Evaluation of different biochemical markers and imaging modalities in type 2 diabetes mellitus patients with and without albuminuria

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Objective
The aim of this study was to evaluate the effects of albuminuria on different biochemical markers, different target organs, and subclinical atherosclerosis in patients with type 2 diabetes mellitus (T2DM).
Patients and methods
Sixty T2DM patients were divided into three equal groups according to their levels of albuminuria – namely, normoalbuminuria, microalbuminuria, and macroalbuminuria. Renal function tests, glycemic status markers, serum electrolytes, high-sensitivity C-reactive protein, fibroblast growth factor 23, vitamin D, intact parathyroid hormone, and fractional excretion of phosphate ($FePO_4$) were measured. Patients also underwent renal arterial duplex, Doppler echocardiography, and estimation of the carotid intima–media thickness.
Results
Blood urea nitrogen and creatinine clearance were significantly higher in patients with albuminuria. Fasting blood glucose, postprandial blood glucose, and glycosylated hemoglobin levels were significantly higher in patients with albuminuria. There were no statistically significant differences among the studied groups as regards serum electrolytes. Fibroblast growth factor 23 levels were significantly higher in patients with albuminuria. In patients with macroalbuminuria, vitamin D levels were significantly lower, whereas intact parathyroid hormone and high-sensitivity C-reactive protein levels were significantly higher. There were no statistically significant differences among the studied groups as regards $FePO_4$. There were no statistically significant differences between the studied groups as regards renal resistive indices, presence or absence of left ventricular hypertrophy, or carotid intima–media thickness. Left ventricular ejection fraction was significantly lower in patients with albuminuria.
Conclusion
In T2DM patients with albuminuria (especially macroalbuminuria), several markers of renal complications are elevated, denoting a high-risk population for the development of end-stage renal disease. Moreover, markers of asymptomatic left ventricular systolic dysfunction were observed, denoting a higher risk for cardiovascular morbidity and mortality.

Keywords:
albuminuria, diabetic nephropathy, fibroblast growth factor 23

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Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defect in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidney, nerves, heart, and blood vessels [1].

The chronic complications of diabetes are thought to be caused by an interaction between hyperglycemia, or other metabolic consequences of insulin deficiency, and independent genetic or environmental factors. Several potentially relevant biochemical sequelae to hyperglycemia have been identified in tissue susceptible to diabetic complications. The most common macrovascular complications are cardiovascular disease, cerebral vascular and peripheral vascular disease [2].

Hyperglycemia can promote vascular complications by multiple postulated mechanisms. It can stimulate

oxidative stress, which has been strongly implicated as a driving force in atherosclerosis by promoting formation of advanced glycation end products, protein cross-linking, and reactive oxygen species formation. In addition, inflammation has been strongly implicated in both atherosclerosis and type 2 diabetes mellitus (T2DM) [3].

There is an incidence of several complications with long-standing diabetes. Diabetes is associated with microvascular complications, such as nephropathy, retinopathy, and neuropathy, and macrovascular complications such as atherosclerosis and stroke. These complications occur in the late stages of diabetes and are chronic [4].

The central pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body [5].

Kidneys are one of the important organs that are involved in diabetes. Diabetes is the most common cause of end-stage renal disease in most parts of the world. Kidney involvement, both directly and indirectly, increases the involvement of other organs and increases morbidity and mortality in diabetic patients [6].

The peak incidence of nephropathy is 10–20 years after diabetes onset and then it occurs as a progressive decrease. Thus, if the patients have the illness for more than 30 years and are still normoalbuminuric, they are likely to have a decreased risk for diabetic nephropathy [7].

Hyperglycemia is a crucial factor in the development of diabetic nephropathy because of its effects on glomerular and mesangial cells, but alone it is not causative [8].

Glycosylation of tissue proteins contributes to the development of diabetic nephropathy and other microvascular complications [9].

T2DM is frequently associated with an inflammatory status; there is a cytokine-associated acute phase reaction, part of the innate immune response. Among several markers of inflammation, high-sensitivity C-reactive protein (hs-CRP) is found to be significant in people with diabetes and remained a significant predictor of diabetes risk [10].

Several novel biomarkers of kidney injury have been shown to increase in diabetes. These changes appear to occur even before microalbuminuria is established. Evidence is currently available, indicating that the following biomarkers may be useful in assessing early nephron injury in patients with diabetes. One of most important markers is fibroblast growth factor 23 (FGF23), and the kidney is the principal target for its action. The major function of this hormone is to regulate phosphate reabsorption and production of 1,25 $(OH)_2$ D [11].

Circulating 1,25 $(OH)_2$ D levels in response to excess FGF23 is due to the regulation of the anabolic and catabolic events through inhibition of 1α-hydroxylase and stimulation of 24-hydroxylase in the proximal tubules of the kidney [12].

Patients with Chronic Kidney Disease (CKD) typically exhibit secondary hyperparathyroidism associated with high serum FGF23 levels, which contradicts the ability of FGF23 to suppress parathyroid hormone (PTH) secretion [13].

Furthermore, low 1,25 $(OH)_2$ D and subsequent hypocalcemia could also be responsible for secondary hyperparathyroidism. Nonetheless, serum FGF23 correlates to PTH in predialysis CKD patients and in patients with early CKD when levels of phosphate and calcium are maintained within normal range. Taken together, these results suggest that FGF23 and PTH may form a regulatory loop similar to FGF23-vitamin D loop, but some of the FGF23–PTH associations could be attributed to alterations of other systemic and local factors [14,15].

Serum FGF23 was positively associated with left ventricular mass index and increased risk of having left ventricular hypertrophy (LVH). It is worth noticing that the associations between FGF23, vascular dysfunction, atherosclerosis, and LVH were all progressively strengthened in patients with a lower estimated glomerular filtration rate (eGFR) despite normal phosphate levels [16].

Most of the recent advances in the understanding of CKD-related cardiovascular disease have focused on atherosclerosis and arteriosclerosis, and much less effort has been dedicated to unveil and evaluate the mechanisms and the impact of interventions related to myocardial dysfunction. Hence, echocardiographic evaluation plays a pivotal role in establishing the diagnosis of myocardiopathy as well as in stratifying risk and defining the impact of interventions [17].

The screening for the detection of subclinical atherosclerosis in asymptomatic diabetic patients is the subject of considerable controversy. There are no prospective studies that support its usefulness and that can modify the natural history of those patients. Even today, there is no consensus on which tests should be performed [18,19].

Carotid intima–media thickness (CIMT) is considered one of the independent predictors of coronary artery disease, a marker of early atherosclerosis and vascular remodeling [20].

Patients and methods

Sixty patients with T2DM for more than 10 years were selected from the outpatient clinic of the Main University Hospital, Faculty of Medicine, Alexandria University. They were divided into three groups: 20 patients with normoalbuminuria (group I), 20 patients with microalbuminuria (group II), and 20 patients with macroalbuminuria (group III).

The protocol of this study was approved by the ethical committee of Alexandria Faculty of Medicine. All patients signed an informed written consent before enrollment into the study. The exclusion criteria were as follows: New York Heart Association (NYHA) class III or IV, heart failure or coronary artery disease, hospitalization in the last 3 months, and nondiabetic glomerulonephritis. All patients were subjected to thorough history taking, full clinical examination, routine laboratory examination in the form serum creatinine, blood urea nitrogen (BUN), estimated creatinine clearance using Cockcroft–Gault equation, fasting plasma glucose, 2-h postprandial glucose, hemoglobin A1c, serum calcium (total and ionized), serum phosphorus, calcium phosphorus product, serum electrolytes (sodium, potassium, and magnesium), and urinary albumin/creatinine ratio (UACR) in early morning urine sample.

Furthermore, they were tested for hs-CRP, plasma intact parathyroid hormone (iPTH) using chemiluminescence, fractional excretion of phosphate, serum intact FGF23 using the enzyme-linked immunosorbent assay technique, and serum vitamin D_3 (25-hydroxycholecalciferol) using liquid chromatography tandem mass spectroscopy.

Estimation of CIMT was carried out using Doppler ultrasound. Left ventricular systolic function and the presence of LVH were assessed using Doppler echocardiography, and renal arterial duplex ultrasound was carried out (using Philips EnVisor C ultrasound machine, Philips Healthcare USA, Bothell, WA). Statistical analysis was carried out using Predictive Analytics SoftWare (PASW) version 18.01 (IBM Company, Chicago, Illinois, USA).

Results

There were no statistically significant differences among the studied groups as regards age (F=0.374; P=0.69).

There was a statistically significant difference among the studied groups as regards weight (F=3.966; P=0.024) between group I and group II (P_1=0.016), between group I and group III (P_2=0.02), but not between group II and group III (P_3=0.921).

As regards BUN, there were no statistically significant differences among the studied groups (F=2.906; P=0.063). There was no statistically significant difference between groups I and II (P_1=0.984). There was a statistically significant difference between groups I and III (P_2=0.04) and between groups II and III (P_3=0.042).

There were no statistically significant differences among the studied groups (F=1.96; P=0.15) for serum creatinine.

There were no statistically significant differences in creatinine clearance among the studied groups (F=2.838; P=0.67). There was no statistically significant difference between groups I and II (P_1=0.15). There was a statistically significant difference between groups I and III (P_2=0.022) but not between groups II and III (P_3=0.37).

As regards fasting blood glucose (FBG), there were statistically significant differences among the studied groups (F=7.83; P=0.001). There was no statistically significant difference between groups I and II (P_1=0.166). There was a statistically significant difference between groups I and III (P_2<0.001) and between groups II and III (P_3=0.015).

There were statistically significant differences among the studied groups in postprandial blood glucose (PPBG) (F=5.656; P=0.006). There was no statistically significant difference between groups I and II (P_1=0.182). There was a statistically significant difference between groups I and III (P_2=0.001) but not between groups II and III (P=0.51).

There were statistically significant differences among the studied groups in HbA1c (F=7.109; P=0.002). There was no statistically significant difference between groups I and II (P_1=0.14). There was a statistically significant difference between groups I and III (P_2<0.001) and between groups II and III (P_3=0.028).

There were statistically significant differences among the studied groups in UACR (F=418.09; P<0.001). There were statistically significant differences between groups I and II (P_1<0.001), between groups I and III (P_2<0.001), and between groups II and III (P_3<0.001).

There were no statistically significant differences among the studied groups as regards serum sodium (F=1.608; P=0.209), serum potassium (F=0.368; P=0.694), or serum magnesium (F=1.162; P=0.32).

There were no statistically significant differences among the studied groups as regards total serum calcium (F=1.112; P=0.336), serum ionized calcium (F=1.618; P=0.318), serum phosphorus (F=1.862; P=0.165), or Ca×P product (F=0.416; P=0.662).

There were no statistically significant differences among the studied groups in hs-CRP (χ^2=5.582; P=0.061). There was no statistically significant difference between groups I and II (P_1=0.126). There was a statistically significant difference between groups I and III (P_2=0.02) but not between groups II and III (P_3=0.457).

There were statistically significant differences among the studied groups in vitamin D level (F=4.694; P=0.015). There was no statistically significant difference between groups I and II (P_1=0.978). There were statistically significant differences between groups I and III (P_2=0.014) and between groups II and III (P_3=0.009).

There were no statistically significant differences among the studied groups as regards serum iPTH (χ^2=5.438; P=0.066). There was no statistically significant difference between groups I and II (P_1=0.099). There was a statistically significant difference between groups I and III (P_2=0.037) but not between groups II and III (P_3=0.279).

There were statistically significant differences among the studied groups as regards serum FGF23 (χ^2=8.86; P=0.012). There were statistically significant differences between groups I and II (P_1=0.03) and between groups I and III (P_2=0.006), but not between groups II and III (P_3=0.291).

There were no statistically significant differences among the studied groups as regards fractional excretion of phosphate ($FePO_4$) (χ^2=2.675; P=0.263).

There were no statistically significant differences among the studied groups (χ^2=3.534; P=0.204) as regards LVH.

As regards left ventricular ejection fraction, there was no statistically significant difference between groups I and II (P_1=0.457). There were statistically significant differences between groups I and III (P_2=0.03) and between groups II and III (P_3=0.02).

There were no statistically significant differences among the studied groups as regards right CIMT (F=0.834; P=0.44) or left CIMT (F=1.791; P=0.176) (Table 1).

There were no statistically significant differences among the studied groups as regards right renal resistive index (F=1.716; P=0.189) or left renal resistive index (F=0.523; P=0.596).

There was a significant positive correlation (r=0.342; P=0.008) between FGF23 and UACR. Moreover, there was a significant negative correlation (r=-0.619; P<0.001) between FGF23 and creatinine clearance using Cockcroft–Gault equation. There was a significant negative correlation between FGF23 and creatinine clearance in the microalbuminuria group (r=-0.528; P=0.017) and the macroalbuminuria group (r=-0.734; P<0.001). A significant positive correlation (r=0.260; P=0.045) was found between FGF23 and BUN. In the macroalbuminuria group, a significant positive correlation (r=0.461; P=0.041) was found between FGF23 and BUN. There was a significant positive correlation (r=0.505; P=0.023) between FGF23 and serum creatinine in the macroalbuminuria group only. There was also a significant positive correlation (r=0.343; P=0.007) between FGF23 and FBG and a significant negative correlation (r=-0.288; P=0.026) between FGF23 and HbA1c. Moreover, a significant negative correlation was found between FGF23 and HbA1c in the normoalbuminuria group (r=-0.642; P=0.002) and in the macroalbuminuria group (r=-0.834; P<0.001).

There was a significant positive correlation (r=0.422; P=0.001) between FGF23 and $FePO_3$. Similarly, a significant positive correlation between FGF23 and $FePO_3$ was found in the normoalbuminuria group (r=0.69; P=0.001) and the macroalbuminuria group (r=0.567; P=0.009). There was a significant negative correlation (r=-0.386; P=0.002) between FGF23 and

Table 1 Difference in studied parameters between groups I (normoalbuminuria), II (microalbuminuria), and III (macroalbuminuria) and their statistical significance

	Group I	Group II	Group III	*P* values
Age [mean (SD)]	48.25 (6.2)	47.75 (7.03)	49.5 (6.53)	0.69
Sex (M/F)	8/12	9/11	11/9	0.724
Weight [mean (SD)]	83 (5.44)	78 (5.69)	78.2 (7.7)	0.024
BUN [mean (SD)]	19.97 (3.5)	20 (5.34)	23.16 (5.37)	0.063
Serum creatinine [mean (SD)]	1.58 (0.16)	1.57 (0.16)	1.66 (0.15)	0.15
CrCl [mean (SD)]	60.87 (5.14)	57.76 (7.22)	55.83 (7.61)	0.067
FBG [mean (SD)]	136.17 (26.47)	151.6 (31.68)	179.17 (43.94)	0.001
PPBG [mean (SD)]	211.9 (65.54)	237.85 (50.24)	276.18 (65.36)	0.006
HbA1c [mean (SD)]	7.7 (1.45)	8.38 (1.48)	9.4 (1.39)	0.002
Na [mean (SD)]	144.85 (4.85)	145.55 (3.76)	143.45 (2.74)	0.209
K [mean (SD)]	4.53 (0.44)	4.53 (0.47)	4.63 (0.28)	0.694
Mg [mean (SD)]	1.95 (0.16)	1.90 (0.19)	1.86 (0.19)	0.32
Total Ca [mean (SD)]	9.47 (0.85)	9.2 (0.74)	9.53 (0.61)	0.336
Ionized Ca [mean (SD)]	4.95 (0.48)	4.79 (0.4)	4.79 (0.4)	0.318
Phosphorus [mean (SD)]	3.88 (0.43)	3.99 (0.43)	3.71 (0.54)	0.165
Ca×P [mean (SD)]	39.69 (5.16)	36.67 (4.61)	35.38 (5.84)	0.622
FGF23 [mean (SD)]	37.58 (48.21)	62.61 (49.33)	78.83 (51.84)	0.012
Vitamin D [mean (SD)]	16.76 (8.07)	16.82 (6.33)	10.2 (3.36)	0.015
iPTH [mean (SD)]	46.13 (29.85)	50.05 (15.78)	54.15 (17.72)	0.066
hs-CRP [mean (SD)]	5.7 (3.58)	7.92 (4.15)	9.34 (5.34)	0.061
$FePO_4$ [mean (SD)]	35.55 (9.02)	38.53 (11.72)	34.02 (9.83)	0.263
RI (Rt) [mean (SD)]	0.67 (0.05)	0.68 (0.04)	0.7 (0.05)	0.189
RI (Lt) [mean (SD)]	0.68 (0.06)	0.67 (0.04)	0.68 (0.06)	0.596
CIMT (Rt) [mean (SD)]	7.25 (1.71)	7.6 (2.01)	7.95 (1.36)	0.44
CIMT (Lt) [mean (SD)]	7.65 (1.57)	7.45 (1.64)	8.3 (1.22)	0.176
LVH (%)	10	25	35	0.204
LVEF [mean (SD)]	64.85 (6.09)	63.56 (4.66)	59.8 (4.27)	0.007

BUN, blood urea nitrogen; Ca, calcium; Ca×P, calcium phosphorus product; CIMT, carotid intima–media thickness; CrCl, creatinine clearance; F, female; FBG, fasting blood glucose; $FePO_4$, fractional excretion of phosphate; FGF23, fibroblast growth factor 23; hs–CRP, high-sensitive C–reactive protein; iPTH, intact parathyroid hormone; K, potassium; Lt, left; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; M, male; Mg, magnesium; Na, sodium; PPBG, postprandial blood glucose; RI, resistive index; Rt, right.

the left ventricular ejection fraction. There was a significant positive correlation (r=0.376; P=0.003) between FGF23 and the resistive index of the right renal artery.

There was a significant positive correlation (r=0.303; P=0.019) between PTH and UACR. There was also a significant positive correlation (r=0.408; P=0.001) between PTH and FBG. Moreover, a significant positive correlation (r=0.333; P=0.009) was found between PTH and PPBG.

There was a significant negative correlation (r=–0.447; P=0.004) between vitamin D and UACR. There was also a significant negative correlation (r=–0.38; P=0.015) between vitamin D and FBG. Furthermore, a significant negative correlation (r=–0.335; P=0.035) was found between vitamin D and PPBG.

There was a relation between LVH and age in the total sample. There was also a relation between LVH and PTH in the macroalbuminuria group only.

Discussion

Several studies have shown the correlation between the serum levels of FGF23 and creatinine, phosphate, and PTH in patients with nondialysis CKD. The level of that hormone increases as renal function decreases. Even in patients with a glomerular filtration rate higher than 80 ml/min, FGF23 levels correlate negatively with 1,25 $(OH)_2$ D_3 and phosphate tubular reabsorption [14,21–23].

Among patients with established CKD, elevated levels of the phosphate-regulating hormone, FGF23, are independently associated with a higher risk for end-stage renal disease, LVH, cardiovascular disease, and mortality [24–27].

In our study, albuminuria (especially macroalbuminuria) was associated with a statistically significant increase in BUN, markers of glycemic status, hs-CRP, FGF23, and iPTH. It was also associated with a statistically significant decrease in creatinine clearance, vitamin D, and left ventricular ejection fraction. The presence of

albuminuria correlated well with BUN, HbA1c, FGF23, and iPTH.

Fliser and colleagues, in a study similar ours, enrolled patients with T2DM and macroalbuminuric diabetic nephropathy. The inclusion criteria were as follows: diabetes mellitus for more than 5 years and proteinuria above 500 mg/day. The exclusion criteria were as follows: serum creatinine above 2.5 mg/dl; serum potassium above 5.5 mEq/l; allergy or intolerance to Angiotensin Converting Enzyme inhibitor (ACEi) or Angiotensin Receptor Blocker (ARB); use of ARB in the last 3 months; class III or IV heart failure or angina; hospitalization in the last 3 months; pregnancy; ongoing chemotherapy; and hematuria or any clinical or laboratorial findings suggestive of associated nondiabetic glomerulopathy. Serum FGF23 showed a significant association with proteinuria, serum creatinine, urinary fractional excretion of phosphate, male sex, and race. It was also inversely and significantly related to estimated and measured creatinine clearance, serum albumin, and glycated hemoglobin. Interestingly, FGF23 was not related to serum calcium, phosphorus, 25 (OH) vitamin D, iPTH, or 24-h urinary phosphorus in this population. In this analysis, FGF23 was significantly related only to estimated creatinine clearance, phosphate fractional excretion, and serum iPTH. A trend of an association was seen with proteinuria, and no relationship was observed among FGF23 and glycated hemoglobin, phosphorus, or 25 (OH) vitamin D. Their data suggest that serum FGF23 is related to the risk for CKD progression in macroalbuminuric diabetic nephropathy. This relationship remained even after adjustments for the main confounding variables, such as sex, race, renal function, proteinuria, and intact PTH level. Similar data had already been shown in nondiabetic kidney disease [28].

Joachim *et al.*, [29] in the Heart and Soul study, studied 792 outpatients with stable cardiovascular disease and normal kidney function to moderate CKD. They evaluated the associations of eGFR and albumin-to-creatinine ratio (ACR) with plasma FGF23 concentrations. They found that mean FGF23 concentrations were higher in those with lower eGFR of 60–89 ml/min/1.73 m^2 in models adjusted for age, sex, race, ACR, blood pressure, diabetes, and BMI. More advanced decrements in eGFR were associated with much higher FGF23 concentrations. Compared with participants with ACR less than 30 mg/g, mean FGF23 concentrations were higher in those with ACR 30–299 mg/g in models adjusted for identical covariates plus eGFR and much higher in individuals with ACR of at least 300 mg/g. Spline analysis demonstrated a linear relationship of ACR with FGF23, independent of eGFR, even among persons with ACR less than 30 mg/g. Participants with eGFR-Cr between 60 and 89 and eGFR less than 60 had significantly higher FGF23 levels compared with participants with eGFR-Cr of at least 90 ml/min/1.73 m^2. Compared with participants with ACR less than 30 mg/g, those with ACR 30–299 mg/g and with ACR greater than 300 mg/g also had higher FGF23 levels in models adjusted for similar covariates [29].

Conclusion

In T2DM patients, the presence of albuminuria (especially macroalbuminuria) identifies a high risk population for the development of renal and cardiovascular complications. This may entail a more aggressive approach toward treatment regimens and a tighter control of other risk factors such as hypertension, cigarette smoking, and dyslipidemia.

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Conflicts of interest

There are no conflicts of interest.

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