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# Plasma Calcium and Phosphorus Levels and Cardiovascular Disease Risks in Egyptian Type 2 Diabetic Patients

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## Abstract

**BACKGROUND:** Cardiovascular (CV) complication of diabetes is considered an important issue that needs deep investigations. The levels of plasma calcium (Ca) and phosphorus (P) have been implicated as having an association to CV diseases (CVDs).

**AIM:** The objective of the present research was to study the plasma levels of both Ca and P and their association to the atherogenic ratio; total cholesterol (TC): High-density lipoprotein-cholesterol (HDL-C) and the plasma albumin in male and female patients with type 2-diabetes. The interrelation between anthropometric parameters represented by body mass index (BMI), waist circumference, and waist/hip ratio with Ca and P was studied. Furthermore, the association between plasma Ca and P with their dietary intake was investigated.

**SUBJECTS AND METODS:** Thirty-one type 2-diabetic male and female patients participated in the study, in addition of ten healthy subjects. Biochemical parameters, anthropometric measurements, and nutrients' intake were assessed. Biochemical parameters include plasma Ca, P, lipid profile, albumin, liver function tests, and creatinine. The patients were classified to different groups according to sex and BMI.

**RESULTS:** Plasma Ca levels of female patients of BMI >30 kg/m$^2$ demonstrated significant increase compared to the control group. All male and female patients showed significant increase in plasma P compared to the control group. Glycosylated hemoglobin of male and females showed significant high values compared to the control group except for diabetic male of BMI >30 kg/m$^2$ that showed insignificant increase. No significant changes in plasma triglycerides and low-density lipoprotein-cholesterol levels were noticed compared to the control. Plasma TC of patients showed significant high levels compared to the control group. The levels of HDL-C of patients were significantly lower than that of the control. The ratios of TC/HDL-C in diabetic patients either male or female and whatever their BMI were significantly higher than that of the control. No significant changes in plasma activities of alanine transaminase and aspartate transaminase and bilirubin levels were observed among the different groups including the control. Plasma albumin levels demonstrated significant reduction compared to the control group whatever the sexes or BMI of the patients. Plasma creatinine levels of the different diabetic groups showed insignificant change from the control group except for the male group of BMI <30 kg/m$^2$ that showed significant elevation. In male, plasma Ca showed significant negative correlation with albumin and positive correlation with creatinine and dietary Vitamin D. In female, a significant positive correlation was noticed between plasma and dietary P while a negative correlation was observed between plasma Ca and dietary iron.

**CONCLUSION:** Elevated plasma P together with reduced plasma albumin and elevated TC/HDL-C may reflect an association of plasma P to CVD in male and female diabetic subjects while high plasma Ca might predict CVD in only female diabetic patients of BMI >30 kg/m$^2$.

## Introduction

Type 2-diabetes imposes a major public health burden worldwide presumably due to such severe complications such as blindness, renal failure, and cardiovascular disease (CVD) risks. The number of diabetic subjects is in continual increase [1], [2], [3]; an issue that require thorough investigation of the disease to prevent the progression and development of such critical complications. Metabolic changes including disturbances in serum calcium (Ca) and phosphorus (P) could exacerbate the progression to type 2-diabetes due to dyslipidemia, hyperglycemia, and hyperuricemia [4].

It is well known that Ca plays a key role in bone mineralization and has a wide range of biological functions [5]. Emerging epidemiologic evidence has proposed an association between elevated serum Ca and metabolic abnormalities including the development of type 2 diabetes [6]. It has been shown that insulin resistance and secretion depend on Ca homeostasis. Insulin secretion as response of elevated blood glucose is a Ca dependent process since increased cytosolic Ca leads to insulin stimulated glucose transporter activity [7], [8]. Since the defects in insulin secretion and action are in intimate relation to type 2-diabetes; therefore, abnormal Ca homeostasis is expected in type-2diabetes. Abnormal Ca regulation may participate in reduced β-cell function, thereby inducing altered glucose homeostasis [9], [10]. It is worthy to investigate whether the increased Ca level is a cause or a result of diabetes and whether the dietary Ca intake may have a

role in this respect. Biomarkers related to Ca metabolism are 25-hydroxy Vitamin D, P, and parathyroid hormone which themselves have been associated with diabetes [11]. The body adjusts Ca transport in the bone, kidney, and intestine to restore its concentration [5]. It was reported that serum Ca correlates with serum cholesterol and blood glucose. Furthermore, serum Ca has been demonstrated to be an independent risk factor for myocardial infarction [12]. Other study showed that circulating Ca is a risk factor for vascular disease which might be mediated by other cardiovascular (CV) risk factor such as circulating lipids, blood pressure, and body mass index (BMI); however, there appears to be a residual independent effect of serum Ca. A causative role of Ca might be related to polymorphisms of Ca-sensing receptors inducing elevated Ca with association to CVD. Ca supplements were shown to accelerate CVD in dialysis patients, in less severe renal failure and in those without overt renal disease. However, dietary Ca has no consistent adverse effect on CV health. Therefore, dietary Ca rather supplements is encouraged [13]. On the other hand, serum Ca is an independent predictor of adverse outcomes 7 years post-acute myocardial infarction [14]. Potential race difference was reported in Ca metabolism where black has stronger association with high serum Ca than for white subjects [15]. Therefore, it is worthy to study Ca metabolism in different races and communities.

Hyperphosphatemia has been demonstrated in uncontrolled diabetes which might likely be due to a transcellular shift. Potential factor responsible for the shift is serum glucose, through its osmotic effect [16]. High serum P contributes to vascular and metabolic disturbances in elderly patients with type 2 diabetes mellitus (DM) and renal impairment [17]. In other study, elevated serum P is recognized as independent predictor for advanced vascular disease especially in chronic kidney disease [18]. Phosphate is essential for life since it participates in cell membrane structure such as nucleic acids, phospholipids, and adenosine triphosphate. P homeostasis is affected by the intestine, parathyroid glands, kidney, and bone interactions. Serum P level is a reflection of dietary P absorption from the gut, its excretion and reabsorption in the kidney and P movement between extracellular and skeletal pools. Parathyroid hormone is important for regulation of serum P by mediating P removal by the kidney [19]. Higher levels of serum P have been reported to be associated with adverse CV outcomes even when within the normal range [20].

Based on the aforementioned facts, more studies are required to understand the factors affecting Ca and P serum levels in type 2 DM and their risks toward CVD. Moreover, the elevated atherogenic ratio of total cholesterol (TC): High-density lipoprotein-cholesterol (HDL-C) together with the reduced plasma albumin might participate in predicting CVDs events in diabetic patients [21], [22].

The objective of the present research was to study plasma Ca and P levels in type 2 Egyptian diabetic patients and their association to CV risk. This would be accomplished by assessing the interrelation of plasma levels of both Ca and P with the atherogenic ratio; TC: HDL-C; and the plasma albumin in male and female diabetic patients. The association between anthropometric parameters represented by BMI, waist circumference (WC), and waist/hip ratio (WHR) with Ca and P was studied. Furthermore, the connotation between plasma Ca and P with their dietary intake was investigated.

## Subjects and Methods

### Subjects

The study included 31 patients (16 males and 15 females) diagnosed with type 2 diabetes recruited as inpatient and outpatient from the clinic of internal medicine, Al-Zahraa University Hospital, Al-Azhar University, Cairo, Egypt. Their ages ranges from 45 to 70 years old. Ten healthy subjects with matched age and sex were enrolled in the study as control healthy subjects. Diabetic patients with hypertension were 63% of the total sample. Excluded patients were those with autoimmune diseases such as rheumatoid arthritis and lupus erythematosus and those with renal failure.

### Design of the study

The study was carried out according to the National Research Centre Ethics Committee, Cairo, Egypt. All participants provided written informed consent. All diabetic subjects were assessed nutritionally through measuring anthropometric parameters and taking questionnaires for dietary intake through 24 h dietary recall and the frequency of consumed food items. The assessed anthropometric parameters were height, weight, WC, and hip circumference. The weight and height were taken while subjects were standing with minimal clothing and no shoes. The waist and hip circumferences were measured by standard non-flexible tape; the individual should stand with feet close together, arms at the side and body weight evenly distributed, the subjects should be relaxed and the measurements were taken at the end of normal respiration [23]. BMI was calculated for each subject according to the equation; weight in kg/square height in meters. WHR was calculated for each subject [24]. The daily nutrients' intakes were calculated using the software of Food Composition table (2006) and the computer program of NutriSurvey program 2007 [25], [26]. The adequacy of nutrients intake was evaluated as percentage of the standard recommended dietary allowance (RDA) [27], [28].

Blood samples were obtained from fasted subjects (patients and control). A part of blood was collected on EDTA for determination of glycosylated hemoglobin using kit supplied from TECO DIAGNOSTICS, USA [29], another part of blood was received on heparin. The heparinized blood samples were centrifuged to obtain the plasma for determination of Ca and P by colorimetric methods using kits obtained from SPINREACT and CHEMELEX (Spain), respectively [30], [31]. Plasma triglycerides (TGs), TC, low-density lipoprotein-cholesterol (LDL-C), and HDL-C were assessed by colorimetric techniques using commercial kits from BioSystem, Spain [32], [33], [34], [35]. The atherogenic ratio TC/HDL-C was calculated for each subject as an indicator of risk factor for CVDs. Plasma alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activities and bilirubin were determined as liver function tests [36], [37], [38]. Plasma albumin was estimated as mentioned previously [39]. Plasma creatinine was determined for assessing kidney function [40].

The study was subjected to two classifications. First, diabetic patients were divided into two groups (male group and female group). The anthropometric parameters of male and female patients were compared to standard values as reported previously [23], [41], [42]. Daily nutrients' intakes of male patients were compared to that of female and both were calculated as percentile of RDA. The different biochemical parameters of male patients were compared to female and both groups were compared with that of the control group. Second, the entire assessed anthropometric and biochemical parameters and nutrients' intake were classified under the male and female groups with BMI> and <30 kg/m$^2$ as obese and non-obese male and female patients to assign the difference of such data in each case.

### Statistical analysis

The data were expressed as mean ± SE and were statistically assayed using one way analysis of variance test followed by *post-hoc* Multiple Comparison, Tukey test when more than two groups were compared. In case of comparing only two groups, Independent-Samples t-test was applied. Pearson correlation was also used for assessing the correlations between different parameters. The values were considered statistically significant when p < 0.05. The SPSS statistical program, version 21 was used.

## Results

The age and anthropometric parameters of male and female diabetic patients are compiled in

**Table 1: Anthropometric parameters of male and female diabetic patients (Mean ± SE)**

| Parameters | Male | Female | Normal values for male | Normal values for female |
|---|---|---|---|---|
| Age (years) | 56.9 ± 2.7 | 56.7 ± 3.4 | - | - |
| Weight (kg) | 84.3 ± 3.6 | 95.3 ± 5.9 | - | - |
| Height (m) | 1.7 ± 0.01 | 1.7 ± 0.01 | - | - |
| BMI (kg/m$^2$) | 29.9 ± 1.4 | 34.3 ± 2.1 | 18.5–24.9$^\Delta$ | 18.5–24.9$^\Delta$ |
| Waist circumference (cm) | 98 ± 2.2 | 107.9* ± 3.7 | 94 or less$^\#$ | 80 or less$^\#$ |
| Hip circumference (cm) | 114.9 ± 2.6 | 131* ± 3.1 | - | - |
| Waist/hip ratio | 0.85 ± 0.01 | 0.82 ± 0.01 | 0.90 or less$^@$ | 0.80 or less $^@$ |

$^\Delta$: [41], $^\#$: [23], $^@$: [42], *: Values statistically significant when diabetic male group were compared with diabetic female group (p < 0.05), BMI: Body mass index.

Table 1. It can be noticed that the mean age and height of the male and female patients showed insignificant changes, they are more or less have the same values. Waist and hip circumference of female were significantly higher than that of male while there were no significant differences concerning BMI and WHR of the two groups. BMI and WC of male and female were higher than their corresponding standard values while their WHR fall more or less within the standard levels.

Different biochemical parameters of male and female diabetic patients are represented in Table 2. Male and female patients demonstrated higher levels of Ca compared to control; the increase was significant in case of females. Plasma P showed significant high values when males and females were compared to control. Glycosylated hemoglobin percentages of both male and female diabetic patients were significantly higher compared to control. Plasma TGs of male and female patients showed higher levels compared to control; although the levels appear very high compared to control; however, the changes were insignificant that could be due to variations of values within the group which were reflected in the high standard errors. Plasma TC levels and TC/HDL-C ratios of male and female diabetics were significantly higher than the control group while LDL-C showed insignificant changes. The plasma levels of HDL-C of both male and female diabetics showed significant reduction compared to the control group. No significant changes in ALT, AST, and ALP activities, bilirubin, and creatinine were present among the different groups including the control. Plasma albumin levels of both male and female patients exhibited significant

**Table 2: Glycosylated hemoglobin % and plasma biochemical parameters of male and female diabetic patients (Mean ± SE)**

| Parameters | Male | Female | Control |
|---|---|---|---|
| Calcium (mg/dl) | 9.24$^{ab}$ ± 0.35 | 9.75$^b$ ± 0.48 | 8.68$^a$ ± 0.18 |
| Phosphorus (mg/dl) | 6.08$^b$ ± 0.39 | 6.25$^b$ ± 0.51 | 3.87$^a$ ± 0.12 |
| Glycosylated hemoglobin % | 10.00$^b$ ± 1.04 | 10.54$^b$ ± 0.67 | 5.63$^a$ ± 0.07 |
| TG (mg/dl) | 159.1$^a$ ± 36.4 | 120.2$^a$ ± 9.4 | 109.4$^a$ ± 2.7 |
| TC (mg/dl) | 170.5$^b$ ± 11.61 | 165.3$^b$ ± 14.52 | 110.00$^a$ ± 2.80 |
| LDL-C (mg/dl) | 95.2$^a$ ± 9.9 | 85.4$^a$ ± 9.9 | 96$^a$ ± 5.6 |
| HDL-C (mg/dl) | 32.21$^b$ ± 2.25 | 34.25$^b$ ± 1.38 | 46.1$^a$ ± 1.08 |
| TC/HDL-C | 5.85$^b$ ± 0.70 | 4.8$^b$ ± 0.36 | 2.4$^a$ ± 0.10 |
| ALT (U/L) | 26.8$^a$ ± 3.9 | 24.8$^a$ ± 3.3 | 31.2$^a$ ± 0.39 |
| AST (U/L) | 27.3$^a$ ± 2.3 | 23.3$^a$ ± 2.9 | 30$^a$ ± 0.4 |
| ALP (IU/L) | 73.6$^a$ ± 2.1 | 76.3$^a$ ± 5.8 | 69.8$^a$ ± 1.6 |
| Bilirubin (mg/dl) | 0.7$^a$ ± 0.11 | 0.6$^a$ ± 0.05 | 0.9$^a$ ± 0.03 |
| Albumin (g/dl) | 3.3$^a$ ± 0.13 | 3.4$^a$ ± 0.14 | 4.3$^b$ ± 0.06 |
| Creatinine (mg/dl) | 1.2$^a$ ± 0.11 | 0.95$^a$ ± 0.08 | 0.89$^a$ ± 0.04 |

In each row; same letters mean non-significant difference while different letters mean significant difference at p < 0.05. TG: Triglycerides, TC: Total cholesterol, LDL-C: Low-density lipoprotein-cholesterol, HDL-C: High-density lipoprotein-cholesterol, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase.

reduction compared to the control group. Although no significant changes were detected between male and female patients concerning all the determined biochemical parameters; a prominent changes in dyslipidemia and TC/HDL-C were seen in male compared to female while Ca and P levels and glycosylated hemoglobin percentage in female were higher than male.

Table 3 collects and compares the anthropometric data of male and female patients that have BMI> and <30 kg/m$^2$, that is, obese and overweight subjects, respectively, in addition to standard normal levels. No significant changes of the ages and heights of the male and female having BMI> and <30 kg/m$^2$ were noticed. Body weights of males having BMI >30 kg/m$^2$ showed significant lower values compared to that of females; while no significant changes were noticed on comparing both sexes having <30 kg/m$^2$. As expected, body weights of patients having BMI >30 kg/m$^2$ were significantly higher than those having BMI <30 kg/m$^2$, this is true when applied on either males or females. BMI of both male and females under the four groups were higher than the corresponding standard normal ranges. It could be noticed that waist and hip circumferences of females were significantly more than that of males having BMI >30 kg/m$^2$ while only insignificant more values were seen when BMI was <30 kg/m$^2$. WC of females having BMI >30 kg/m$^2$ was significantly higher than those having BMI <30 kg/m$^2$, this is not true when applied on males. Hip circumference of females having BMI >30 kg/m$^2$ showed insignificant change from those having BMI <30 kg/m$^2$, this is also true concerning male patients. No significant changes in WHR were observed between male and female whatever the BMI was.

The biochemical parameters of male and female diabetic patients classified according to BMI< or >30 kg/m$^2$ are compiled in Table 4. Plasma Ca levels of female patients of BMI >30 kg/m$^2$ demonstrated significant increased values compared to those of BMI <30 kg/m$^2$ and that of the control. All male and female patients showed significant increase in plasma levels of P compared to the control group whatever the BMI was. No significant changes were noticed among the four male and female groups however both male and female groups of BMI <30 kg/m$^2$ showed higher Ca levels than those of BMI >30 kg/m$^2$. Glycosylated hemoglobin showed insignificant changes when male and female diabetic patients of BMI> or <30 kg/m$^2$were compared with each other's while such groups demonstrated significant high values compared

to the control group except for diabetic male of BMI >30 kg/m$^2$ that showed insignificant change. No significant changes in plasma TG and LDL-C levels were noticed among the different groups including the control. No significant changes in plasma TC levels were observed among the different diabetic groups, while the values of the four groups showed significant high levels compared to the control group. It could be noticed that the TC levels of male and female patients of BMI >30 kg/m$^2$ were appreciably higher than those of BMI <30 kg/m$^2$ but insignificantly. The levels of HDL-C of the four diabetic patients groups were significantly lower than that of the control, while male and female diabetic patients of BMI >30 kg/m$^2$ showed lower values than those having BMI <30 kg/m$^2$ but with insignificant differences. The ratio of TC/HDL-C of the four diabetic patients groups was significantly higher than that of the control; meanwhile, male and female diabetic patients of BMI >30 kg/m$^2$ showed higher values than those having BMI <30 kg/m$^2$ but with insignificant differences. No significant changes in plasma activities of ALT and AST and bilirubin levels were observed among the different groups including the control. Plasma ALP activities of female patients having BMI >30 kg/m$^2$ demonstrated significant increase compared to the control group and compared to female of BMI <30 kg/m$^2$ while did not have significant change from male patients under both body mass indices. All male patients and only the females of BMI <30 kg/m$^2$ did not show any significant changes in ALP when compared to each other and compared to the control group. Plasma albumin levels of the four different groups with different sex and BMI showed insignificant changes when compared to each other while demonstrated significant reduction compared to the control group. Plasma creatinine levels of the different diabetic groups showed insignificant change from the control group except for the male group of BMI <30 kg/m$^2$ that showed significant elevation.

The different nutrients' intakes are represented by Tables 5 and 6. Table 5 showed the dietary intake of female and male patients without any further classification, however, Table 6 demonstrated such dietary intake when male and female were classified as those having either BMI >30 kg/m$^2$ or <30. In Table 5, female patients showed higher intake of carbohydrate, fat, Ca, and total calories than those of males, meanwhile males' intakes from protein, fibers, P, iron and Vitamin D were higher than that of females with significant differences in case of fibers and iron. It can be

**Table 3: Anthropometric parameters of diabetic patients divided under male and female according to the BMI<or>30 kg/m$^2$ (Mean ± SE)**

| Parameters | Male BMI>30 | Male BMI<30 | Female BMI>30 | Female BMI<30 | Normal values for male | Normal values for female |
|---|---|---|---|---|---|---|
| Age (years) | 56.3[a] ± 2.6 | 55.5[a] ± 4.8 | 57.2[a] ± 2.8 | 57.6[a] ± 7.2 | - | - |
| Weight (kg) | 94.9[b] ± 2.7 | 73.9[a] ± 2.8 | 111[c] ± 7.2 | 80.4[a] ± 2.1 | - | - |
| Height (m) | 1.7[a] ± 0.02 | 1.7[a] ± 0.02 | 1.7[a] ± 0.01 | 1.7[a] ± 0.02 | - | - |
| BMI (kg/m$^2$) | 34.4[b] ± 1.1 | 25.7[a] ± 0.6 | 39.7[c] ± 2.7 | 29.3[a] ± 0.1 | 18.5–24.9* | 18.5–24.9* |
| Waist circumference (cm) | 98.7[a] ± 3.4 | 97.4[a] ± 2.7 | 115.3[b] ± 5.9 | 101[a] ± 2.4 | 94 or less[#] | 80 or less[#] |
| Hip circumference (cm) | 113.4[a] ± 3.5 | 117[a] ± 3.6 | 137.5[b] ± 4.03 | 125[ab] ± 3.9 | - | - |
| Waist/hip ratio | 0.87[a] ± 0.02 | 0.83[a] ± 0.01 | 0.84[a] ± 0.02 | 0.81[a] ± 0.02 | 0.90 or less[@] | 0.80 or less[@] |

*: [41], #: [23], @: [42]. In each row; same letters mean non-significant difference while different letters mean significant difference at p < 0.05, BMI: Body mass index.

**Table 4: Biochemical parameters of male and female diabetic patients classified according to BMI<or>30 kg/m² (Mean ± SE)**

| Parameters | Male BMI >30 | Male BMI <30 | Female BMI >30 | Female BMI <30 | Control |
|---|---|---|---|---|---|
| Calcium (mg/dl) | 8.9[a] ± 0.3 | 9.57 [a] ± 0.6 | 11.04 [b] ± 0.6 | 8.47[a] ± 0.1 | 8.68[a] ± 0.2 |
| Phosphorus (mg/dl) | 5.93[b] ± 0.7 | 6.23 [b] ± 0.4 | 5.85 [b] ± 0.6 | 6.65[b] ± 0.8 | 3.87[a] ± 0.1 |
| Glycosylated hemoglobin % | 8.3[ab] ± 0.97 | 11.1[b] ± 1.8 | 10.95 [b] ± 1.1 | 10.4[b] ± 0.6 | 5.6[a] ± 0.1 |
| TG (mg/dl) | 167.7[a] ± 56.1 | 157.4[a] ± 51.03 | 113.7 [a] ± 13.3 | 127.7[a] ± 10.9 | 109.4[a] ± 2.7 |
| TC (mg/dl) | 177.7[b] ± 10.03 | 163.3[b] ± 14.52 | 173.8[b] ± 14.80 | 156.7[b] ± 12.03 | 110[a] ± 2.80 |
| LDL-C (mg/dl) | 94.9[a] ± 10.08 | 95.6[a] ± 18.04 | 83.4[a] ± 10.9 | 91.7[a] ± 16.4 | 96[a] ± 1.8 |
| HDL-C (mg/dl) | 31.27[a] ± 3.2 | 33.1 [a] ± 3.4 | 33.67[a] ± 1.9 | 34.8[a] ± 2.2 | 46.1[b] ± 1.1 |
| TC/HDL-C | 6.13[b] ± 0.9 | 5.57[b] ± 1.2 | 5.15[b] ± 0.5 | 4.27[b] ± 0.6 | 2.4[a] ± 0.1 |
| ALT (U/L) | 22.86[a] ± 3.9 | 30.7[a] ± 6.9 | 20.3[a] ± 3.6 | 30.7[a] ± 4.3 | 31.2[a] ± 0.39 |
| AST (U/L) | 25.8[a] ± 1.9 | 27.9[a] ± 4.5 | 20.3[a] ± 3.4 | 28.2[a] ± 4.1 | 30.0[a] ± 1.4 |
| ALP (IU/L) | 77.1[ab] ± 2.6 | 70[ab] ± 2.9 | 84[b] ± 8.7 | 68.7[a] ± 6.9 | 69.8[a] ± 1.6 |
| Bilirubin (mg/dl) | 0.9[a] ± 0.2 | 0.6[a] ± 0.2 | 0.6[a] ± 0.1 | 0.7[a] ± 0.1 | 0.9[a] ± 0.03 |
| Albumin (g/dl) | 3.5[a] ± 0.14 | 3.1[a] ± 0.2 | 3.5[a] ± 0.2 | 3.2[a] ± 0.2 | 4.3[b] ± 0.06 |
| Creatinine (mg/dl) | 1.03[ab] ± 0.1 | 1.3[b] ± 0.2 | 0.9[a] ± 0.1 | 0.97[a] ± 0.1 | 0.89[a] ± 0.04 |

In each row; same letters mean non-significant difference while different letters mean significant difference at p < 0.05, TG: Triglycerides, TC: Total cholesterol, LDL-C: Low-density lipoprotein-cholesterol, HDL-C: High-density lipoprotein-cholesterol, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, BMI: Body mass index.

noticed that the nutrients that showed low percentage from RDA in case of male and female patients were the fibers and Ca while those showed higher percentages were carbohydrates, proteins, P, iron, and Vitamin D. In Table 6, as expected, the total calories intakes of both diabetic male and female having BMI >30 kg/m² were significantly higher than those of BMI <30 kg/m². Carbohydrate and fat intakes of diabetic male and female having BMI >30 kg/m² were significantly higher than patients with BMI <30 kg/m². No significant changes were observed on comparing male with female having either BMI <30 or >30 kg/m² concerning carbohydrates and fat intakes, however female having BMI >30 kg/m² consumed significant higher fat than the corresponding male. Protein intakes showed insignificant changes among the four groups, while it was higher in male having BMI >30 kg/m² than those with BMI <30 kg/m², the reverse was demonstrated in case of female. Fibers' intakes of diabetic male and female patients having BMI >30 kg/m² were significantly lower than patients with BMI <30 kg/m². Intakes of fibers by male patients were significantly higher than that of females on both body mass indices. Ca intakes of diabetic male and female having BMI <30 kg/m² were higher than patients with BMI >30 kg/m², the changes were insignificant. No significant changes were observed on comparing male with female having either BMI <30 or >30 kg/m² concerning Ca intakes. It can be observed that P intakes of both diabetic male and female having BMI >30 kg/m² were higher than those of BMI <30 kg/m² but without statistical significance. No significant changes in P were demonstrated on comparing female with male of either BMI <30 or >30 kg/m². Iron and Vitamin D intakes of diabetic male having BMI >30 kg/m² were insignificantly higher than those of BMI <30 kg/m² while the reverse occurred in case of females. Iron intake of male patients

having BMI >30 kg/m² was significantly higher than that of females having BMI of either > or <30 kg/m².

Pearson correlation test was applied on male and female patients separately to detect the correlations of plasma Ca with plasma P, lipids, TC/HDL-C, creatinine, and albumin, in addition to glycosylated hemoglobin. Furthermore, correlation study was applied on P with the same aforementioned parameters. Glycosylated hemoglobin correlations with plasma albumin and TC/HDL-C were studied. Plasma albumin correlations with TC/HDL-C and WC were also investigated. The correlations of WC, WHR, and BMI with plasma Ca, P, lipids, and TC/HDL-C were also assessed. Dietary Vitamin D, P, Ca, and iron correlations with plasma Ca and P were studied. Furthermore, the correlations between different plasma lipids were investigated. It was noticed that there was a significant positive correlation between plasma creatinine and plasma Ca in males (r = +0.6) but not in females. A significant negative correlation was noticed between plasma Ca and plasma albumin (r = –0.703) in males. Plasma TC showed significant positive correlation with TG and LDL-C (r = 0.704 and 0.807, respectively) and demonstrated significant negative correlation with HDL-C (r = –0.557) in male and female patients. Plasma TC/HDL-C showed significant negative correlation with HDL-C (r = –0.863) and significant positive correlation with LDL-C, TG, and TC (r = 0.596, 0.766, and 0.85, respectively) in males and females. Dietary Vitamin D showed significant positive correlation with plasma Ca (r = 0.631) in males. In female patients, dietary P demonstrated significant positive correlation with plasma P (r = 0. 607) while dietary iron showed significant negative correlation with plasma Ca (r = –0.676). All other parameters studied within the correlation test did not demonstrate any significant correlations.

**Table 5: Nutrients' intakes of male and female diabetic patients (mean ± SE) and their percentage from the RDA**

| Parameters | Nutrition intake for male | % RDA | Nutrition intake for female | % RDA |
|---|---|---|---|---|
| Energy (kcal) | 2002 ± 824 | - | 2432 ± 870 | - |
| Carbohydrate (g) | 275.3 ± 32.02 | 211 | 359.1 ± 30.7 | 276.2 |
| Protein (g) | 87.2 ± 6.9 | 155.7 | 78.5 ± 6.4 | 170.7 |
| Fat (g) | 58.8 ± 4.9 | - | 74.4 ± 8.5 | - |
| Fibers (g) | 11.3* ± 1.1 | 37.7 | 6 ± 1.02 | 28.6 |
| Calcium (mg) | 750.3 ± 66 | 75.03 | 929.3 ± 90.2 | 77.4 |
| Phosphorus (mg) | 1060.9 ± 84.7 | 151.6 | 898.4 ± 86.4 | 128.3 |
| Iron (mg) | 17.7* ± 1.7 | 221.3 | 9.9 ± 1.5 | 123.8 |
| Vitamin D (µg) | 35.1 ± 9.1 | 234 | 30.7 ± 14.4 | 204.7 |

*: Values statistically significant when diabetic male group were compared with diabetic female group (p < 0.05). RDA: Recommended dietary allowance.

**Table 6: Nutrients' intakes of of male and female diabetic patients classified according to BMI<or>30 kg/m² (values are means ± SE) and their percentages from RDA**

| Parameters | Male of BMI >30 kg/m² | % RDA | Male of BMI <30 kg/m² | % RDA | Female of BMI >30 kg/m² | % RDA | Female of BMI <30 kg/m² | % RDA |
|---|---|---|---|---|---|---|---|---|
| Energy (kcal) | 2450ᵃ ± 160.5 | - | 1614ᵇ ± 239.8 | - | 2928ᵃ ± 111 | - | 1856ᵇ ± 364.2 | - |
| Carbohydrates (g) | 357.9ᵇ ± 44.5 | 275.3 | 202.5ᵃ ± 23.1 | 155.8 | 441.4ᵇ ± 24.9 | 339.5 | 263.1ᵃ ± 24.4 | 202.4 |
| Protein (g) | 87.9ᵃ ± 9.3 | 156.9 | 86.6ᵃ ± 10.81 | 154.6 | 74.7ᵃ ± 9.9 | 162.4 | 82.9ᵃ ± 8.2 | 180.2 |
| Fat (g) | 72.3ᵇ ± 4.9 | - | 47.2ᵃ ± 4.7 | - | 95.0ᶜ ± 10.4 | - | 50.4ᵃ ± 3.2 | - |
| Fibers (g) | 7.6ᵇ ± 0.76 | 25 | 14.6ᶜ ± 0.8 | 48.7 | 3.6ᵃ ± 0.24 | 17.1 | 8.8ᵇ ± 1.5 | 41.9 |
| Calcium (mg) | 636.6ᵃ ± 76.6 | 63.7 | 847.7ᵃᵇ ± 92.5 | 84.8 | 840.8ᵃᵇ ± 109.9 | 70.1 | 1032.6ᵇ ± 146.5 | 86.1 |
| Phosphorus (mg) | 1227.4ᵇ ± 144.1 | 175.3 | 918.2ᵃᵇ ± 66.9 | 131.2 | 1045.3ᵃᵇ ± 128.5 | 149.3 | 726.9a ± 69.2 | 103.8 |
| Iron (mg) | 20.8ᶜ ± 2.5 | 260 | 15.1ᵇᶜ ± 1.8 | 188.8 | 7.7ᵃ ± 0.5 | 96.3 | 12.5ᵃᵇ ± 2.9 | 156.3 |
| Vitamin D (µg) | 42.4ᵃ ± 23.8 | 282.7 | 28.9ᵃ ± 16.2 | 192.7 | 13.4ᵃ ± 9.6 | 89.3 | 50.9ᵃ ± 59.9 | 339.3 |

In each row; same letters mean non-significant difference while different letters mean significant difference at p < 0.05. BMI: Body mass index, RDA: Recommended dietary allowance.

## Discussion

Uncontrolled DM certainly increased the risks of complications. To reduce the development of complications, it is advised to follow the right medicaments, the correct dietary regimen, and physical activity. An extra important issue to minimize the risk of the complications is the continual biochemical blood analysis to discover any unwanted change and try to manage as fast as possible. The present research was established to study the changes in plasma P and Ca in male and female patients with type 2-diabetes as being risk factors for CVDs. The study also included assessing lipid profile, albumin, glycosylated hemoglobin, liver and kidney function, anthropometric parameters, and nutrients' intake and how these parameters may interrelate with plasma Ca and P as risk factors for induction of CVD.

The present study showed elevated plasma Ca in type 2-diabetic patients which was more prominent in female having BMI >30 kg/m² compared to the normal control group. The highest level of plasma Ca was present in female patients having the highest significant WC (113 cm) which is very high compared to standard values (80 cm or less) recommended by WHO [23]. Dietary Ca in the present study showed reduced intake by diabetic patients compared to RDA which may indicate that the elevated plasma Ca is due to pathological condition and not related to dietary Ca. The present result showed no significant correlation between glycosylated hemoglobin and plasma Ca in diabetic subjects. It is worthy to mention that plasma Ca demonstrated significant positive correlation with creatinine in male patients with the consideration that creatinine was within the normal range. It was reported that serum Ca levels in diabetic subjects are higher than normal [43]. Elevated serum Ca was shown to be associated with fasting blood glucose, insulin resistance, a decrease in insulin sensitivity and impaired glucose tolerance [10], [44], [45]. *In vitro* study demonstrated that cytosolic Ca may contribute to insulin resistance within adipocyte [46]. Also, elevated serum Ca was reported to be associated with the risk of developing diabetes [47], [48]. A question is raised here about elevated Ca, is it a participating factor for developing diabetes or is it a complication of diabetes that might have a hand in developing CVD. It is also worthy to

understand the role of dietary Ca in this respect. Ca homeostasis is controlled by Ca sensing receptors present in the parathyroid gland, and kidneys. The changes in circulating Ca trigger such receptors to adjust parathyroid hormone and Ca absorption to restore Ca concentration [15]. It has been reported that elevated serum Ca might be a predictor of CVD in men [49]. A significant positive association was demonstrated between serum Ca and systolic and diastolic blood pressure and cholesterol. It has also been reported that serum Ca levels were significantly higher in men having a history of myocardial infarction than those without such history in all age groups. Therefore, it appears likely that serum Ca is a predictor of CVD in men [49]. In the present study, no significant correlation was noticed between plasma Ca and plasma lipids, however female patients with BMI >30 kg/m² that showed significant elevation in Ca exhibited also significant high TC/HDL-C and TC with significant reduction of HDL-C which may predict a risk factor for CVDs in such group. It is to be noted that Ca present in three major forms in plasma, 50% in the ionized form, a physiologically important fraction, 40% is bound to albumin and 10% is in soluble complexes with anion [50]. Therefore, serum total Ca does not only reflect Ca physiology but also it is a function of serum albumin level [51]. It has been reported that serum albumin had a strong and consistent association with systolic and diastolic blood pressure [52]. Plasma Ca demonstrated significant positive correlation with dietary vitamin D in male and significant negative correlation with dietary iron in female. This is may indicate to what extent dietary nutrients may have association with plasma Ca.

The current study demonstrated significant increases in plasma P in all diabetic patients whether male or female and whatever the values of BMI or WC in comparison to the control group. The diabetic patients also demonstrated significant high TC/HDL-C and TC with significant reduction of HDL-C indicating CVDs risks. It was reported that high serum P level was demonstrated in type 2 DM which has been ascribed to renal dysfunction [17] since the elimination of P depends on renal function. Meanwhile, a previous study showed reduced serum P in type 2 DM due to disturbance in metabolism [53]. Raikou *et al*., 2020 [17] demonstrated positive correlation between serum P and serum glucose in both diabetic and non-diabetic. Raikou *et al*., 2020 [17] reported a significant association between high serum P

and received vitamin D supplements where Vitamin D mediates the absorption and metabolism of P. In the present study, no Vitamin D supplement was given to the patients; however, they consumed very high dietary vitamin D compared to RDA as shown from the dietary sheet. Furthermore, dietary P demonstrated high levels compared to RDA. No significant correlations were present between dietary Vitamin D and plasma P in the current study; however, a significant positive correlations were noticed between plasma and dietary P.

It is well documented that P has multiple biological functions such as cellular signal transduction, energy exchange, and mineral metabolism. About 80% or more of total body P exists in teeth and bone; however, intracellular P is present in form of organic compounds such as adenosine triphosphate and as free anions like $H_2PO_4^-$ which is known as phosphate. Serum P primarily occurs as inorganic phosphate which is maintained within the physiological range through regulation of dietary absorption, bone formation, renal excretion, and equilibration with intracellular stores [54], [55]. Considerable interest has been developed in the relation between increasing serum phosphate levels and adverse CV outcomes [56]. Available studies showed relation between phosphate and CV outcomes in patients with impaired kidney function that have hyperphosphatemia and abnormal serum Ca that result from secondary hyperparathyroidism [57], [58]. It is also, reasonable that higher levels of serum P may also be associated with adverse outcomes even in the absence of kidney disease since it has been reported that the increase of blood phosphates level increased the risk of coronary atherosclerosis in normal subjects [59]. The present study showed elevated plasma P in diabetic patients without kidney failure.

In normal condition, high serum phosphate stimulates directly or indirectly the osteoblasts fibroblast growth factor-23 (FGF-23) which could regulate phosphate metabolism [60] through inhibiting P reabsorption by renal tubular epithelial cells. Furthermore, elevated serum P may reduce serum calcitriol mediated increment of blood phosphate. Calcitriol receptor is mainly expressed in the small intestine, bone, kidney, and parathyroid gland to increase blood phosphate and Ca. In the intestine, calcitriol receptor expression increases the absorption of phosphate [61]. In parathyroid cells; binding of P to the Ca-sensitive receptors stimulate parathyroid hormone secretion [62]. Parathyroid hormone increases blood Ca and reduces blood phosphates through mainly an action on kidney and bone. Parathyroid hormone promotes reabsorption of $Ca_2^+$ from the kidney thereby reducing Ca excretion while inhibits the reabsorption of P and promotes P excretion, therefore reduces blood P. Parathyroid hormone facilitates the formation of calcitriol which indirectly promotes the absorption of Ca and P by intestinal epithelial cells. In addition, parathyroid hormone activates osteoclast, enhances its osteolytic effect and releases Ca and P. The binding of parathyroid hormone to parathyroid hormone-related protein receptor1 promotes FGF-23 secretion [63]. Moreover, serum iron, erythropoietin and insulin-like growth factor-1 can regulate phosphate metabolism. Iron deficiency may promote FGF-23 synthesis. Erythropoietin can induce FGF-23 mRNA in bone marrow erythroid cells. Insulin-like growth factor-1 negatively regulates FGF-3 [64], [65], [66]. One or more of the aforementioned elements that could reduce plasma P and Ca might be inhibited in type 2-diabetic patients thereby lead to sustained elevation of P and Ca.

Ca, phosphate, and Ca -phosphate product levels have an association to CV risk factors and outcomes and may be potential modifiable risk factors for stroke and death in adults [67]. Diabetes is considered as an influential risk factor of atherosclerotic disease, therefore management of hypertension and dyslipidemia is crucial in diabetic patients [68]. It was reported that diabetic patients are exposed to two- to four-fold increase in coronary heart disease [69]. It was also demonstrated that diabetic dyslipidemia consists of low HDL-C and increased TGs with normal LDL-C while LDL is converted to more atherogenic lipoprotein termed small dense LDL [70]. The high TC/HDL-C is more important in predicting the CV risk of type 2 diabetic patients than the non-HDL-C [71]. The TC/HDL-C has been recognized as being more significantly associated with CVD and the main lipid predictor of coronary heart disease and was ascribed as being a useful tool in the treatment guidelines in diabetic patients [71], [72]. An association was reported between fasting blood sugar and TC/HDL-C which was suggested as being a contributory factor to the increased prevalence of coronary artery disease in patient with type 2 DM [21]. In the present study, the lipid profile and TC/HDL-C of diabetic patient were similar to that of Goldberg, 2001 [70]. Furthermore, in the current study Plasma TC showed significant positive correlation with TG and LDL-C and significant negative correlation with HDL-C in patients. Plasma TC/HDL-C showed significant negative correlation with HDL-C and significant positive correlation with LDL-C, TG, and TC in patients.

The main risk factors for development and progression of chronic diabetic kidney disease are hyperglycemia and hypertension [73]. However, in spite of controlling both hyperglycemia and hypertension, still the residual risk of diabetic nephropathy remains high among diabetic patients [74], [75]. Diabetic dyslipidemia especially elevated TGs and reduced HDL-C are not only associated with CV risks but also has been reported to have a link to the risk of chronic diabetic kidney disease [75]. The previous studies suggested that internal lipid accumulation may participate to glomerular injury through induction of oxidative stress and the release of pro-inflammatory cytokines [76], [77]. It was demonstrated that the expression of key protein of HDL metabolism in mesangial and tubular cells was reduced in mediated cellular cholesterol efflux in the development of diabetic kidney disease [78]. Therefore

cardio-renal syndrome is expected as diabetic complications.

The present study demonstrated reduced plasma albumin in all diabetic patients. A significant negative correlation was observed between plasma Ca and albumin in diabetic male patients in the current study while no correlation was noticed between plasma albumin and glycosylated hemoglobin. Low albumin was reported to predict severe cardiac events in stable coronary artery disease [22]. Furthermore, low serum albumin is associated with elevated heart failure risk in elderly independent of inflammation and incident coronary events [79]. In addition, a strong independent association was demonstrated between reduced plasma albumin and CVDs, partly explained by plasma albumin as a negative acute phase reactant [80]. Several studies have authenticated serum albumin as a prognostic biomarker in severe illness like diabetes since albumin synthesis is dependent on sufficient insulin reserve [81], [82], [83]. Serum albumin level was noticed to be inversely associated with glycosylated hemoglobin [84] which confirms that hypoalbuminemia could reflect insulin deficiency and subsequent hyperglycemia. Therefore, serum albumin may be a sensitive indicator of insulin secretory reserve, which controls ketosis risk and glycemic control, where serum albumin concentration is inversely correlated with ketosis risk [85]. Therefore, serum albumin can be used by the treating physician as a marker of insulin reserve in beta-cells of patients with acute hyperglycemia and to identify those at risk of ketosis to avoid or prevent diabetic complications [86]. It has been also shown that reduced albumin is associated with an unfavorable metabolic profile, characterized by elevated adipose tissue inflammation, adiposity, and glucose, with increased risk for type 2 diabetes [87].

In the present research, it must not be ignored that non-alcoholic fatty liver (NAFLD), if present, might participate as risk factor for CVDs, but unfortunately abdominal ultrasonography was not carried out on the diabetic patients for diagnosis of such condition. NAFLD affects more than 20% of populations worldwide and most patients are with type 2 DM [88]. It has been reported that two thirds of patients with BMI ≥30 kg/m$^2$ have steatosis (fatty infiltration of the liver) [89]. The most common risk factors for developing steatosis are obesity, diabetes and hypertriglyceridemia [89], [90], [91]. Patients with NAFLD have been reported to have high glycosylated hemoglobin, obesity, increased or normal ALT, normal AST, AST/ALT of <0.8 (>0.8 in more advanced stage), elevated TG, and reduced LDL-C [88]. The present research showed that WC of both male and female are higher than normal values and BMI ranged from 29.9 in male to 34.3 in female reflecting overweight and obesity. Furthermore, in the current study ALT and AST were in the normal range with the ratio AST/ALT ranged from 0.94 in female to 1.02 in male, this ratio reached 1.3 in male of BMI >30 kg/m$^2$. The TG level was insignificantly high than normal with significantly reduced HDL-C in diabetic patients in the present study. The current research might suggest but not confirm the presence of fatty liver that might contribute as risk factor for developing CVDs.

WHR is an indicator of health and the risk of developing serious health conditions. The WHO states that abdominal obesity is defined as WHR above 0.90 for males and above 0.85 for females or a BMI above 30 [23]. It is to be noted that abdominal obesity defined as WHR of ≥0.8 for women and ≥1.0 for men indicates hypercholesterolemia in such persons according to the National Institute of Diabetes and Digestive and Kidney Diseases [92]. WHR has been reported to be a better predictor of CVD than WC and BMI [93]. However, other studies have demonstrated that WC, not WHR, to be a good index of CV risk factors [94], body fat distribution [95], and hypertension in type 2 diabetes [96]. The present study demonstrated WC and BMI to be more associated with CVDs in diabetic patients than WHR.

## Conclusion

The elevated plasma P together with the increased TC/HDL-C and reduced plasma albumin in type 2-diabetic patients might be risk factors for the development of diabetic complications represented by CVDs. High plasma Ca might predict CVD in only female diabetic patients of BMI >30 kg/m$^2$.

## Acknowledgment

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