



## Case-controlled Study

## Prevalence and Correlates of Hypophosphatemia Among Type 2 diabetic patients attending the National Center for Diabetes, Endocrinology and genetics (NCDEG)

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## ARTICLE INFO

## Keywords:

Type 2 diabetes mellitus  
Hypophosphatemia  
Prevalence  
Associated factors

## ABSTRACT

**Objectives:** To estimate the prevalence of hypophosphatemia and its associated factors among type 2 diabetic patients attending (NCDEG) in Amman-Jordan, and compare the prevalence of hypophosphatemia between diabetics, nondiabetic subjects.

**Patients and methods:** A case-control study was carried out at (NCDEG). A total of 1580 diabetic patients (59.7% females, 40.3% males), mean age (SD) of  $55.15 \pm 15.3$  attended this center from January 1st, 2020 till March 31st, 2020 were included. Our study included 2155 non-diabetic from the national population-based multi-purpose study in Jordan in 2017, to compare serum inorganic phosphate between diabetic, nondiabetic. Pregnant, those aged <18 or >80 years, GFR below 30 ml/min or those on hemodialysis were excluded. The data included patient's age, gender, smoking and medication, HbA1c. Statistical analysis were performed using the Package for Social Sciences (SPSS) version 21.

**Results:** The overall prevalence of hypophosphatemia in the diabetic patients was significantly higher (10.5% vs. 3.2%, P-value 0.001). Multivariate logistic regression analysis showed that in diabetic: males, current smokers, diabetic patients with HbA1c between 7 and 9% and >9%, those who on thiazide diuretics were 2, 1.9, 1.8, 1.7, and 1.9 times, more likely to have hypophosphatemia than their counterparts (P-values 0.001, 0.001, 0.006, 0.018 and 0.003), respectively, and it was found those on statin were less likely to have hypophosphatemia.

**Conclusion:** The prevalence of hypophosphatemia among type 2 diabetic patients is high. Factors independently related to hypophosphatemia in diabetic patients: male gender, smoking, poor glycemic control, taking thiazides and not being on statin.

## 1. Introduction

Inorganic phosphate is a basic element found in a wide range of biological products as potassium is the major intracellular cation, inorganic phosphate is the major intracellular anion, and the majority of intracellular phosphate is organic with only a small percentage being inorganic [1]. It has a significant impact on many physiological pathways, both synthetic and catabolic, including skeletal mineralization, cellular signaling, energy storage, and acid-base balance maintenance [1,2]. A normal adult has approximately 712 g of phosphorous, about 89% of phosphorous is present in the skeleton and 60–70% of it is passively absorbed by the jejunum and ileum [2].

Free inorganic phosphate is the most common form of inorganic phosphate in plasma, and the normal plasma concentration of inorganic phosphate in adults ranges between (2.5–4.5 mg/dl or 0.75–1.45 mmol/l) [2]. Inorganic phosphate level varies according to several factors; infants and young children for example, have almost double the serum inorganic phosphate level as compared to adults, due to greater need of phosphorous for rapid mineralization of the skeleton [3]. Such increase in phosphate requirement during growth is provided by the increase in growth hormone activity that will in turn maximize tubular phosphate reabsorption [3]. Ingestion of a rich carbohydrate meal will result in a decrease in serum inorganic phosphate level by (0.3–0.5 mmol/l or 1.0–1.5 mg/dl), due to the increase in the secretion of insulin needed to

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<https://doi.org/10.1016/j.amsu.2022.103770>

Received 12 April 2022; Received in revised form 8 May 2022; Accepted 8 May 2022

Available online 11 May 2022

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increase cellular uptake and utilization of inorganic phosphate [2]. There is also a circadian variation in inorganic phosphate concentration with the lowest concentration in the morning and the highest concentration in the evening [3].

Hypophosphatemia classified into mild (serum inorganic phosphate of 0.6–0.8 mmol/L; 1.8–2.5 mg/dL), moderate (0.4–0.5 mmol/L; 1.0–1.7 mg/dL), or severe (serum inorganic phosphate lower than 0.3 mmol/L; 0.9 mg/dL). Its clinical presentation can vary according to its onset, severity and patient's age. The most important clinical symptoms of hypophosphatemia are bone pain, muscle weakness and cardiac dysfunction [4].

Data from clinical studies had shown that the prevalence of hypophosphatemia might be common in certain situations: Major trauma (75%), sepsis (65–80%), intensive care units' patients (29–34%), chronic alcoholism (2.5–3.1%), chronic pulmonary disease (21.5%), hospitalized patients (2.2–3.1%) and inpatients receiving ferric carboxymaltose (29.1%) [5].

Data on the prevalence of hypophosphatemia and its associated factors in subjects with diabetes are scarce [6]. The main objective of our study is to estimate the prevalence of hypophosphatemia and its associated factors among type 2 diabetic patients.

## 2. Materials and methods

### 2.1. Sampling and data collection

A retrospective clinical case-control study was carried out at (NCDEG), which treats patients with diabetes mellitus from all over the country [7]. All type 2 diabetic patients aged 18–80 years who attended NCDEG during the period January 1st through March 2020 and had their serum inorganic phosphate level assessed were eligible for inclusion in the study. Patients who was excluded: pregnant, patients with GFR below 30 ml/min, patients on hemodialysis, and patients on chemotherapy. Data were abstracted from the medical records using a data sheet prepared for the purpose of this study.

We have also obtained data for comparison of hypophosphatemia between diabetic and nondiabetic subjects from a multipurpose national population-based survey conducted in Jordan in 2017 [8], a sample was selected from the 12 governorates of Jordan. These 12 governorates belong to the 3 regions of the country, i.e. the north, middle, and south. A complex multistage sampling technique was used to select the households, in each selected area, 1 day before data collection, 2-membered teams (a male and a female in each) visited the selected households, explained the purpose of the study, and invited all members aged  $\geq 7$  years to report to the health center the next day after an overnight fast. Subjects on regular medications were asked not to take their medications early on that day and to bring all of their medications with them to the survey site [8]. We choose them age and gender matched healthy control subjects 2155 out of 4590, in which serum inorganic phosphate was measured, and any one with HbA1c more than 6.5% according to (ADA) 2018 guidelines, was excluded.

### 2.2. Definitions of the study variables

#### 2.2.1. Measurements and laboratory analyses

Anthropometric measurements, including weight, height, and waist circumference were measured while the subjects were wearing light clothing and no shoes this was according to the anthropometric cutoff values for detecting metabolic abnormalities in Jordanian adults. BMI was expressed as the quotient between weight (kg) and height squared (m<sup>2</sup>) [9]. Patients were classified according to BMI according the recommendation of the World Health Organization as adopted by the American Diabetes Association [10]. Smoking was classified into three categories according to WHO guidelines 1998; current smoker was defined as: a person who smokes cigarettes daily or occasionally; past-smoker: a person who formerly was a daily or occasional smoker,

but currently does not smoke at all; nonsmoker: a person who has never smoked before [11].

Diabetes mellitus was diagnosed according to ADA criteria, if the patient had FPG  $\geq 126$  mg/dL (7.0 mmol/L) in two occasions or if the patient had a random plasma glucose  $\geq 200$  mg/dL (11.1 mmol/L) in the presence of classical symptoms of hyperglycemia, or if he or she had HbA1c  $\geq 6.5\%$ . Moreover, diabetes was considered to be controlled if the patient had HbA1c  $< 7.0\%$  according to the American Diabetes Association (ADA) 2018 guidelines [12].

Readings of systolic and diastolic blood pressures were taken while the subjects were seated and the arm was kept at the heart level, after at least 5 min of rest, using a standardized mercury sphygmomanometer, high blood pressure was defined as systolic blood pressure  $\geq 130$  mmHg or diastolic blood pressure  $\geq 80$  mmHg or if the patient was already on antihypertensive drugs [13].

Metabolic abnormalities were defined according to the American Diabetes Association 2021 as follows: total serum cholesterol  $\geq 200$  mg/dL, serum LDL  $\geq 100$  mg/dL, serum triglyceride  $\geq 150$  mg/dL, serum HDL  $\leq 40$  mg/dL in men, and  $\leq 50$  mg/dL in women, or if the patient was already on antidiabetic agents [13].

Inorganic phosphate was measured by "Colorimetric Endpoint Method" using Roche/Cobas Integra C501 automated system. The imprecision, with run (CV) was 1.38 and 1.12% between runs as judged by internal quality-control systems [14]. Our operational definition of hypophosphatemia was the occurrence of inorganic phosphate level below 2.5 mg/dL (normal range 2.5–4.5 mg/dL). This normal range of inorganic phosphate (2.5–4.5 mg/dL) is the normal value provided by our laboratory as there is no published data regarding the normal inorganic phosphate values for Jordanians yet.

Serum 25(OH)D concentrations were determined using the Alinity i 25-OH vitamin D assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of (25-OH vit D) in human serum and plasma on the Alinity i analyzer. And vit D range defined as Sufficient ( $\geq 30$ ) ng/ml, Insufficient (20–29.9), Deficient ( $< 20$ ) ng/ml [15].

Serum Parathyroid was measured by: the electrochemiluminescence immunoassay (ECLIA) for use on Elecsys and cobas e 601 immunoassay analyzer, for quantitative determination of intact parathyroid hormone in human serum and plasma, with normal range value 15–65 pg/ml [16].

### 2.3. Ethical considerations

The study used routinely available data from patients' medical records. Identifying information was not collected and the data were kept confidential and used only for scientific purposes by researchers. Thus, the study carries no harm what so ever to patients. The study was approved by the Institutional Review Board (IRB) of the NCDEG, Amman, Jordan. The study conforms with the 1975 Helsinki declaration, as revised in 2008 and its later amendments or comparable ethical standards.

### 2.4. Statistical analysis

Data were entered and analyzed using the Statistical Package for Social Sciences (SPSS version 21). The prevalence rates of hypophosphatemia among diabetic patients, overall and by relevant variables, were obtained and assessed for statistical significance using the chi square test. The mean (SD) concentrations of serum inorganic phosphate by different levels of relevant variables were derived and assessed for statistical significance using the independent *t*-test or ANOVA as appropriate. Multivariate logistic regression was used to assess the independent effect of a given variable after adjusting for potential confounders. A P-value of  $< 0.05$  was considered statistically significant.

To determine whether the prevalence of hypophosphatemia is

significantly lower in diabetic patients as compared to the population sample of nondiabetics, we used the chi square test. We further conducted multivariate logistic regression to control for potential confounding. Hypophosphatemia (yes/no) was considered as the outcome variable and diabetes (yes/no) was entered as an independent variable together with a number of potential confounders. Model fit was assessed using the -2-log likelihood. The most parsimonious model was presented.

### 3. Results

#### 1 Sociodemographic, clinical, and anthropometric characteristics of the study sample.

This study included 1580 diabetic patients, 944 (59.7%) females and 636 (40.3%) males, aged between 18 and 80 years with a mean age (SD) of  $55.15 \pm 15.3$ . The sociodemographic, clinical and anthropometric characteristics of the study population are presented in Table (1). Patients were (21%) below the age of 45 years, (35%) between 45 and 60, and (44%) more than 60 years. (90%) of patients were either obese or overweight and the mean (SD) of BMI was ( $30.56 \pm 6.0$ ). A total of 72% had elevated waist circumference  $WC \geq 91.8$  cm for males and  $\geq 88.5$  cm for females, 78% were nonsmokers, 51% were hypertensive, and 88% had dyslipidemia, 34% had controlled diabetes with  $HbA1c < (7\%)$ , 37% had  $HbA1c$  between (7–9%) while 29% had  $HbA1c \geq (9\%)$ . The study showed that 83% of the diabetic patients had a parathyroid level 15–65 pg/ml, 94% had a Mg level 1.6–2.4 mg/dl, and 60.3% had

vitamin D level more than 30 ng/ml.

#### 2 Prevalence of Hypophosphatemia in diabetics according to Relevant Demographic and Clinical Characteristics:

The overall prevalence of hypophosphatemia in the diabetic group was (10.5%) as compared to (3.2%) in the non-diabetic group (p-value 0.001). As shown in Table (2), hypophosphatemia was more prevalent in male diabetic patients (15.3%) as compared to females (7.3%) (P-value 0.001), and (23%) of diabetic patients with hypophosphatemia were more than 45 years old. Also, it was obvious that hypophosphatemia was more prevalent in overweight diabetic patients (13.5%), compared to obese (9%), and patients with normal BMI (8%) (P-value 0.013).

Regarding  $HbA1c$ , hypophosphatemia existed in (7.7%) of patients with  $HbA1c$  less than 7%, (12.6%) of those with  $HbA1c$  between 7.9% and 9%, and (11%) of those with  $HbA1c \geq 9.0\%$  (P-value 0.025). Moreover, 15.6% of diabetic patients who were taking thiazide diuretics were hypophosphatemic (P-value 0.002), as it was more frequent in smokers (16.7%) compared to non-smokers (8.8%) (P-value 0.001), but there was no significant difference in hypophosphatemia in diabetic patients related to insulin type or using the loop diuretics.

This study also found that cases with  $HbA1c$  less than 7% ( $HbA1c$

**Table 2**

Prevalence of Hypophosphatemia in Diabetic patients attending the NCDEG according to Relevant Demographic and Clinical Characteristics (n = 1580).

Variables	Hypophosphatemia in diabetic patients		P-value
	Yes n (%)	No n (%)	
Total	166 (10.5)	1414 (89.5)	
<b>Gender</b>			
Female	69 (7.3)	875 (92.7)	0.001 <sup>a</sup>
Male	97 (15.3)	539 (84.7)	
<b>Age, years</b>			
<45	28 (7.5)	344 (92.5)	0.093
45–60	71 (11.8)	532 (88.2)	
>60	67 (11.1)	538 (88.9)	
<b>BMI, (Kg/m<sup>2</sup>)</b>			
Normal	12 (7.9)	140 (92.1)	0.013 <sup>a</sup>
Overweight	76 (13.5)	486 (86.5)	
Obese	78 (9.0)	788 (91.0)	
<b>Waist circumference</b>			
Normal	42 (9.6)	397 (90.4)	0.450
Elevated	124 (10.9)	1017 (89.1)	
<b>Smoking</b>			
Not smoker	108 (8.8)	1124 (91.2)	0.001 <sup>a</sup>
Current smoker	58 (16.7)	290 (83.3)	
<b>Hypertension</b>			
Yes	90 (11.2)	712 (88.8)	0.346
No	76 (9.8)	702 (90.2)	
<b>Dyslipidemia</b>			
Yes	101 (10.5)	865 (89.5)	0.976
No	14 (10.4)	121 (89.6)	
<b>Patients on statin</b>			
Yes	85 (9.7)	795 (90.3)	0.218
No	81 (11.6)	619 (88.4)	
<b>HbA1c, %</b>			
<7	41 (7.7)	491 (92.3)	0.025 <sup>a</sup>
7–9	74 (12.6)	512 (87.4)	
$\geq 9$	51 (11.0)	411 (89.0)	
<b>Patients on insulin</b>			
Yes	62 (10.9)	505 (89.1)	0.678
No	104 (10.3)	909 (89.7)	
<b>Insulin type</b>			
Mixtard	16 (7.8)	189 (92.2)	0.071
Basal	46 (12.7)	315 (87.3)	
<b>Patients on loop diuretics</b>			
Yes	10 (13.0)	67 (87.0)	0.467
No	156 (10.4)	1347 (89.6)	
<b>Patients on thiazide diuretics</b>			
Yes	46 (15.6)	249 (84.4)	0.002 <sup>a</sup>
No	120 (9.3)	1165 (90.7)	

<sup>a</sup> Donates for significant p-value (<0.05).

**Table 1**

Sociodemographic, clinical, and anthropometric characteristics of the study participants, Jordan, 2021 (N = 1580).

Variable	n (%)
<b>Gender</b>	
Female	944 (59.7)
Male	636 (40.3)
<b>Age, years, mean <math>\pm</math> SD (<math>55.15 \pm 15.3</math>)</b>	
<45	229 (20.8)
45–60	391 (35.5)
>60	481 (43.7)
<b>BMI, (Kg/m<sup>2</sup>), mean <math>\pm</math> SD (<math>30.56 \pm 6.0</math>)</b>	
Normal	152 (9.6)
Overweight	562 (35.6)
Obese	866 (54.8)
<b>Waist circumference (cm) <sup>a</sup></b>	
Normal	439 (27.8)
Abnormal	1141 (72.2)
<b>Smoking</b>	
Not smoker	1232 (78.0)
Current smoker	348 (22.0)
<b>Hypertension</b>	
Yes	802 (50.8)
No	778 (49.2)
<b>HbA1c, %, mean <math>\pm</math> SD (<math>7.45 \pm 1.7</math>)</b>	
<7	532 (33.7)
7–9.0	586 (37.1)
$\geq 9$	462 (29.2)
<b>Dyslipidemia</b>	
Yes	966 (87.7)
No	135 (12.3)
<b>PTH level (pg/ml)</b>	
Normal (15–65)	910 (82.7)
Low (<15)	14 (1.3)
High (>65)	177 (16.1)
<b>Mg</b>	
Normal (1.6–2.6)	1497 (94.7)
Low (<1.6)	83 (5.3)
<b>Vitamin D level (ng/ml)</b>	
Sufficiency ( $\geq 30$ )	953 (60.3)
Insufficiency (20–29.9)	237 (15.0)
Deficiency (<20)	390 (24.7)

<sup>a</sup> Waist circumference: males: more than 91.8, females: more than 88.5.

$6.18 \pm 0.45$ ) had the highest concentration of serum inorganic phosphate ( $3.41 \pm 0.61$  mg/dl). On the contrary, cases with HbA1c  $\geq 9.0\%$  (mean HbA1c  $10.41 \pm 1.44$ ) had the lowest concentration of serum inorganic phosphate level ( $3.32 \pm 0.64$  mg/dl). Therefore, cases with lower values of HbA1c had higher values of serum inorganic phosphate level and vice versa. However, these differences were not statistically significant (P-value = 0.533).

### 3 Factors independently related to hypophosphatemia in diabetic patients using multivariate logistic regression analysis:

In the diabetic group, as shown in Table (3), male patients were found to be 2 times more likely to have hypophosphatemia than female patients (P-value 0.001). Current smokers were 1.9 times at more risk to have hypophosphatemia as compared to non-smokers (P-value 0.001). Additionally, patients with uncontrolled DM (HbA1c between 7 and 9% and  $\geq 9\%$ ) were 1.8 times and 1.7 times at more risk of hypophosphatemia as compared to those with HbA1c less than 7% (P-values 0.006 and 0.018 respectively). Moreover, diabetic patients taking thiazide diuretics were 1.9 times more likely to have hypophosphatemia in comparison with non-thiazide users (P-value 0.003). It is also found that patients on statin were less likely to have hypophosphatemia (P-value 0.005).

On the other hand, Lower serum inorganic phosphate level in the diabetic subjects was most obvious in diabetic patients with age group between 45 and 60 years and more than 60 years, 2.3 and 2.5 times (P-value 0.001, 0.001), respectively. Male participants were 2.2 times more likely to have hypophosphatemia than females (P-value 0.001).

### 4 Hypophosphatemia in diabetic patients compared to hypophosphatemia in non-diabetic subjects:

As mentioned earlier, the prevalence of hypophosphatemia in diabetic patients was much higher than that in nondiabetic subjects (10.55 VS 3.2%). As it shown in Table (4), to remove the effect of potential confounding, we used multivariate logistic regression which showed that hypophosphatemia was independently and significantly associated with diabetes (odds ratio = 2.2, P = 0.001). The overall mean serum inorganic phosphate level in diabetic group was  $3.39 \pm 0.63$  mg/dl and that in non-diabetic group was  $3.49 \pm 0.52$  mg/dl, and the difference was statistically significant, (P-value 0.001).

**Table 3**

Factors Independently Related to Hypophosphatemia among Diabetic Patients attending the NCDEG Using Multivariate Logistic Regression.

Variable <sup>a</sup>	Diabetic group	
	Odds Ratio (95% Confidence Interval)	P-value
<b>Gender</b>		
Female	1	0.001
Male	2.00 (1.41–2.79)	
<b>Age</b>		
<45	1	
45–60	1.88 (1.15–3.07)	0.011
>60	1.90 (1.13–3.21)	0.016
<b>Smoking</b>		
Not smoker	1	0.001
Current smoker	1.91 (1.32–2.76)	
<b>HbA1c %</b>		
<7	1	
7–9	1.78 (1.18–2.68)	0.006
$\geq 9$	1.71 (1.09–2.68)	0.018
<b>Patients on thiazide diuretics</b>		
No	1	0.003
Yes	1.91 (1.22–2.71)	
<b>Patients on statin</b>		
No	1	
Yes	0.60 (0.42–0.86)	0.005

<sup>a</sup> Every variable in this table is adjusted for all other variables in the table.

**Table 4**

Hypophosphatemia in diabetics and nondiabetics after controlling for potential confounders using multivariate logistic regression.

Variable <sup>a</sup>	Diabetic group	
	OR (95% CI)	P-value
<b>Study group</b>		
Non-diabetic	1	0.001
Diabetic	2.22 (1.61–3.07)	
<b>Gender</b>		
Female	1	0.001
Male	2.19 (1.65–2.91)	
<b>Age</b>		
<45	1	
45–60	2.29 (1.60–3.29)	0.001
>60	2.49 (1.66–3.72)	0.001
<b>Smoking</b>		
Not smoker	1	0.001
Current smoker	1.84 (1.36–2.50)	

<sup>a</sup> Each variable in this table is adjusted for all other variables in the table.

### 5 Gender difference in Serum Inorganic Phosphate level according to age

As shown in Table (5), there was a gender difference that could be clinically important, females less than 45 years had serum inorganic phosphate level ( $3.37 \pm 0.63$ ), compared with those above 60 years, whose serum inorganic phosphate level was ( $3.55 \pm 0.61$ ) (P-value 0.030), while in males less than 45 years the serum inorganic phosphate level was ( $3.38 \pm 0.61$ ) compared with those above 60 years whose serum inorganic phosphate level was ( $3.20 \pm 0.59$ ), (P-value 0.041).

## 4. Discussion

In our study, the prevalence of hypophosphatemia among diabetic patients attending the (NCDEG) was found to be (10.5%) which was much higher than the prevalence observed among non-diabetics in the population –based national study (3.2%).

Our study showed the significant association between uncontrolled diabetes and hypophosphatemia. Diabetic patients with HbA1c between 7 and 9% and  $\geq 9\%$  were 1.8 and 1.7 times, respectively, more likely to have hypophosphatemia as compared to those with HbA1c less than 7%. Bora et al. also reported the same result [17]. Vorum et al. also found that at HbA1c between 6 and 7.5%, a maximum inorganic phosphate level of ( $3.26 \pm 0.45$  mg/dl) was documented while a minimum inorganic phosphate level of ( $2.06 \pm 0.15$  mg/dl) was seen with HbA1c more than 10.5% [18]. In contrast to our finding, Dalili et al. and Galli-Tsinopoulou et al. found no significant association between hypophosphatemia and HbA1c level [19,20].

The mechanisms responsible for hypophosphatemia in diabetic patients are not fully understood, osmotic diuresis associated with hyperglycemia is obviously the major contributor for the disturbance in phosphate handling by the renal tubules where the excessive sodium – dependent glucose reabsorption depolarizes the brush border membrane for phosphate reabsorption leading to hyperphosphaturia [21].

**Table 5**

Gender difference in serum level according to age.

Age group	Serum Inorganic Phosphate level			
	Diabetic group		Non-diabetic group	
	3.39 $\pm$ 0.63		3.49 $\pm$ 0.52	
	Female Mean $\pm$ SD	Male Mean $\pm$ SD	Female Mean $\pm$ SD	Male Mean $\pm$ SD
<45	3.37 $\pm$ 0.63	3.38 $\pm$ 0.61	3.54 $\pm$ 0.51	3.51 $\pm$ 0.52
45–60	3.52 $\pm$ 0.61	3.26 $\pm$ 0.66	3.54 $\pm$ 0.52	3.18 $\pm$ 0.54
>60	3.55 $\pm$ 0.61	3.20 $\pm$ 0.59	3.42 $\pm$ 0.48	3.00 $\pm$ 0.53
P-value	0.030	0.041	0.058	0.001



Additionally, the abnormally high blood glucose in patients with type 1 or type 2 diabetes is linked to a state of non-physiological variations in plasma insulin throughout the 24-h period. During hyperglycemic-hyperinsulinemic conditions, large amount of glucose enters insulin-sensitive tissues (muscle and fat) where it will be metabolized by phosphorylation leading to lower plasma levels of inorganic phosphate [22].

Our study found a gender difference in inorganic phosphate levels, with females in both diabetic and non-diabetic groups having higher serum inorganic phosphate levels than males. This sex difference in inorganic phosphate metabolism has been reported in different studies (Koek et al., Dominguez et al. and Schwarz et al.) [23–25].

An association between cigarette smoking and hypophosphatemia was noticed in our study. In agreement with our finding, Hussein also reported that cigarette smoking can lead to a decrease in inorganic phosphate levels as well as an increase in calcium levels [26]. Other studies (Villabance AL et al., Murraroy CL, Lopez AD) also reported the same finding. The most likely explanation for the association between cigarette smoking and lower inorganic phosphate levels is the inhibition of parathyroid hormone action on the renal tubules by one or more of the chemicals found in the cigarettes leading to increase in serum inorganic phosphate levels and decrease in serum calcium levels [27, 28].

In our study, hypophosphatemia was associated with the use of thiazide diuretics. Consistent with our result, Liamis et al. in his study on 204 patients presented with hyponatremia, 12.5% of them were treated with thiazide diuretics and demonstrated concurrent hypophosphatemia. Thiazide diuretics can produce an increase in renal clearance of phosphate and might have direct effect on distal renal tubular reabsorption of phosphate [29].

Hypophosphatemia is observed to be less common in diabetic patients who are taking statin, which was consistent with Aronson, Daron, et al., that showed the use of statin was lower among patients with elevated inorganic phosphate level (p-value 0.04) [30]. Statin can elevate the skeletal muscle phosphodiesterase which elevate the skeletal muscle metabolites phosphocreatine (PCr), adenosine triphosphate (ATP), and inorganic phosphate (Pi) [31].

The serum inorganic phosphate levels in diabetic group were found to be significantly lower than that in the non-diabetic group (P-value 0.001). Consistent with our finding, Bora et al. also found that the mean serum inorganic phosphate level concentration in cases of type 2 diabetes was significantly lower than non-diabetics ( $2.68 \pm 0.56$  Vs  $3.64 \pm 0.42$  mg/dl) (P-value less than 0.01) [17]. Linyan et al. also found that serum level of inorganic phosphate is obviously decreased in type 2 diabetic patients than in the non-diabetic group (P-value less than 0.05) [32]. Moreover, Ugwuja El et al. assessed serum inorganic phosphate level along with other electrolytes (Na, K, HCO<sub>3</sub>, and Cl) in 60 patients with diabetes and compared them with 60 apparently healthy, age and sex matched individuals and concluded, that serum inorganic phosphate level was significantly lower in diabetics than in non-diabetic (P-value less than 0.05) [33]. Hamad et al. reported that the levels of inorganic phosphate were significantly lower among diabetics in comparison to nondiabetics (P-value 0.001) [34].

Another gender difference in our study was the increase in serum inorganic phosphate level with age in females, in contrast to the decrease with age in males. Consistent with our finding, Koek et al. also found gender differences in serum calcium and inorganic phosphate levels that were age dependent with females above 45 years having higher calcium and inorganic phosphate levels compared to males [23, 35]. Several other studies also showed that postmenopausal women usually have higher serum inorganic phosphate levels than men of similar age (Keating et al., Sinton et al., Boyd et al., Tonelli et al., and de Boer et al.) [36–39].

Hypophosphatemia's physiologic consequences are likely to differ between patient groups. Although evidence suggests a link between hypophosphatemia and markers of poor respiratory, cardiovascular, and

postsurgical outcomes, studies fail to account for confounding variables. Overall, evidence suggests that hypophosphatemia is associated with poor clinical outcomes, implying that hypophosphatemia is a poor prognostic indicator [40].

To determine whether treatment is necessary, the cause of the hypophosphatemia must be identified, for example, hypophosphatemia in diabetic ketoacidosis patients will usually resolve on its own with normal dietary intake, while patients with evidence of renal or gastrointestinal phosphate loss, replacement therapy is required [2].

## 5. Conclusion and recommendation

The overall prevalence of hypophosphatemia in diabetic patients was significantly higher than non-diabetic subjects. Factors independently related to hypophosphatemia in diabetic patients included: male gender, smoking, poor glycemic control, taking thiazides and not being on statin. Our data also showed that patients with diabetes had significantly and independently a higher rate of hypophosphatemia as compared to nondiabetic subjects. Further studies are needed to assess the exact relationship between hypophosphatemia and uncontrolled diabetes.

## 6. Limitations of the study

The main strength of our study its relatively large sample size (1580 patients) and the fact that it was the first study conducted in Jordan to assess the prevalence of hypophosphatemia among type 2 diabetic patients.

One of the limitations of our study was the lack of data on hormonal levels such as testosterone, estradiol and FGF-23, which might affect calcium and inorganic phosphate metabolism and could contribute to the observed gender difference in serum inorganic phosphate levels [23, 41].

## Financial disclosure

The authors have no financial relationship relevant to this article to disclose.

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The authors have no financial relationship relevant to this article to disclose.

## Ethical approval

All procedures performed in this study which involved human participants were in an accordance with the National Center for Diabetes Endocrinology and Genetics (NCDEG) Ethics Committee, which is accredited by the National Ethics Committee.

## Consent

The study used routinely available data from patients' medical records. Identifying information was not collected and the data were kept confidential and used only for scientific purposes by researchers. Thus, the study carries no harm what so ever to patients.

## Author statement

**Rula Rashed** wrote the manuscript, originator of the manuscript subject, acquisition of data, supervised the research.

**Dana Hyassat** contributed in writing the manuscript, contributed to the conception and design of the study, supervised the research.

**Anwar Batieha**: performed the statistical analysis, approved the protocol from statistical point of view, analyzed the data and approved the results.

**Mohammad Aldabbas** Entered the data on SPSS.

**Faiq Aldarabah** assisted in the data collection.

**Mohammed EL-Khateeb** was responsible for lab analysis.

**Kamel Ajlouni**: originator of the manuscript subject, and supervised the research, helped in developing the idea.

## Registration of research studies

1. Name of the registry: Prevalence and Correlates of Hypophosphatemia Among Type 2 Diabetic Patients Attending the National Center for Diabetes, Endocrinology and Genetics (NCDEG)
2. Unique Identifying number or registration ID: 7805
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): <https://www.researchregistry.com/browse-the-registry/#home/>

## Guarantor

Prof. Dr. Kamel Ajlouni.

## Declaration of competing interest

The authors declare that they have no conflict of interest.

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