IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
**Research Article**
February 2017 Vol.:8, Issue:3


# The Relationship between Vit D3 and Certain Bone Biochemical Markers in Obese and Non-Obese Type 2 Diabetic Patients in Babylon

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

***Hadeel Luay kareem,**Dr. Shaymaa Zahraw Nada,*** Dr. Muneam MakkiAL.Shawk**

**&**College of medicine/Karbala University/Iraq*

*&***Prof. College of medicine/ Babylon University/Iraq*

**Submission:** 27 January 2017
**Accepted:** 1 February 2017
**Published:** 25 February 2017



**Keywords:**25(OH) Vitamin D; ALP; Ca; PO4; T2DM; obesity.

**ABSTRACT**

**Background:** vitamin D, regulate the bone mineralization in human body, alteration of vitamin D may play a role in the development of T2DM.Vitamin D also has been recognized to regulate endocrine pancreatic function; it stimulates pancreatic beta cells proliferation and insulin secretion. And several studies suggest that vitamin D status may have a significant role in glucose homeostasis in general, and on the path physiology and progression of metabolic syndrome and type-2 diabetes in particular. **Aim:** The present study was planned to measure the levels of 25-hydroxy vitamin D, HbA1c and other biochemical markers of bone in obese and non-obese type 2 diabetic patients and compared their levels with healthy control subjects.**Material and Methods**: Sixty patients with T2DM (30 obese and 30 non-obese) and 30 age matched healthy controls were enrolled in this study. 25-hydroxy vitamin D was measured in serum by ELISA. HbA1c, phosphorus, total calcium and alkaline phosphatase was measured in plasma by colorimetric method**Results:** By using SPSS program the data were analyzed. its shows highly significant decrease in serum 25(OH)Vitamin D, $PO_4$ in patients group when compared with healthy control group ($p<0.001$), also there was highly significant decrease in their levels in obese with T2DM when compared with non-obese T2DM patients ($p<0.001$), HbA1c shows significantly increase in patient groups, and there was no significant different in total Calcium as well as in Alkaline phosphatase between patient and control($p>0.05$),.And there was a negatively significant correlation ($p<0.001$) among vitamin D and age, diabetic duration, blood pressure and smoking.**Conclusion:** The most important findings of this study suggest that the level of vitamin D which is considered as important markers of bone health was decreased in T2DM and especially in obese patient when compared with non-obese T2DM as well as when compared with healthy control group and it's significantly correlated with diabetic duration.

**INTRODUCTION:-**

Type 2 diabetes mellitus (T2DM) (also called non-insulin-dependent diabetes mellitus) causes abnormal carbohydrate, lipid and protein metabolism associated with insulin resistance and impaired insulin secretion. Insulin resistance is a major contributor to progression of the disease and to complications of diabetes (1). Insulin resistance is a key feature of obesity, metabolic syndrome, and T2DM (2) Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. Obesity is the final consequence of chronic positive energy balance, regulated by a complex network between endocrine tissue and the central nervous system (3). Body mass index (BMI) was defined as an individual of body weight divination by square of their height in meters that applies to both adult men and women. BMI was classified as (Underweight <18.50, Normal weight 18.50 - 24.99, overweight ≥25.00 and obese ≥30.00) (4). GLlycated hemoglobin (HbA1c) is a widely accepted marker for monitoring diabetes. It is important because it gives an idea to the risk of illness and the complication of diabetes (5). Vitamin D is a fat-soluble steroid hormone responsible for increasing intestinal absorption of calcium, iron, magnesium, phosphate, and zinc. Vitamin D is unique among hormones because it can be made in the skin from exposure to sunlight (6).

Vitamin D comes in two forms. Vitamin D2 is obtained from the UV irradiation of the yeast sterol ergosterol and is found naturally in sun-exposed mushrooms. Vitamin D3 is synthesized in the skin and is present in oil-rich fish such as salmon, mackerel, and herring; commercially available vitamin D3 is synthesized from the cholesterol precursor 7-dehydrocholesterol naturally present in the skin or obtained from lanolin (7). Obesity was associated with vitamin D deficiency because the body fat sequesters the fat-soluble vitamin (8).

Vitamin D has also been recognized to regulate endocrine pancreatic function; it stimulates pancreatic beta cells proliferation and insulin secretion. And several studies suggest that vitamin D status may have a significant role in glucose homeostasis in general, and on the pathophysiology and progression of metabolic syndrome and T2DM.The deficiency in vitamin D was associated with a reduced insulin secretion, which might be an important factor for the susceptibility of developing diabetes. Vitamin D has been proposed also as a possible therapeutic agent in the prevention and treatment of type-1 and type-2 diabetes (9).

The main mechanism of vitamin D is to enhance absorption of calcium from the intestine. Therefore insufficient calcium results from insufficient vitamin D from low intake or from low calcium intake. hypocalcemia was associated with both the development of metabolic syndrome and T2DM (10).

**SUBJECTS AND METHODS -**

**Subjects:** A case-control study was carried on 60 diabetic persons who were admitted to diabetic and endocrinology care center in Merjan hospital / Babylon province/Iraq and registered in the center as T2DM patients from May 2016 to August 2016. Thirty healthy persons were selected as a control group. The patients were subdivided into thirty non-obese and thirty obese T2DM patients.

All the population, that were included in this study, were informed about the aim of the study and they are convinced to participate in this study.

**Exclusion Criteria:** In this study, all patients with Type1 diabetic mellitus, hepatitis, patient with thyroid problem (hyper or hypothyroidism), renal failure, heart disease, liver disease, malignant disease, patients on chemotherapy, or chronic inflammatory diseases, based on clinical and laboratory investigations and alcoholic patients were excluded.

**Blood sample:** Venous blood was taken from all cases and control groups using disposable syringes. Five milliliters of blood was obtained from each subject, Two milliliters were added to an ethylene diamine tetra acetic acid (EDTA) tube for Hemoglobin A1C measuring, and Three milliliters were separated by centrifugation at (3000rpm) for 15min. the sera were stored frozen at (-20ºC) until assayed. Each serum sample was analyzed for 25-hydroxyvitamin D, phosphorus, total calcium and alkaline phosphatase.

**Methods:** Determination of serum 25-hydroxy vitamin D were analyzed by using ELISA technique by Calbiotech Company. This analysis was done in the laboratory department of Merjan medical city/ immunology unit using semi-automated ELISA according to corresponding associated manual principle of Calbiotech Company, HbA1c, phosphorus, total calcium and alkaline phosphatase was measured in serum by colorimetric method.

**Statistical analysis:** The data throughout this work was reported in the form of (mean value ± standard deviation). Quantitative differences between groups were determined by student

F-test, where differences considered as highly significant when ($p<0.001$). The data were processed with SPSS 19 version

**RESULTS:** The results in table (1) showed that serum 25(OH) Vitamin D and PO4 concentration were significantly lower in patients group when compared with control group. Also, there were significant differences between obese and non-obese T2DM patients. The results of ALP and Ca were non-significant different in obese and non-obese patient in comparison with control ($P>0.05$), and there was highly significant increase in HbA1c level in T2DM patient groups compared with control group. Table (2) shows highly significant negative correlation between vitamin D and age, smoking, Diabetes mellitus duration and blood pressure in T2DM patient. This study revealed that the mean of serum vitamin D concentration in all patient groups with age have lower level than in control ($P< 0.001$), as well the levels of non-obese group in age >65 seems to be higher than the levels of obese groups of patients at same age, as shown in table (3). The results in table (4) showed highly significant increase of vitamin D levels in control groups in male when compared with female groups, and the obese patients in both genders have Low levels of vitamin D when compared with other groups, Female patient groups showed higher vitamin D levels than its level in male patients group. However, ALP, Ca and $PO_4$ showed no significant results. The results in table (5) showed that vitamin D levels of smoking patient groups were highly significant decrease ($P< 0.001$) in comparison with healthy control.

This study revealed that the mean of vitamin D concentration of all studied groups have lower level than control at highly significant ($P< 0.0001$), as well the levels of newly dignosedT2DM patients group(>1 month) seems to be higher than the levels of other groups of patients, as shown in figure(1).

**Table (1): The difference between patient and control in the studded markers.**

| Total patients and control | | N | Mean± SD | P- Value of F-Test |
|---|---|---|---|---|
| Vitamin D | Control | 30 | 46.20±6.60 | 0.000 |
| | Obese | 30 | 37.03±7.49 | |
| | Non-Obese | 30 | 38.33±8.50 | |
| | Total | 90 | 40.52±8.52 | |
| ALP | Control | 30 | 135.80±29.18 | 0.066 |
| | Obese | 30 | 150.30±28.76 | |
| | Non-Obese | 30 | 151.76±28.55 | |
| | Total | 90 | 145.95±29.41 | |
| Ca | Control | 30 | 8.66±0.55 | 0.283 |
| | Obese | 30 | 8.89±0.81 | |
| | Non-Obese | 30 | 8.94±0.77 | |
| | Total | 90 | 8.83±0.72 | |
| PO4 | Control | 30 | 6.11±1.58 | 0.022 |
| | Obese | 30 | 4.63±1.56 | |
| | Non-Obese | 30 | 5.49±2.73 | |
| | Total | 90 | 5.41±2.10 | |
| BMI | Control | 30 | 28.59±3.56 | 0.000 |
| | Obese | 30 | 34.64±5.62 | |
| | Non-Obese | 30 | 26.12±2.73 | |
| | Total | 90 | 29.78±5.46 | |
| HbA1C | Control | 30 | 5.32±0.59 | 0.000 |
| | Obese | 30 | 6.41±1.10 | |
| | Non-Obese | 30 | 6.63±1.43 | |
| | Total | 90 | 6.12±1.23 | |

**Table (2): Correlation state of demographic features of patients and vitamin D.**

| | | Vit. D | Age | Smoking | Duration | B. P. |
|---|---|---|---|---|---|---|
| **Vit. D** | Pearson Correlation | 1 | | | | |
| | Sig. (2-tailed) | | | | | |
| **Age** | Pearson Correlation | -0.40** | 1 | | | |
| | Sig. (2-tailed) | 0.000 | | | | |
| **Smoking** | Pearson Correlation | -0.40** | 0.954** | 1 | | |
| | Sig. (2-tailed) | 0.000 | 0.000 | | | |
| **Duration of disease** | Pearson Correlation | -0.39** | 0.947** | 0.953** | 1 | |
| | Sig. (2-tailed) | 0.000 | 0.000 | 0.000 | | |
| **B P** | Pearson Correlation | -0.44** | 0.928** | 0.925** | 0.914** | 1 |
| | Sig. (2-tailed) | 0.000 | 0.000 | 0.000 | 0.000 | |

**Table (3): Relationship of vitamin D in DM patients and Control with age.**

| | | Age Groups | N | Mean ±SD | P. Value of F- Test |
|---|---|---|---|---|---|
| Vitamin D | Obese T2DM | 25- 34 years | 2 | 29.50±2.12 | 0.000 H S |
| | | 35 -44 years | 9 | 38.22±7.62 | |
| | | 45 -54 years | 11 | 37.09±7.85 | |
| | | 55 - 64 years | 6 | 40.00±7.15 | |
| | | > 65 years | 2 | 30.00±2.82 | |
| | Non-Obese T2DM | 25 - 34 years | 2 | 30.50±0.70 | |
| | | 35 -44 y | 5 | 44.00±9.51 | |
| | | 45 - 54 y | 10 | 35.20±5.51 | |
| | | 55 - 64 years | 8 | 38.37±10.05 | |
| | | > 65 y | 5 | 42.0±8.71 | |
| | Control | | 30 | 46.20±6.60 | |

**Table (4): Relationship of gender in Vit. D, ALP, Ca and PO4 levels in study group.**

| Parameters | | Male | | | Female | | |
|---|---|---|---|---|---|---|---|
| | | Obese T2D.M | Non-Obese T2DM | Control | Obese T2DM | Non-Obese T2DM | Control |
| Vitamin D | No | 13 | 19 | 15 | 17 | 11 | 15 |
| | Mean ± SD | 36.23 ± 6.86 | 36.73 ± 8.20 | 50.26 ± 4.81 | 37.64± 8.09 | 41.09 ± 8.67 | 42.13± 5.64 |
| | P. Value | 0.000 | | | | | |
| ALP | Mean ± SD | 156.38±26 .72 | 156.73±28 .57 | 133.13±31. 30 | 145.64±30.1 7 | 143.18±2 7.69 | 138.46± 27.73 |
| | P. Value | 0.147 | | | | | |
| Ca | Mean ± SD | 8.78±0.93 | 9.01±0.87 | 8.84±0.60 | 8.98±0.72 | 8.82±0.61 | 8.48±0.45 |
| | P. Value | 0.371 | | | | | |
| PO4 | Mean ± SD | 4.86±1.97 | 5.69±2.73 | 6.08±1.75 | 4.45±1.19 | 5.15±2.82 | 6.13±1.45 |
| | P. Value | 0.141 | | | | | |

**Table (5): Relationship Vit. D in Diabetes Mellitus patients and Control with smoking.**

| | | Smoking | N | Mean ±SD | P. Value of F-Test |
|---|---|---|---|---|---|
| Vitamin D | Obese T2DM | Smoker | 5 | 35.00±5.38 | 0.000 H.S |
| | | Non Smoker | 25 | 37.44±7.87 | |
| | Non-Obese T2DM | Smoker | 8 | 39.62±7.06 | |
| | | Non Smoker | 22 | 37.86±9.07 | |
| | | Control | 30 | 46.20±6.60 | |
| | | Total | 90 | 40.52±8.52 | |

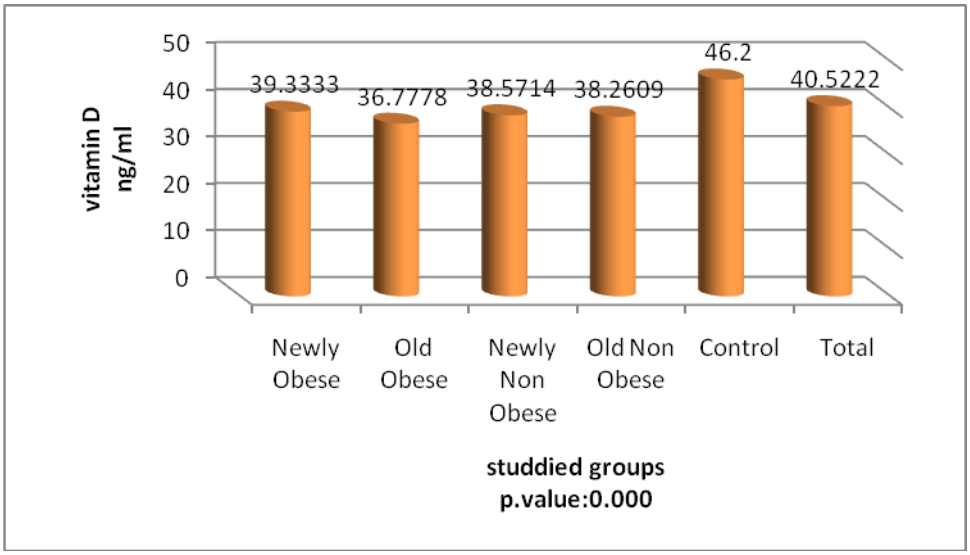


**Figure (1): The change in vitamin D concentration in studied group according to D.M duration**

**DISCUSSION:**

The deficiency of vitamin D causes multiple risk factors for increased skeletal fragility. This study showed that vitamin D levels were significantly decreased in patient groups compared with control group, several studies suggest that vitamin D status may have a significant role in glucose homeostasis in general, this result similar to Rana, *et al.* results who found that levels of vitamin D, calcium and parathyroid hormone were significantly less in diabetic patients as compared to healthy controls (11).

The level of vitamin D was significantly decreased in obese patient group when compared with non-obese patient group, this may be caused by the decreased exposure to sunlight (limited mobility of obese patients) excessive storage of 25-OH-D3 in adipose tissue, and inhibition of its synthesis in liver by the increased level of 1,25-dihydroxyvitamin D, this is in accordance with the results obtained by Holecki *et al*. who found that in obese subjects, serum concentration of 25-(OH)-D3, total calcium, and phosphorus were significantly lower in comparison with normal weight controls (6). Vogt *et al.* suggest that the association between lower 25(OH) D levels and an unfavorable lipid profile is stronger in individuals with abdominal obesity than in those with abdominal overweight or a normal waist circumference (12).

The present results show that PO4 levels were significantly decreased in patient groups compared with control group, decrease PO4 level may be because disorder in phosphorus

metabolism in diabetic patient, this result conform by Fang, and Li. who found that serum level of phosphate in type 2 diabetic group was significantly lower than that in the control group (P<0.05). and their results showed no significant correlation between the level of blood glucose and the serum level of phosphorus but in control group, the blood glucose and serum levels of phosphorus were positively correlated (r=0.226, P=0.042) (13 ). And this results relatively not agree with Yetkin *et al.* who found no significant difference in calcium and phosphorus between the diabetic group and controls (14).

This study was appeared non-significant difference in the level of total Ca between patient groups and control group may be because the measuring total Ca instead of the ionic Ca form, this result conform by Fang and Li. who found non-significant difference in the level of serum calcium and magnesium between patients with type 2 diabetes mellitus and control ( 13). However, the study by Rana, *et al*. not conform the present result who found that serum calcium were significantly lower in patients with T2DM when compared with healthy controls (11).

ALP concentration in serum of patient and control gives no significant different in comparison between them, may be because total ALP was measured not bone ALP isoenzyme, study by Kemink, *et al.* conform this result who found no differences in the mean serum ALP, BAP and osteocalcin levels between the diabetic patients and the controls (15).

In Joergensen *et al.* study found that Vitamin D levels were not associated with age, but low levels of vitamin D were weakly associated with elevated systolic blood pressure. This results weakly conform the present results because there was a significant correlation between age and blood pressure (16).

This study showed significantly different between male and female in vitamin D level this result not conform by Yetkin *et al*. study who found that men and women did not significantly differ in the prevalence of 25-OHD deficiency (14 ).

## REFERENCES

1-Gæde, P., Oellgaard, J., Carstensen, B., Rossing, P., Lund-Andersen, H., Parving, H.H. and Pedersen, O., Years of life gained by multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: 21 years follow-up on the Steno-2 randomized trial. *Diabetologia*.2016; *59*(11), 2298-2307.

2-Danve, A., Sehra, S., Jaykumar, D. and Kulkarni, S., Tumor necrosis factor inhibitors may improve glycemic control in patients rheumatoid arthritis and concomitant diabetes mellitus. *Osteol Rheumatol Open.* 2016; *J, 1*(1), 17-19.

3-Kushner. and Robert. Treatment of the Obese Patient (Contemporary Endocrinology). Totowa, NJ: Humana Press. 2007; 158.

4-Kanazawa, M., Yoshiike, N., Osaka, T., Numba, Y., Zimmet, P. and Inoue, S., Criteria and classification of obesity in Japan and Asia-Oceania. Asia Pacific journal of clinical nutrition.2002; 11(s8), S732-S737.
5-Titchener, J., A patient-centred clinical approach to diabetes care assists long-term reduction in HbA1c. Journal of primary health care. 2014;6(3),195-202.
6-Holick, M.F. and Chen, T.C., Vitamin D deficiency: a worldwide problem with health consequences. The American journal of clinical nutrition. 2008; 87(4), 1080S-1086S.
7-Holick MF Vitamin D deficiency. 2007; N Engl J Med 357:266–281.
8- Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF, Decreased bioavailability of vitamin D in obesity. Am J ClinNutr.2000; 72:690–693.
9-Parra, E.G., González-Casaus, M.L. and Villa-Bellosta, R., Vitamin D and Diabetes in Chronic Kidney Disease. In *Vitamin D in Chronic Kidney Disease*. 2016; 267-283.
10-Pittas, A.G., Lau, J., Hu, F.B. and Dawson-Hughes, B., The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. The Journal of Clinical Endocrinology & Metabolism.2007; 92(6), 2017-2029.
11-Rana, S., Morya, R.K., Malik, A., Bhadada, S.K., Sachdeva, N. and Sharma, G.A. relationship between vitamin D, parathyroid hormone, calcium levels and lactose intolerance in type 2 diabetic patients and healthy subjects. *Clinica Chimica Acta*. 2016; *462*,174-177.
12- Vogt, S., Baumert, J., Peters, A., Thorand, B. and Scragg, R., 2016. Effect of waist circumference on the association between serum 25-hydroxyvitamin D and serum lipids: results from the National Health and Nutrition Examination Survey 2001–2006. *Public Health Nutrition*, 1-10.
13-Fang, L. and Li, X., [Level of serum phosphorus and adult type 2 diabetes mellitus]. *Zhong nan da xue xue bao. Yi xue ban= Journal of Central South University. Medical sciences*. 2016; *41*(5), 502-506.
14-Yetkin, D.O., Kucukkaya, B., Turhan, M. and Oren, M., The effect of 25-hydroxyvitamin D levels on QT interval duration and dispersion in type 2 diabetic patients. *Croatian medical journal*. 2015; *56*(6), 525-530.
15-Kemink, S.A.G., Hermus, A.R.M.M., Swinkels, L.M.J.W., Lutterman, J.A. and Smals, A.G.H., Osteopenia in insulin-dependent diabetes mellitus; prevalence and aspects of pathophysiology. Journal of endocrinological investigation. 2000; 23(5), 295-303.
16-Joergensen, C., Gall, M.A., Schmedes, A., Tarnow, L., Parving, H.H. and Rossing, P., Vitamin D levels and mortality in type 2 diabetes. *Diabetes care*. 2010; *33*(10), 2238-2243.