FIBROBLAST GROWTH FACTOR-23 AND HYPOPHOSPHATEMIA IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS

FAKTOR RASTA FIBROBLASTA 23 I HIPOFOSFATEMIJA KOD PACIJENATA SA HRONIČNOM OPSTRUKTIVNOM BOLEŠĆU PLUĆA

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Summary: Impaired serum phosphate levels may contribute to respiratory muscle weakness that further negatively impacts Chronic Obstructive Pulmonary Disease (COPD) patients. Recently, Fibroblast Growth Factor 23 (FGF-23) has been shown to play an important role in the regulation of body phosphate. The current study includes 2 groups: 70 COPD patients and 34 control subjects. Blood samples were taken for a panel of routine lab tests. FGF-23 was measured using a commercially available ELISA kit. Plasma FGF-23 levels were significantly higher in the patient group compared to the control group (P= 0.000). Tubular maximum absorption of phosphate was significantly reduced in COPD patients compared to the control group (P=0.04). Plasma FGF-23 negatively correlated with FEV1 and serum albumin. Elevated plasma FGF-23 levels found in COPD patients correlated with disease severity and may represent an additional factor causing low serum phosphate.

Keywords: FGF-23, hypophosphatemia

Kratak sadržaj: Poremećeni nivoi fosfata u serumu mogu dodatno oslabiti respiratorne mišiće, što negativno utiče na pacijente sa hroničnom opstruktivnom bolešću pluća (HOBP). Nedavno je pokazano da faktor rasta fibroblasta 23 (FGF-23) ima važnu ulogu u regulisanju nivoa fosfata u telu. Ova studija obuhvatila je dve grupe: 70 pacijenata sa HOBP i 34 kontrolna subjekta. Uzeti su uzorci krvi za panel rutinskih laboratorijskih testova. FGF-23 meren je pomoću komercijalnih ELISA testova. Nivoi FGF-23 u plazmi bili su značajno viši u grupi pacijenata nego u kontrolnoj grupi (P=0,000). Maksimalna tubularna apsorpcija fosfata bila je značajno snižena kod pacijenata sa HOBP u poređenju sa kontrolnom grupom (P=0,04). FGF-23 u plazmi bio je u negativnoj korelaciji sa FEV1 i albuminom u serumu. Povišeni nivoi FGF-23 u plazmi kod obolelih od HOBP u korelaciji su sa stadijumom oboljenja i možda predstavljaju dodatni faktor odgovoran za nizak nivo fosfata u serumu.

Ključne reči: FGF-23, hipofosfatemija

Introduction

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases (1).

COPD is an important co-morbidity in those dying from other smoking related diseases, most commonly ischemic heart disease and lung cancer (2). COPD is the fourth leading cause of death worldwide (3, 4). Moreover, due to an aging population, increases in its prevalence and mortality are expected in the coming decades (4). COPD is set to become the third leading cause of death worldwide by the year 2020, surpassed only by heart disease and stroke (5).

The most important complaints of patients with COPD are dyspnoea and impaired exercise performance. Both are related to diminished muscle

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function, especially muscle weakness in both respiratory and skeletal muscles (6).

Low serum phosphate levels may be responsible for this state of muscle weakness that further negatively impacts COPD patients (8). Phosphate plays a crucial role in the different cellular biochemical processes (8) that control lipid, protein and carbohydrate metabolism (9). Moreover, phosphate is a major constituent of the ATP (Adenosine Triphosphate) high energy bonds that is required for the execution of different biochemical processes with subsequent effects on muscle contractility, cellular integrity and other biological functions (6). In addition to erythrocytes, leukocytes and platelets dysfunctions, clinical features of hypophosphatemia and phosphate depletion include neuromuscular abnormalities, possible rhabdomyolysis and muscle weakness (10). Previous studies highlighted the importance of early detection of phosphate depletion in COPD patients and the association between the correction of hypophosphatemia and improvement in respiratory muscle function (11, 12).

Fibroblast Growth Factor-23 (FGF-23) is a recently discovered circulating phosphaturic factor that plays a crucial role in renal phosphate reabsorption (13). FGF-23 is a member of the Fibroblast Growth Factors family of humoral factors which include other FGF peptides (e.g. FGF-19, FGF21) that share a common homology sequence (14). FGF-23 is produced as a peptide with 251 amino acids by bones (15, 16) that consists of a signal peptide with 24 amino acids, and a secreted FGF-23 protein consisting of 227 amino acids which is approximately 32-kD (11).

Recently Fibroblast Growth Factor 23 has been shown to play an important role in the regulation of body phosphate. FGF-23 induces phosphaturia and inhibits renal 1-α hydroxylase leading to decreased calcitriol synthesis (17). FGF-23 was investigated in different diseases and recent studies have illustrated a prognostic role in patients with ESRD (18). Very few researchers investigated phosphate status in patients with COPD, its etiology and impact on COPD patients. Furthermore, to our knowledge there is no data about FGF-23 in patients with COPD, its relation to plasma phosphate levels in this group of patients and any possible relation to the severity of COPD. The aim of this study is to evaluate the FGF-23 level in patients with COPD and investigate whether FGF-23 levels may correlate with any of the disease severity indicators and other factors controlling phosphate metabolism in COPD patients.

Patients and Methods

The study was approved by the King Fahad Specialist Hospital Ethics and Research committee. All patients and control subjects gave their written informed consent before participating in the study.

The current study included 2 groups: 70 COPD patients aged (mean±SD) 63.0±4.60 years seen at the Pulmonology Department of King Fahad Specialist Hospital in Dammam, Kingdom of Saudi Arabia, and 34 age and sex matched randomly selected control subjects with no pulmonary disease aged (mean±SD) 62.6 ± 6.1 years. None of the patients included were taking any medications that could affect bone metabolism (e.g. vitamin D supplements, antacids, phosphate supplements, TPN or corticosteroids). All subjects included in this study were on a phosphate unrestricted diet. For all subjects participating in this study, the history of the dietary intake of phosphorous for the last three days prior to testing was recorded. All subjects participating in the study were evaluated with a complete history, physical examination, chest x-ray, arterial blood gases and pulmonary function tests (PFT). PFT criteria for defining COPD were, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) Guidelines 2009 Update[1]: an FEV1/FVC ratio (forced expiratory volume in 1 second/functional vital capacity) of less than 70% post bronchodilator, with severity categorized into mild (FEV1 ≥ 80% of predicted), moderate (FEV1 ≥ 50% to < 80%), severe (FEV1 ≥ 30% to < 50%) and very severe (FEV1 < 30%).

Exclusion criteria comprised DM, other concomitant chronic diseases, hepatic or renal diseases, malignancy, endocrine disorders, pregnancy and acute infections. The use of other treatments, such as short/long acting beta agonists, anticholinergics, theophylline, and as-needed antibiotics, was unrestricted in this study because they have no known effect on calcium metabolism. Patients with a history of any other disease or under any other medication (including oral and parenteral corticosteroid treatment during the previous 6 months) that may have influenced phosphate metabolism were excluded from the study. All female patients were postmenopausal. Cigarette smoking status, age, body mass index (BMI), and the total cumulative doses of the medications were recorded for all patients. Diagnosis of COPD was based on previous history, radiologic features and standard measurements of pulmonary function tests criteria according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) Guidelines 2009 Update (4), as previously mentioned.

The following investigations were carried out for all subjects involved in the study: respiratory function tests performed for all subjects participating in the study according to a standard protocol described by the American Thoracic Society/European Respiratory Society (19) using Vmax29 Sensor Medics (VIASYS).

Venous blood samples were extracted from each subject after an overnight fast of 12 hours. Serum samples were analyzed for fasting serum glucose, urea, creatinine, albumin, total protein, sodium, potassium, chloride, alkaline phosphatase, liver enzymes

(ALT, AST), calcium, phosphorous, magnesium, iron and ferritin. These parameters were measured using a Siemens Diagnostics (Marburg GmbH, Germany) RxL Dimension Clinical Chemistry analyzer. Paired urine and blood samples were used to measure creatinine and phosphate levels and the results were utilized to calculate the maximum tubular absorption of phosphate, as described previously (20). 25-OH vitamin D was measured using the Liasion chemiluminescent immunoassay analyzer (Diasorin, Italy); the intra-assay coefficient of variation was 11.6 %. Serum-intact PTH level was measured using the Architect 2000i immunoassay analyzer (Abbott Diagnostics, IL, USA). An EDTA plasma sample was taken for FGF-23 testing. This was measured using a commercially available Elisa kit from PromoKine, Catalogue No: PK-EL-KI6000 (Bioscience alive), Heidelberg, Germany. The assay has a detection limit of 3.0 RU/mL. The intra-assay coefficient of variation (CV) was 5% at both 50.9 Ru/mL and 140 Ru/mL respectively, while the inter-assay CV was 5% at 50.9 and 7.3% at 153 Ru/mL.

Statistical analysis

Comparison among the groups was conducted using the Student's t-test for normally distributed variables. The nonparametric Mann-Whitney U test was used for variables with a non-Gaussian distribution. Normally distributed variables were expressed as mean ± SD, while variables with non-Gaussian distribution were expressed as median, range, and 25 to 75 percentiles. For correlation studies, the Pearson correlation test was used. A P value of <0.05 was considered statistically significant. The commercial statistical software package used was SPSS 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Pulmonary function data are summarized in *Table I*. Clinical and biochemical data of the studied groups are illustrated in *Table II*. There was no significant difference regarding age or blood pressure in the two groups. Similarly, there was no difference between the patient and control group regarding the serum level of corrected calcium, sodium, potassium, chloride, urea, creatinine, iron or ferritin (P>0.01). COPD patients had a significantly lower hemoglobin level (P=0.021) suggesting anemia due to a chronic disease state. The patient group had a significantly lower serum level of phosphate compared to the control group (P<0.01). Hypophosphatemic patients represented 34% of the COPD patient group. Both serum PTH and alkaline phosphate levels were significantly higher in the patient group compared to the control group (P values 0.009 and 0.000) respectively. However, serum vitamin D levels did not differ significantly between the patients and controls (P=0.575).

Plasma FGF-23 was significantly higher in the patient group compared to the control group (P= 0.000). Plasma FGF-23 Medians (range) were 280.5 (51–968) and 140 (21–200) in patients and controls respectively (*Figure 1a*).

On the other hand, maximum tubular absorption of phosphate was significantly reduced in COPD patients compared to the control group (P=0.04). TMP/GFR values (mean ±SD) were 0.84 ± 0.09 and 0.96 ± 0.26 mmol/L in patients and controls respectively (*Figure 1b*).

The lower limit of the reference range for serum phosphate of 0.7 mmol/L was used to stratify subjects as normophosphatemic and hypophosphatemic and the plasma FGF-23 levels were evaluated in both

Table I Respiratory function tests for COPD patients and controls.

Parameter	Controls	Patients	P value
Carbon Monoxide Diffusing Capacity	86.88 ± 3.6	83.44 ± 6.8	0.000
Total Lung Capacity, L	86.41 ± 3.6	88.30 ± 5.4	0.001
FEV1/FVC, %	82.32 ± 5.7	56.49 ± 7.5	0.013
FEV1, %	90.85 ± 3.9	65.33 ± 12.8	0.000
FVC, %	84.73 ± 3.4	84.77 ± 11.05	0.016
Lactate, mmol/L	0.39 ± 0.24	1.43 ± 0.63	0.003
Bicarbonate (HCO_3), mmol/L	23.75 ± 2.3	30.59 ± 4.39	0.03
Oxygen Saturation (SO_2), mmHg	97.18 ±1.5	92.08 ± 2.92	0.01
Partial arterial CO2 ($PaCO_2$), mmHg	41.24 ± 3.1	60.28 ± 10.32**	0.000
Partial arterial Oxygen (PaO_2), mmHg	95.88 ± 1.5	69.8 ± 14.23**	0.000
pH	7.38 ± 0.023	7.3 ± .04284**	0.002
FIO_2, %	21.0 ± 0.01	22.67 ± 5.09**	0.000

FIO_2: Fractionated inspired oxygen
FEV1: Forced Expiratory Volume (1st second)
FVC: Forced Vital Capacity
* P value < 0.05 significant difference between the controls and patient group
** P value< 0.01 highly significant

Table II Clinical and biochemical parameters of the studied groups.

Parameter	Controls (n: 34)	Patients (n:70)	P value
Age (years)	63.0 ± 4.60	62.6 ± 6.1	0.9
Males/females	30/14	47/23	0.1
BMI (kg/m^2)	32.8 ± 6.2	26.9 ± 3.6	0.02
Albumin (g/L)	42.21 ± 2.13	35.14 ± 4.83*	0.03
Corrected calcium (mmol/L)	2.13 ± 0.08	2.19 ± 0.12	0.1
Sodium (mmol/L)	139.53 ± 3.26	137.1 ± 3.54	0.73
Potassium (mmol/L)	4.12 ± 0.32	4.40 ± 0.45	0.1
Chloride (mmol/L)	102.47 ± 3.3	115.19 ± 2.5	0.2
BUN (mmol/L)	5.07 ± 1.19	5.25 ± 1.08	0.6
Creatinine (μmol/L)	83.21 ± 12.9	77.97 ± 12.96	0.7
Iron (μmol/L)	20.59 ± 4.2	8.55 ± 4.7	0.001
Ferritin (ng/mL)	138.0 (80.0–230.0)	123.0 (11.28–882.00)	0.7
Hemoglobin (g/L)	146.9 ± 11.947	133.0 ± 219*	0.021
Leukocytic count	5.57 ± 1.29	7.42 ± 2.9	0.002
Magnesium (mmol/L)	0.817 ± 0.151	1.97 ± 9.58	0.2
Phosphorous (mmol/L)	1.31 ± 0.22	0.92 ± 0.29*	0.01
ALP (U/L)	84.82 ± 22.15	118.90 ± 40.8**	0.009
25 OH Vitamin D (ng/mL)	10.41 ± 4.35	12.55 ± 4.18050	0.575
PTH (pg/mL)	46.15 (21.20–86.10)	69.90 (28.10–280.10)**	0.000

Data are presented as Mean ± SD for normally distributed variables.
SBP: Systolic blood pressure
DBP: Diastolic blood pressure
BMI: Body mass index
Hb: Hemoglobin
BUN: Blood urea nitrogen
ALP: Alkaline phosphatase
PTH: parathyroid hormone
* = Significant difference versus the control group (P<0.05)
** = Significant difference versus the control group (P<0.05)

groups (*Figure 1c*). Plasma FGF-23 levels were significantly increased in subjects with lower phosphate values compared to normophosphatemic subjects (P =0.000). FGF-23 values were 673 (278–968) median (range) and 173 (21–512) RU/mL in the hypophosphatemic and normophosphatemic group respectively.

In the patient group, as expected, plasma FGF-23 levels correlated negatively with serum phosphorous (r: –0.799 and P=0.000). Furthermore, plasma FGF-23 correlated negatively with FEV1 (r: –0.352 and P= 0.003) and serum albumin (r: –0.476 and P= 0.000).

On the other hand, both serum albumin and phosphate correlated positively with FEV1 (r: 0.338 and P=0.004 and r: 0.465 and P=0.000 respectively).

When the FGF-23 results for COPD patients were classified into mild (9 patients), moderate (53), severe (8 patients) and very severe (no patients) COPD according to the GOLD criteria, there was no significant difference between FGF-23 among the 3

groups (p>0.05) (data not shown). This may be due to the low number of patients included in the different groups, and the fact that all our patients were outpatients and none of them had very severe COPD (as defined by the GOLD criteria) that necessitated hospitalization.

Discussion

Respiratory and skeletal muscle weakness is considered a major factor in patients affected with chronic obstructive lung disease (COPD) (21). Improvement in muscle power was found to have a positive impact on improving the overall status of COPD patients (8). Phosphorous is an important electrolyte that plays a significant role in different physiological processes, especially muscle contraction, production of high energy phosphate bonds, and is also important for enzymes involved in different cellular mechanisms (22). Serum phosphorus levels are influenced by a recently discovered peptide FGF-23 (fibro-
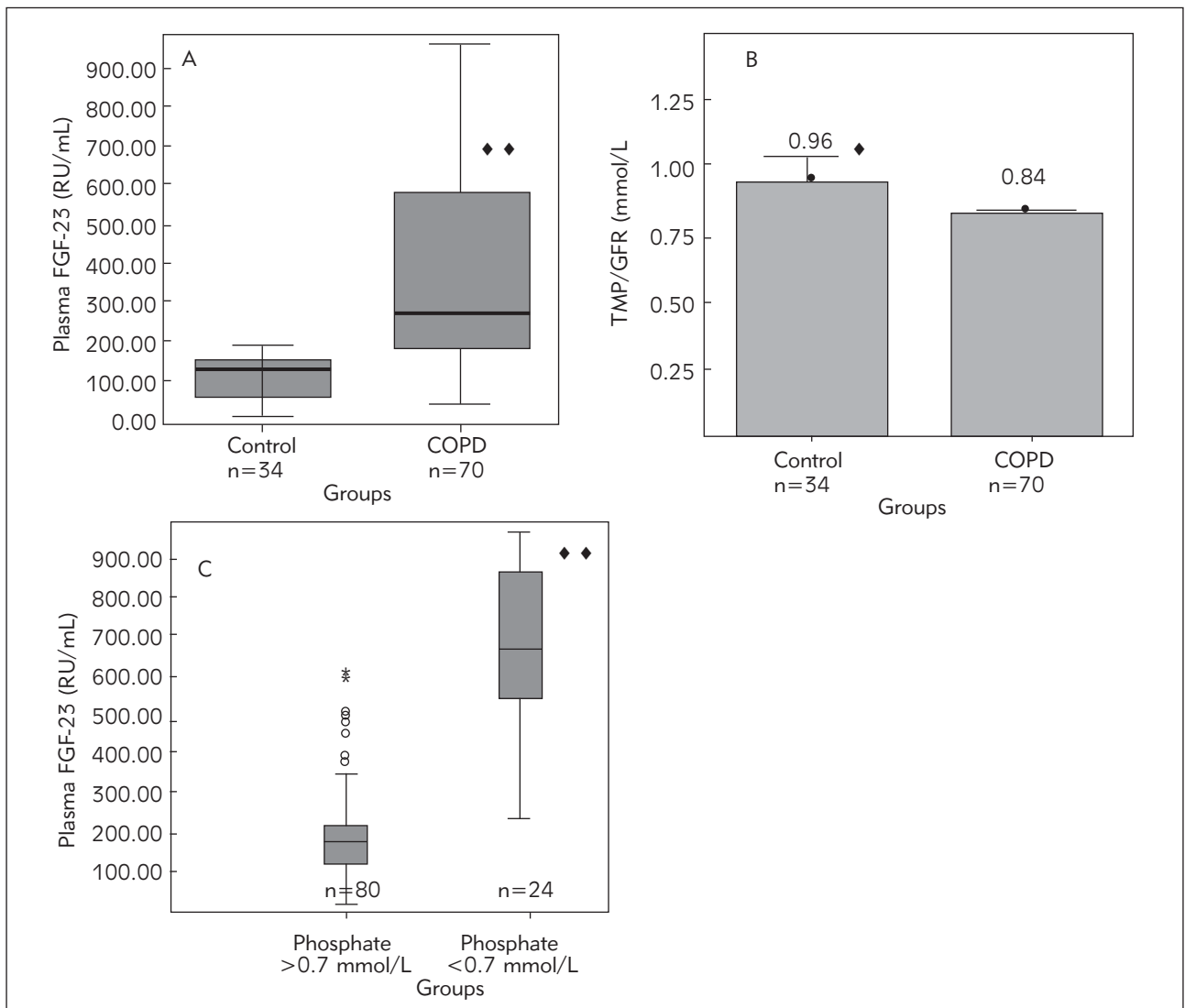
Figure 1 Fibroblast growth factor and tubular maximum absorption of phosphates in COPD patients and control subjects.
The box plots represent the interquartile ranges 25th–75th percentile. The whiskers above and below the box plots represent the minimum and maximum values. The line across the box plots represents the median. Figures above the bar represent the mean values.
n = number of subjects in each group.
♦ ♦: P Value<0.01
♦   : P value<0.05
0: Outliers: Values > the upper quartile and 1.5 times the interquartile range
*: Extreme: Values > the upper quartile and 3 times the interquartile range

blast growth factor-23) (23). To our knowledge, the relationship between serum phosphate and FGF-23 was not investigated before in COPD patients. The current study evaluated plasma FGF-23 and its possible relationship to different respiratory parameters in a cohort of patients affected with COPD compared to age and sex matched healthy control subjects.

In the current study a substantial percentage (34%) of the COPD patients had a low serum phosphate level, which may influence COPD severity. Different authors reported an increased incidence of hypophosphatemia in COPD (9). They attributed low

phosphate in COPD patients to different predisposing factors including: poor nutritional intake, medications, renal or intestinal excretion, and cellular redistribution (24). A recent study illustrated a widespread prevalence of undernutrition among COPD patients, which ranged between 4 and 49 percent (25). The current study found significantly increased plasma FGF-23 levels in COPD patients compared to controls. This elevated FGF-23 negatively correlates with FEV1, serum albumin and serum phosphorous in the patient group. Conversely, TMP/GFR was significantly lower in COPD patients than in healthy controls. These findings of elevated plasma FGF-23 levels

and low urinary TMP/GFR in COPD patients provide an additional explanation for the low serum phosphate detected in COPD. High FGF-23 levels exert a phosphaturic effect on the renal tubules increasing the loss of phosphate and resulting in reduced TMP/GFR values. Low serum phosphate levels may have a negative impact on respiratory muscle function with a subsequent decrease in FEV1.

The interesting finding of the current study is the strong negative correlation between FGF-23 and FEV1, serum albumin and phosphate. Reduced levels of both serum albumin and phosphate (in the absence of renal or liver disease) most probably reflect poor nutritional intake of both elements. This nutritional deficiency is manifested by the significantly decreased serum albumin and phosphorous values in COPD patients compared to healthy controls. As both albumin and phosphate play an important role in different biological processes within muscle cells, decreased levels of phosphate and albumin may result in impaired muscle function which is manifested by reduced FEV1 in our COPD patients group. The reduced FEV1 may not only be due to the possible poor nutritional intake of phosphate, but also due to increased plasma FGF-23 levels that exert a phosphaturic effect in COPD patients

Elevated plasma FGF-23 levels may be explained as a protective mechanism to the noxious irritant factors causing the COPD (e.g. cigarette smoking). Previous studies on FGF-23 deficient mice indicated an important protective role for FGF-23 to guard against emphysema. FGF-23 knockout mice developed spontaneous emphysema as part of a »rapid aging« phenotype that is potentially related to perturbations of vitamin D metabolism (26, 27). In the present study, the patients had marked vitamin D deficiency that may provide a contributory factor for the raised plasma FGF-23 in COPD patients, however

we do not think it is the sole factor because 25 OH vitamin D levels in the patients did not differ significantly from the 25 OH vitamin D levels in the control subjects. The decreased vitamin D levels in both groups could be explained by the widespread vitamin D deficiency in Saudi Arabia. Different groups including ours reported a high prevalence of vitamin D deficiency in Saudi Arabia (28, 29).

The current study also showed a significant increase in PTH and alkaline phosphatase levels when patients were compared to controls. This finding is most probably related to the low phosphate levels found in COPD patients.

Collectively, the results of our study illustrate a significant increase in plasma FGF-23 levels that may contribute to the low phosphate levels in the studied group of COPD patients. These elevated levels of FGF-23 correlated with reduced lung function in COPD patients as measured by FEV1 in our patient group.

Future work may involve the evaluation of FGF-23 and pulmonary function tests before and after phosphate supplementation in COPD patients. Furthermore, studies could evaluate other essential nutrients needed for muscle function (e.g. plasma carnitine) in patients with COPD.

Conflict of interest statement

The authors declare no conflict of interest. Dr Mohamed Elsammak was responsible for the lab work, writing the manuscript and statistical work. Dr Adel Attia was responsible for carrying out pulmonary function tests, collecting the clinical details and sampling from patients. Dr Moosa Soliman revised and corrected the final draft of the manuscript.

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