiency and supplementation and relation to cardiovascular health. Am J Cardiol. (**2012**) ;109:359–63.
14. Schleicher & Pfeiffer .Vitamin D Testing: How Will We Get it Right? American Association for Clinical Laboratory.(**2013**).
15. Heins, M, Heil W, Withold W. Storage of Serum or Whole Blood Samples? Effect of Time and Temperature on 22 Serum Analytes. Eur J Clin Chem Clin Biochem. (**1995**); 33(4): 231-238.
16. Endres, D,B., Rude, R.K . Mineral and Bone Metabolism In: Burtis CA, Ashwood ER, Bruns ED, (Eds.), Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4^{th} (Edn.), St. Louis (MO): Saunders Elsevier.(**2006**) pp: 1891-1965.
17. Henry, R.Clinical Chemistry: Principles and Technics, 2^{nd} (Edn.), NY: Harper & Row, New York. (**1994**) pp:723.
18. Mann, C.K, Yoe, J.H. Spectrophotometric determination of magnesium with sodium 1-azo-2- hydroxy-3-(2,4-dimethyl-carboxanilido)- napthalene- 1'-(2-hydroxy-benzene-5-sulfonate) Anal Chem. (**1956**); 28(2): 202-205.
19. Kunst A . Bergmeyer Methods of Enzymatic Analysis, 3^{rd} (Edn.), Tietz NW (Ed.) Metabolites 1: Carbohydrates. 5 Clinical Guide to Laboratory Tests. (**1984**); 6: 163-172.
20. Bischoff,Ferrari, T. Dietrich., E.J., Orav, F.B., Hu, Y., Zhang, E.W., Karlson, *et al.*Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y .Am J Clin Nutr. (**2004**) ; 80 : 752-758.
21. Merlino, J., Curtis, T.R., Mikuls, J.R., Cerhan, L.A., Criswell, K.G .Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study Arthritis Rheum. (**2004**) ; 50 : 72-77.
22. AL-yassin, H. A. M, Nimnim Talip Ali and Challab Dohan Eqbal.Calcium And Diabetes Mellitus Type Two a prospective study done on people with Type 2 Diabetes in Diwanya teaching hospital. Kufa Med.Journal. (**2009**); VOL.12.No1.
23. Taheri Ehsaneh, Saedisomeolia Ahmad, Djalali Mahmoud, Qorbani Mostafa and Civi Madani Manouchehr.The relationship between serum 25-hydroxy vitamin D concentration

and obesity in type 2 diabetic patients and healthy subjects. Journal of Diabetes & Metabolic Disorders. (**2012**); 11:16.

24. Fukumoto, S. Phosphate metabolism and vitamin D. Bonekey Reports. (**2014**) ;3:497. DOI: 10.1038/bonekey.2013.231.
25. DeLuca, H.F.“Overview of general physiologic features and functions of vitamin D,” American Journal of Clinical Nutrition. (**2004**); 80(6): 1689S–1696S.
26. Carpenter, T.O., DeLucia, M.C., Zhang, J.H et al . “A randomized controlled study of effects of dietary magnesium oxide supplementation on bone mineral content in healthy girls,” The Journal of Clinical Endocrinology & Metabolism. (**2006**) ; 91(12): 4866–4872.
27. Anne, M.U and Mohammed, S. Role of Magnesium in Vitamin D Activation and Function. J Am Osteopath Assoc. .,(**2018**) ;118(3):181-189. Phosphorus, Magnesium, Vitamin D and Fluoride. Washington, DC: National Academy Press; 1997.
28. Subramanian ,K., Bergman,P .,and Henriques-Normark,B. Vitamin D Promotes Pneumococcal Killing and Modulates Inflammatory Responses in Primary Human Neutrophils .(**2017**) ;9(4):375-386. doi: 10.1159/000455969.
29. Hyppönen, E., Laara, E., Reunanen, A., Jarvelin, M. R. & Virtanen, S. M. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet. (**2001**) ;358:1500–1503.
30. Maestro, J., Campión, N., Dávila., and Calle,C.“Stimulation by 1,25-dihydroxyvitamin D_3 of insulin receptor expression and insulin responsiveness for glucose transport in U-937 human promonocytic cells,” *Endocrine Journal*. (**2000**) ; 47 (4): 383–391.
31. Williams, I. D., Caterson, G. J., Cooney, R. R., Zilkens., and Turtle,J.R. “High affinity insulin binding and insulin receptor-effector coupling: modulation by Ca2+,” Cell Calcium. (**1990**); 11(8): 547–556.
32. Ayat, B, Al-Ghafari, Khadijah S, Huda A. "Relationship between Serum Vitamin D and Calcium Levels and Vitamin D Receptor Gene Polymorphisms in Colorectal Cancer", *BioMed Research International*. (**2019**) vol. 2019, Article ID 8571541.
33. Ahmed, F, Mahmoud M. El Nokeety , A. Heikal et al . Serum 25-hydroxyvitamin D level is negatively associated with serum phosphorus level among stage 3a-5 chronic kidney disease patients. (**2022**) ; 42 (3) : 223-262.
34. Seo JW , Park TJ. Magnesium metabolism. *Electrolyte Blood Press.* (**2008**) ;6(2):86-95.
35. Tran H, Dang T, Nguyen T,Bao et al. Relationship between vitamin D status and the relevant parameters of glucose in patients with type 2 diabetes. J of diabetes, metabolic syndrome, and obesity. (**2021**) ; 14 (4) : 179-186.