

## Evaluation of Calcium, Phosphorus and Magnesium Level Among Vitamin D Deficient Diabetes Mellitus Patients in Khartoum State

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**Abstract:** Diabetes mellitus (DM) is increasing at an alarming rate both nationally and worldwide, there is increased evidence in correlation between vitaminD(vitD),calcium, phosphorus and magnesium, altered vitamin D and calcium homeostasis may play role in the development of type2 diabetes mellitus (T2DM) this study aimed to evaluate calcium, phosphorus and magnesium in vit D deficient T2DM. A cross-sectional study Was Conducted in Khartoum state, 120 type2 diabetic patients enrolled, aged between 25-80 years,then classified in to two groups based on vitamin D level (<30 ng/ml represent study group (deficient),while >30ng/ml represent control),Anthropometrics parameters (age, gender, and body mass index),diabetes duration were examined.Glucose,25hydroxyvitamin(25-OHvitD), calcium, phosphorus and magnesium level was measured. Prevalence of type 2 DM and BMI>26.5 more frequent in females (63%, 77.60%) respectively compared to males (37%, 65.90%).Diabetic females have highly vitD deficiency compared to males (75% vs. 45.45%),study showed correlation between vitD, calcium and magnesium ( $r = -0.2365, 0.4467$ ) respectively and inversely correlated with phosphorus ( $r = 0.4467$ ), although there was no different in mean of calcium, phosphorus and magnesium between two group (blood glucose<180 mg/dl and blood glucose>180mg/dl)*P*-value <0.05. our preliminary findings conclude calcium, magnesium and phosphate are essential nutrients in vitamin D deficient T2DM patients.

**Keywords:** diabetes mellitus, vitaminD, calcium, phosphorus, magnesium.

### INTRODUCTION

In Sudan knowledge of DM epidemic is limited. The most recent data come from a small-scale study that was carried out in 1996. The results of the study indicated a prevalence of 3.4%.4, but recent estimates place the diabetes population at around one million – around 95% of whom have t2DM[1].The term DM describes a metabolic disorder of multiple a etiology characterized by chronic hyperglycemia, resulting from defect in insulin secretion, insulin action or both [2]. Type 2 DM which accounts for 90–95% of those with diabetes (non–insulin-Dependent diabetes) type DM, or adult-onset diabetes, encompasses individuals Who have insulin resistance and usually have relative (rather than absolute) insulin Deficiency at least initially, and often throughout their lifetime, these individuals donot need insulin treatment to survive[3]. Most patients with this form of DM are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria May have an increased percentage of body fat distributed predominantly in the Abdominal region [4]. vitD is characterized as a regulator of homeostasis of bone and mineral Metabolism, but it can also provide non skeletal actions because vitD receptors have been found in various tissues including the brain, prostate, breast, colon, Pancreas, and immune cells VitD may

play an important role in modifying the Risk of DM. The incidence of t2 DM is increasing worldwide and results from a lack of insulin or inadequate insulin secretion following increases in insulin resistance, and it has been proposed that vitD deficiency plays an important role in insulin resistance resulting in diabetes[5]. The potential role of vitD deficiency in insulin resistance has been proposed to be associated with inherited gene polymorphisms including vitD-binding protein, vitD receptor, and vitD 1 alpha-hydroxylase gene[6]. VitD, calcium, phosphorus and magnesium are completely different structurally and while they cooperate with each other in the body for many functions, they each also have functions separate from each other. VitD has a direct role in regulating the amount of calcium found in the bloodstream which is available for the body to use. Therefore, problems with vitD deficiency often have symptoms that are related to the proper use of calcium[7]. There is evidence to suggest that altered vitD and calcium homeostasis may play role in the development of t2DM[8].

The role of vitD in t2DM suggested by cross sectional studies showing that low serum concentration of 25-hydroxy vitamin [25(OH)D] are associated with impaired glucose tolerance and diabetes [9]. The role of calcium in the development of type 2 DM is suggested

indirectly by Cross sectional studies in which high calcium intake has been found to be inversely associated with body weight and fatness[10].

### MATERIALS AND METHODS

Descriptive cross-sectional study was conducted during the period from (march to June 2014) in Khartoum state, 120 t2DM patients, aged between 25-80 years, patients were classified in to two groups based on vitD level, <30ng/ml considered as study group and >30ng/ml as control group after an overnight fasting 6 ml of overnight fasting peripheral blood was taken, the blood samples were centrifuged at 3000 rpm for 10 min and serum stored at -20°C, till utilized for different metabolic parameters

#### Estimation of vitD

Quantities of vitD level a solid phase competitive inhibition enzyme immunoassay was used to determine vitD(in use the vitD Elisa kit (lot E 140116AE) (EuroIMMUN AG) Germany according to the manufacturer protocols. 200 ML of sample diluted with Biotin Micro plate well which coated with monoclonal anti vitD antibodies .during incubation antigen antibodies reaction occurred. then unbound 25-OH vitD was removed by washing. 100 µl of streptavidin-peroxidase will added to detect bond Biotin labeled 25-OH vitD, 100 µl tetramethylbenzidine promotes a color reaction, the color intensity is inversely proportional 25-OH vitD. Concentration of the sample calculated using standards curve (Sunrise-TECAN) [11].

#### Estimation of glucose

##### Principle

Glucose oxidase catalyzes the oxidation of beta D- glucose present in the plasma to glucono-1,5 lactone with the formation of hydrogen peroxide. The action is hydrolysed to D-glyconic acid by peroxidase enzyme the hydrogen Peroxides broken down to water and oxygen, oxygen react with oxygen which Converted to colored compound measured spectrophotometry[12]. Briefly according to manufacturer 10 µl of plasma was added to 1ml of working reagent, and then incubated for 10 minute absorbance was read at 520nm.

#### Estimation of calcium

##### Principle

By using 8hydroxyquinoline-5-sulfonic acid to eliminate the interference of Magnesium, calcium ions combine with arsenazoIII to produce blue colored complex at a neutral solution the absorbency increase is directly proportional to the Concentration of calcium[13]. 10 µl of serum added to 1ml of reagent mix thoroughly at 37°C and read the Absorbance, 5 min later by Mindray BS-380.

#### Estimation of phosphorus

##### Principle

Ammonium molybdate combine with phosphate in present of sulphuric acid to produce A phosphomolybdate complex .the absorbency increase is directly proportional to the Concentration of phosphate[14]. 10 µl of serum added to 1ml of reagent mix thoroughly at 37c and read the absorbance 3 min later by Mindray BS -380.

#### Estimation of magnesium

##### Principle

By using the EGTA to eliminate the interference of calcium, magnesium ions combine with xylydyl blue magnesium complex at an alkaline solution, the absorbency increase is directly proportional to the concentration of magnesium [15]. 10 µl of serum added to 1ml of reagent mix thoroughly at 37 C and read the absorbance 5min later by Mindray BS-380.

#### Measurement of BMI

Weight and height were measured and BMI was calculated by dividing weight in (Kg) by square of height in (m).

#### Statistical Analysis

Data from all patients were presented as percentage and (mean±SD), differences between means of patients and control groups were considered statistically significant with *p*-value threshold <0.05 using independent *T*-test. Significant correlation (*r*) was calculated using linear correlation test.

### RESULTS

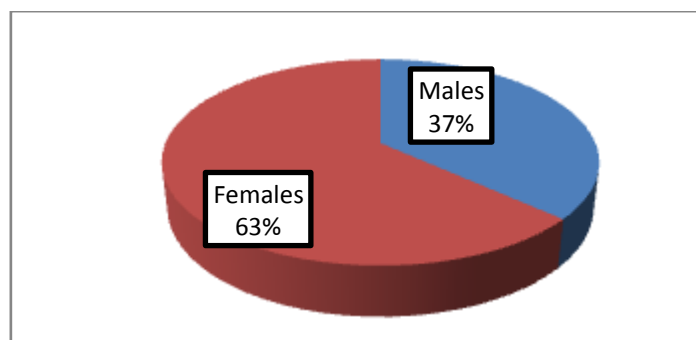


Fig-1: Shows percent of male and female among t2DM (n=120)

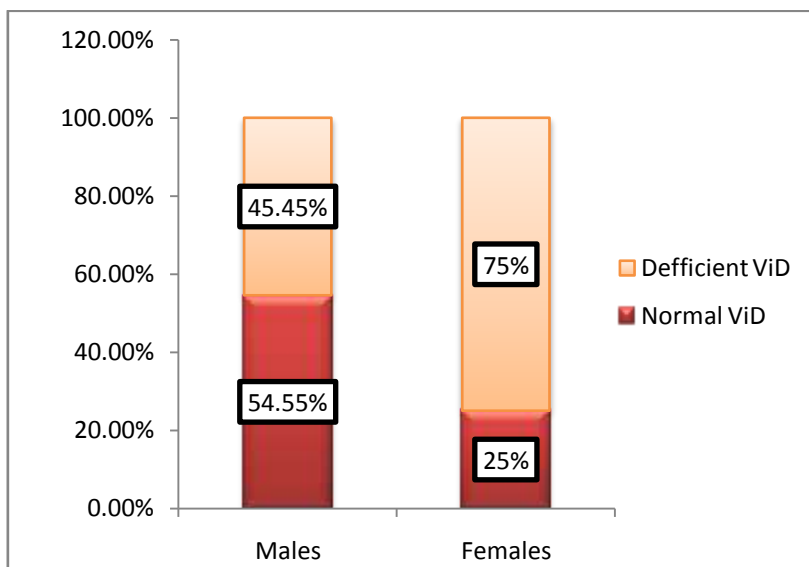


Fig-2: Representing vitD deficiency among type2 DM males and females results expressed as percentage (%) in (n=120).

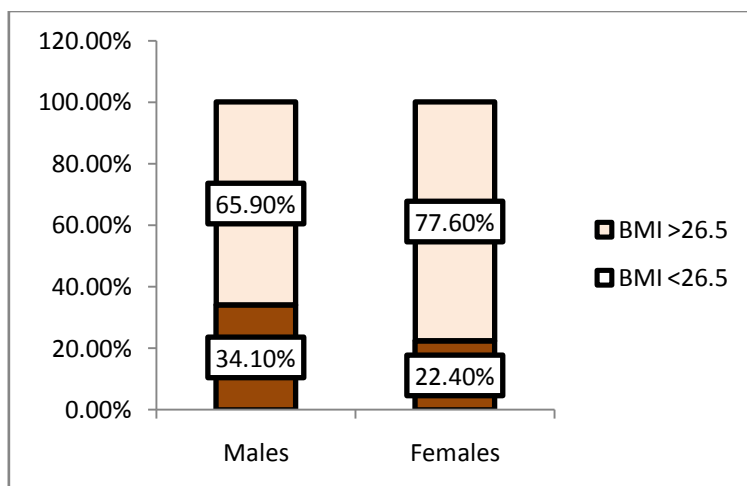


Fig-3: Shows the percentages of BMI (<26.5 and > 26.5) among gender, results presented as percentages (%).

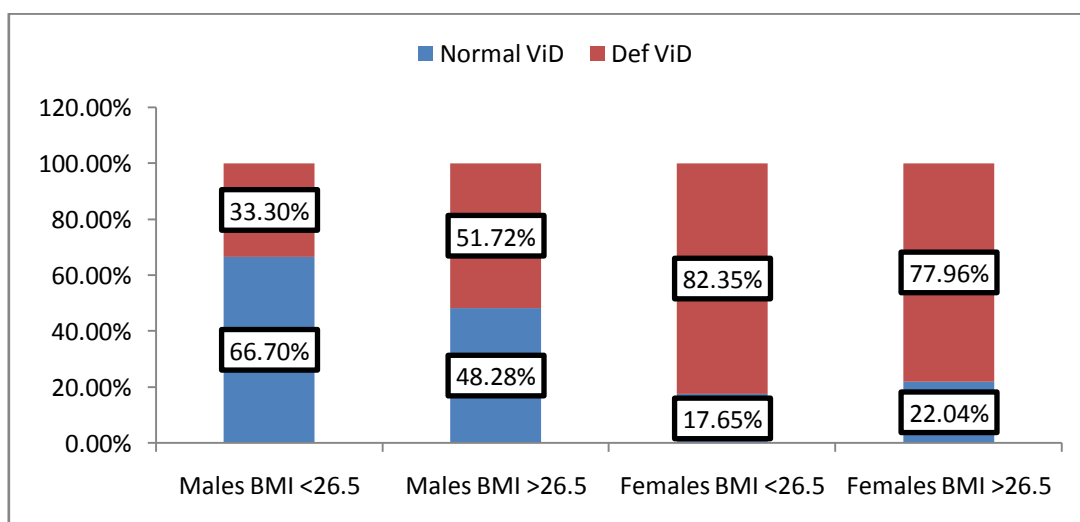


Fig.4: Presented association between vitD, BMI and gender among type2 DM patients, results expressed as percentage (%).

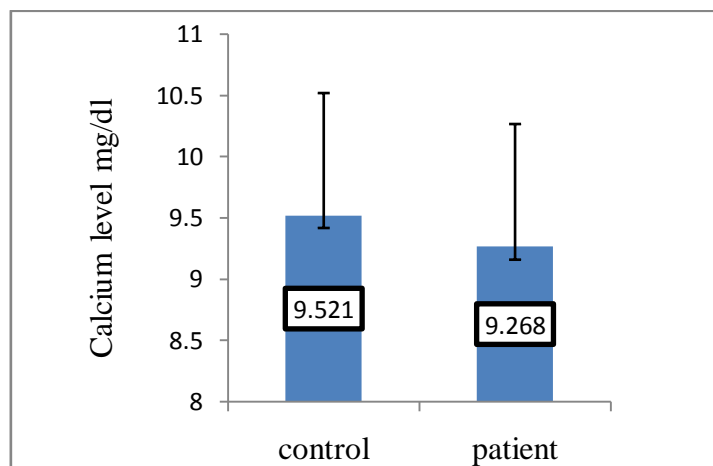


Fig-5: Show mean calcium in study group (control and patient), result presented as (mean±SD) and P value <0.05.

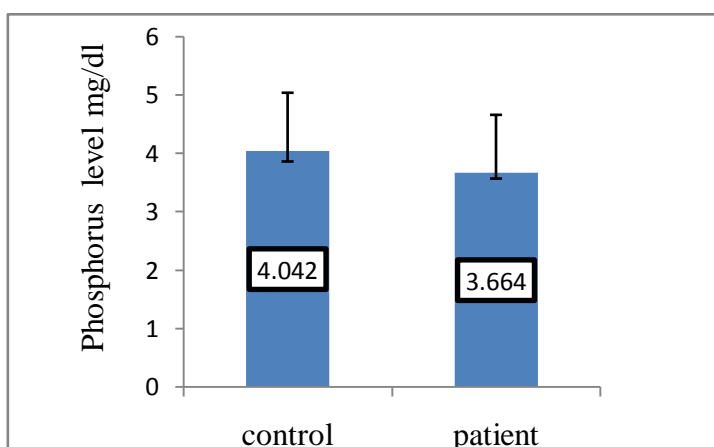


Fig-6: Show mean phosphorus level in study group (control and patient), result presented as (mean±SD) and P value <0.05

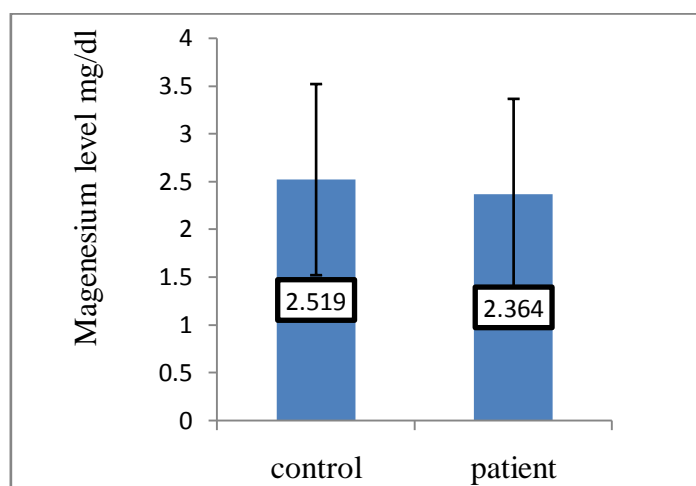


Fig-7: Show mean magnesium level in study group (control and patient), results presented as (mean±SD) and P value <0.05.

Table-1: Showed correlation between vitD and calcium, phosphorus and magnesium

Parameters	r	Sig
Calcium	0.2365	0.0147
Phosphorus	-0.0942	0.3367
magnesium	0.4467	0.0000

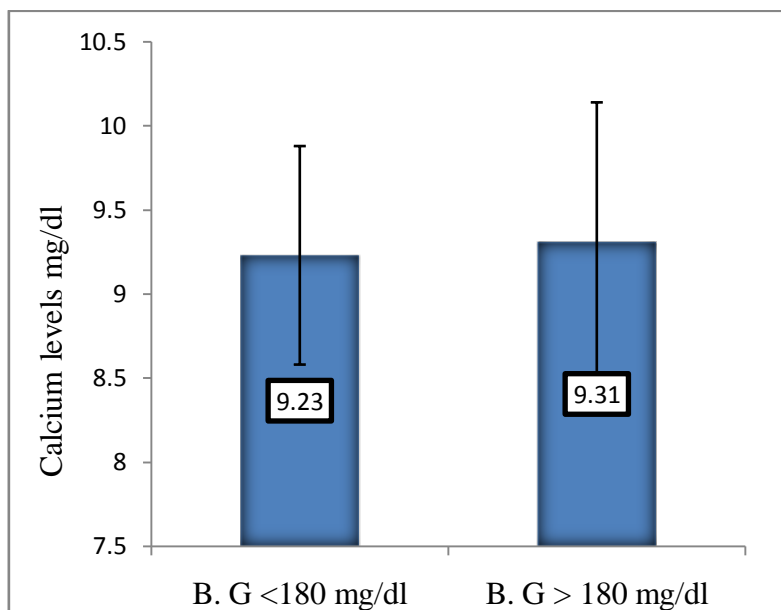


Fig-8: Shows mean of calcium level in group one (blood glucose <180 mg/dl) Group two (blood glucose >180mg/dl), results express as (mean±SD) and *P*-value <0.05.

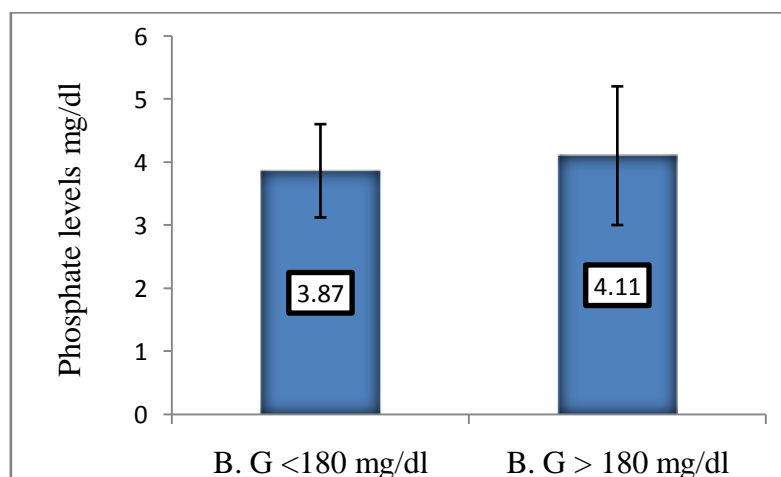


Fig-9: Show mean of phosphorus level in group one (blood glucose <180 mg/dl) Group two (blood glucose >180mg/dl), result express as (mean±SD) and *P*-value <0.05.

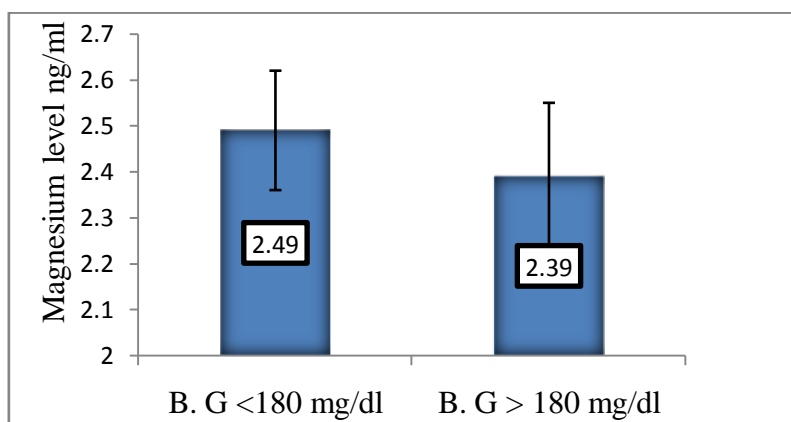


Fig-10: Show mean of magnesium level in group one (blood glucose <180 mg/dl) Group two (blood glucose >180mg/dl), result express as (mean±SD) and (*P*-value <0.05).

## DISCUSSION

DM is major health problem that is approaching epidemic proportions globally. Worldwide, the prevalence of chronic non communicable disease is increasing at alarming rate due to change in life style. Recent studies have demonstrated correlation between low vitD level and development of type 2 DM. In addition there is association between D.M ,calcium according to result of new study low level of serum calcium and vitD could be linked to type 2 D.M. The present study aimed to evaluate calcium, phosphorus and magnesium among vitD deficient type2 DM patient

The present study showed that, the prevalence of type2 D.M are more frequent in female (63%) than male (37%) this finding agreed with previous study done by Esayas Hare got who reported that in many countries of sub- Saharan Africa women are more likely to be expected to have higher prevalence of DM[16].

Also in the present study showed that, obesity are more common in female (77.6%) than in male (65.9%) this result agreed with previous study done by R martorell who stated that the obesity effect women more than men[17].

This study showed that ,vit D deficient are more frequent in type2 D.M female (75%) than male (45%) this finding confirmed by Monsour Al zahariani who reported that type2 DM female were more prone for vitD deficiency[18].

In our study, there was no significant different in the calcium, phosphorus level but there is significant different in magnesium level between those who had a normal vitD and deficient vitD. This finding agreed with previous study done by Hashemipours who reported that the calcium and phosphorus level don't change in mild vitD deficiency[19]. another study confirm our finding done by Deng who stated that there is significant different in the level of magnesium between those who had a normal vitD and deficient vitD[20]. In our study there was a correlation between calcium, magnesium and vitD .phosphorus was inversely correlated to vitD ,this result is similar to study done by Chih. chien sung [5].

Anastassios Pittas reported that by observational study show relatively consistent association between low vitD, calcium status and development of type 2 D.M or dairy intake and prevalent type2 D.M this result is similar to our study [21]. However the available human data are limited because most observational studies are cross-sectional while prospective studies have not measured vitD concentration future research should focus on studies within prospective observational cohort to clarify and quantify the association between calcium intake and vitD concentration rather than self-reported intake of

vitD and incident type2 D.M and define the individual contribution of each nutrient on type2 D.M.

## CONCLUSION

vitD and calcium insufficiency may influence glycemia while combined supplementation with both nutrient may be beneficial optimizing glucose metabolism

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