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# Fibroblast Growth Factor 23 Can Serve as an Early Biomarker of Type 2 Diabetic Nephropathy Progression

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**Abstract**

Fibroblast growth Factors (FGFs) are multifunctional proteins with a wide variety of effects. (1) Aim: The aim of the present study was to examine FGF23 levels in patients with type II diabetic nephropathy (DN) and its correlation with bone metabolism biomarkers (2) Methods-material: Demographic, history, clinical examination and laboratory data were recorded from 80 patients with type II diabetes mellitus and diabetic nephropathy, 31 patients with type II diabetes mellitus and normal renal function and 31 healthy volunteers. (3) Results: GFR in negatively related to FGF23 levels, especially in the progression form early (I, II) to late stage (II, IV) of diabetic nephropathy, while a moderate relation between 1.25$(OH)_2D_3$ and FGF23 was found only in stage III and IV of diabetic nephropathy. Weak or no correlation was noticed among other parameters. (4) Conclusions: FGF23 is related to early progression of diabetic nephropathy and to markers of bone metabolism in stage III and IV of DM related chronic renal disease. The latter is not valid for patients with DM with normal renal function. Future research is needed to clarify FGF 23 role as prognostic and therapeutic index.

**Keywords**: fibroblast growth factor 23, diabetic nephropathy.

## 1. Introduction

Fibroblast growth Factors (FGFs) are multifunctional proteins with a wide variety of effects. Today, FGFs (over 22) are classified as intracrine, paracrine, and endocrine FGFs by their action mechanisms (Itoh et al., 2015:154).

FGG23 belongs structurally to FGF family and specifically to endocrine FGFs which include FGF19, FGF21, and FGF23. The latter is a 32KD protein (251 amino acids), encoded in FGF23 gene, which in turn is located in chromosome 12p13 and composed by three exons. However, it is functionally included in a group of hormones that regulate phosphorus metabolism called phosphatonins (Amin, 2014).

Several tissues express FGF-23, such as bone tissue, bone marrow vessels, ventrolateral thalamic nucleus, thymus, and lymph nodes. Its principal target is kidney, where it regulates phosphate reabsorption and production of 1,25$(OH)_2D_3$ (Liu et al., 2007:18)

In the past decade, FGF23 has emerged as a possible marker (both diagnostic and prognostic) and therapeutic target in several conditions: hereditary diseases such as a) syndromes

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of FGF23 excess and syndromes of FGF23 deficiency; and b) hypophosphatemic and hyperhosphatemic disorders, acute renal failure and chronic kidney disease (CKD), stoke and subarachnoid hemorrhage, several types of neoplasm and psoriasis (Kendrick J ,2011;11).

High FGF23 levels in patients with CKD are related with progression to ESRD, cardiovascular disease, transfusion needs, infection susceptibility and death (Myrou et al.,2016:16; Tsai et al.,2016:95). In patients with diabetes mellitus (DM), FGF23 has been proposed as possible new marker for gestational DM (Tuzun et al. 2018:62) and has been positively related to resistin in patients with Type II DM (T2DM) (Nakashima et al.,2018:8; Reyes-Garcia ,2014:37, National Kidney Foundation. 2012:60). A previous report relates serum FGF23 to bone metabolic disease and preclinical vascular disease in Type II DM patients (Ketteler et al.2017:92).

The aim of the present study was to examine FGF23 levels in patients with type II diabetic nephropathy (DN) and its correlation with bone metabolism biomarkers [25(OH)D3, 1,25$(OH)_2D_3$, parathormone, calcium, phosphorus, alkaline phosphatase].

## 2. Materials and Methods

This prospective study was performed in the 1st Propaedeutic Clinic of Internal Medicine at "AHEPA" University Hospital, Thessaloniki, Greece. It was part of a thesis project (Reference No 3377/Academic Year 2017-2018, National Archive of PhD Theses No 44124), approved by AHEPA General University Hospital Research Committee.

In total, 140 persons were included. Eighty (80) patients with T2 DM and renal disease were divided into two groups, depending on the stage of the renal disease, i.e. Group 1 (n1=48): stage I and II DN and Group 2 (n2=32): stage III and IV DN. Another sixty-two (62) volunteers served as control groups: Group 3 (n3=31), patients with T2 DM and normal renal function; and Group 4 (n4=31), healthy individuals. Diagnosis and staging of CKD were in accordance with National Kidney Foundation Disease Outcome Quality Initiative guidelines12-13. All participants were informed about the study's goal and procedures.

Exclusion criteria were: other than T2 DM type of diabetes (e.g. Type 1 DM, drug-induced, pregnancy-related, pancreas diseases related, etc.),presence of acute renal failure, chronic renal failure in renal replacement therapy, hepatic failure, cardiac failure, malignancies, women in reproductive age, thyroid gland disease, systemic inflammatory disease or immunosuppression state.

Demographic, history and clinical examination data were recorded. Creatinine clearance was estimated via 24h urine collection and Glomerular Filtration Ration (GFR) measurement was performed via 51Cr-EDTA (ethylenediamine tetra acetic acid) method. Microalbuminuria (Microalb) level was considered as the average between two 24h urine measurements, collected 10day apart from each other. In case of large difference between the two results, a third urine collection was ordered. FGF23 examination was carried out with sandwich ELISA (Enzyme-linked Immunosorbent Assay) method (ELISA Kits, AMS Biotechnology Ltd®, UK) and the rest of parameters (parathyroid hormone-PTH, serum Phosphorus – P, calcitriol -1,25 $(OH)_2D_3$, calcifediol-25,$(OH)D_3$ , alkaline phospatase- ALP and total serum Calcium – Ca) was performed in biochemistry analyst.

Data analysis was performed with SPSS v.21 (IBM® Corp. Armonk, NY, USA) and included initially descriptive statistics analysis, followed by Kolmogorov-Smirnov and Shapiro-Wilk normality tests. After that, multiple comparisons among the 4 groups was carried out via non-parametric Kruskal-Wallis technique; while in case of significant difference ($p<0.05$), further post-hoc analysis was performed. Finally, relation between parameters was examined.

## 3. Results

Main demographics parameters are displayed in Table 1, while descriptive statistics of the measured parameters are shown in Table 2.

**Table 1.** Selected demographic characteristics in the four groups. BMI – Body Mass Index, DN-Diabetic Nephropathy. (*p > 0.02)

| | Sex (No of male/female) | Age* (mean/standard deviation) | BMI (mean/standard deviation) |
|---|---|---|---|
| Group I (DNI, DNII) | 27/21 | 63 (7) | 30(4) |
| Group II (DNIII, DNIV) | 19/13 | 66.72(6.60) | 27.9(4) |
| Group III (DM) | 11/20 | 64.77(5.31) | 31.31(6.25) |
| Group IV (Healthy) | 13/18 | 60.68(6.11) | 28.62(5.1) |

**Table 2.** Descriptive statistics, in the form of mean (standard deviation), of the measured parameters

| Parameter | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| ClCr (ml/min) | 92.33(23.93) | 34.96(14.96) | 123.01(14.93) | 124.61(18.59) |
| Ca (mg/dl) | 9.26(0.45) | 8.66(0.54) | 9.37(0.64) | 9.42(0.47) |
| P (mg/dl) | 3.92(0.43) | 4.92(1.05) | 3.64(0.5) | 3.55(0.5) |
| ALP (IU/l) | 77.46(25.78) | 88.00(20.27) | 68.71(16.07) | 59.74(14.42) |
| PTH (pmol/l) | 8.08(4.11) | 18.41(8.15) | 4.70(1.13) | 3.99(1.41) |
| Microalb (mg/l) | 1423.88 (2218.53) | 4235.47 (2294.08) | 9.29(5.85) | 7.74(6.02) |
| $25(OH)D_3$ (ng/ml) | 20.67(7.64) | 11.86(3.6) | 40.63(11.24) | 50.98 (16.1) |
| $1.25(OH)_2D_3$ (pg/ml) | 14.56(3.55) | 9.51(3.33) | 43.33(10.53) | 52.45(10.31) |
| FGF23 (ng/ml) | 1.00(0.27) | 2.55(1.42) | 0.49(0.08) | 0.44(0.05) |

Kruskal-Wallis test results for all measured parameters and age, revealed significance $p<0.05$, while the results (p) of the post analysis are displayed in Table 3*:*

Relation between change of parameters revealed weak negative relation between $25(OH)D_3$ and FGF23 (Kendall $\tau_b = -0.377$, $p < 0.01$) in Group I, a moderate relation between $1.25(OH)_2D_3$ and FGF23 ($\tau_b=-0.631$, $p < 0.01$) in Group II and a weak negative relation $1.25(OH)_2D_3$ and FGF23 ($\tau_b = -0.453$, $p < 0.01$) Group IV. The rest of the results were either non-significant or without correlation.

**Table 3.** Adjusted significance (p) in post-hoc comparisons among groups. Group I: DNI+DNII, Group II: DNIII+DNIV, Group II: DM and Group IV: Healthy volunteers.

| Parameter | Group IV-Group I | Group IV-Group III | Group IV-Group II | Group I-Group III | Group I-Group II | Group III-Group II |
|---|---|---|---|---|---|---|
| Age | 0.13 | 0.31 | 0.01 | 1.00 | 1.00 | 1.00 |
| ClCr | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.97 |
| Ca | <0.001 | <0.001 | <0.001 | 1.00 | 1.00 | 1.00 |
| P | 1.00 | 0.055 | <0.001 | 0.33 | <0.001 | <0.001 |
| ALP | 0.29 | 0.007 | <0.001 | 1.00 | 0.005 | 0.06 |
| PTH | 1.00 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Microalb | 1.00 | <0.001 | <0.001 | <0.001 | <0.001 | 0.004 |
| $25(OH)D_3$ | 0.002 | <0.001 | <0.001 | <0.001 | <0.001 | 0.97 |
| $1.25(OH)_2D_3$ | 0.006 | <0.001 | <0.001 | <0.001 | <0.001 | 0.89 |
| FGF23 | 1.00 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Calculated Kendal $\tau_b$ between FGF23 and the others parameters in all Groups reveals a moderate relation with ClCr ($\tau_b$ = -0.584, p < 0.001), PTH ($\tau_b$ = 0.583, p < 0.01) , Microalb ($\tau_b$ = 0.653, p < 0.01), 25(OH)$D_3$ ($\tau_b$ = -0.667, p < 0.01) and 1.25$(OH)_2D_3$ ($\tau_b$ = -0.697, p < 0.01).

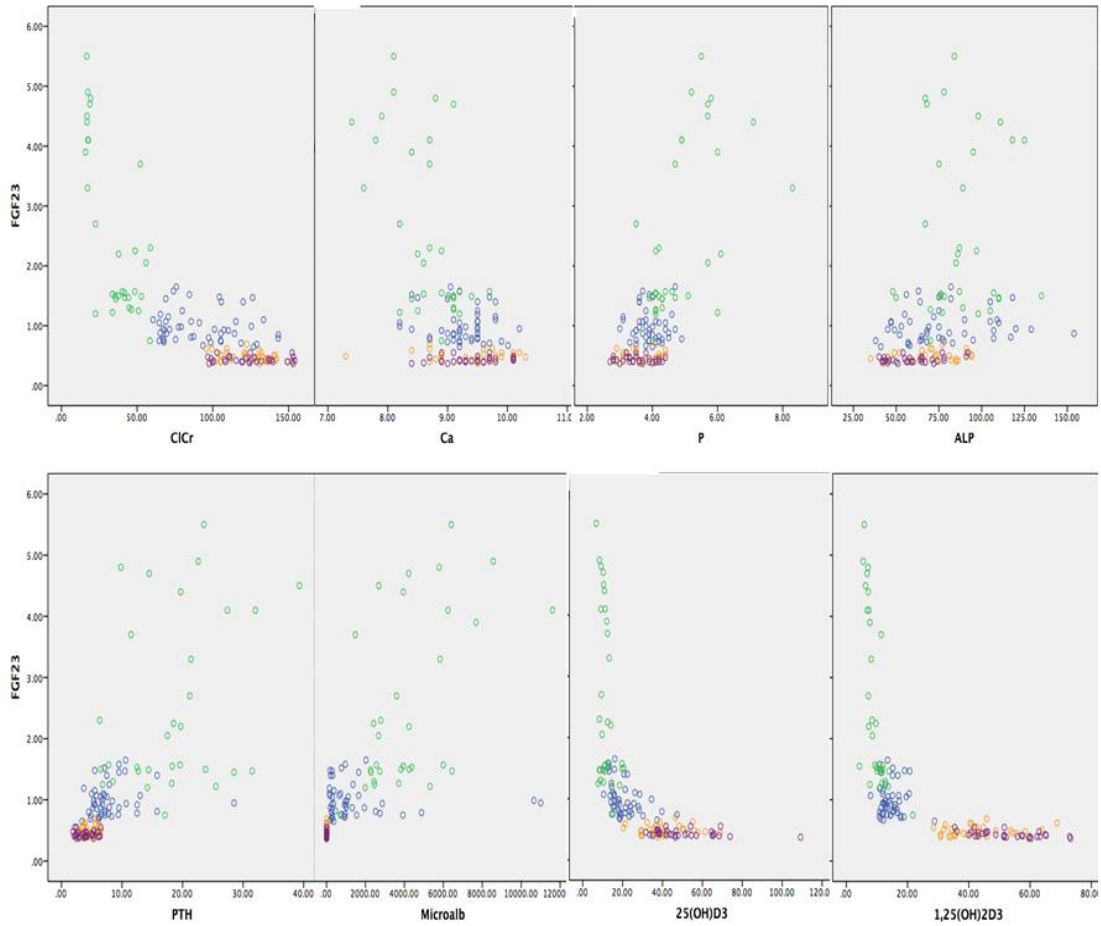


**Fig. 1.** Scatter plot of FGF23 and other parameters, in all four Groups (blue-Group I, green- Group II, orange-Group III, purple-Group IV)

## 4. Discussion

From the above as GFR decreases, FGF23 levels increase; the relation is increasingly expressed in stage III and IV DN. Moreover, in these two stages, there is also a weak positive relation between microalbuminuria and FG23. Previous reports have also supported that FGF-23 is a significant independent predictor of renal outcome in patients with macroalbuminuric DN (Titan et al., 2011: 6). The rest of the results seem also to agree with the available literature data.

Recently, there is ever growing research interest about the subject and new data, confirming the present study, are becoming available. In a similar study with 30 type II DM normoalbuminuric patients and 30 sex and age matched healthy individuals as a control group, negative correlation was found between FGF23 and GFR (El-Saeed et al., 2017: 24). Moreover, higher concentration of FGF-23 reduced the odds of early nephropathy in patients with type 2 DM, in comparison with those in more advanced nephropathy (Farías-Basulto et al., 2018: 49).On the contrary, a recent report suggests that other biomarkers, such as tumor necrosis factor receptor 1 (TNFR1), kidney injury molecule-1 (KIM-1) and 3) urinary markers: albumin/creatinine ratio (ACR) may be better indices of renal function decline (Nowak et al., 2018: 93).

Positive correlation was recorded between FGF21 and FGF23 and between each of them and other biochemical parameters, such as cholesterol, triglycerides, LDL cholesterol, creatinine, and urinary albumin excretion (Farías-Basulto et al.,2018: 49). Other studies report strong positive correlation was found between soluble Klotho (s-Klotho) levels and FGF23 levels in DN (Nowak et al., 2018: 93; Inci et al., 2016: 20; Inci et al., 2016: 64). In the same type of patients (DN) FGF23

levels is related also to diastolic dysfunction (Dogan et al., 2016: 48). However, there is no data of which FGF23 increase could predict the presence of diastolic dysfunction (Silva et al., 2019: 20). Also, there is no direct feedback loop between volume status and FGF-23 in hypertension or DN (Humalda et al., 2016: 95); even though that in early stages of CKD, FGF23, as well as lower magnesium levels were significantly and independently associated with higher pulse pressure levels, an established biomarker of cardiovascular morbidity and mortality (Fragoso et al., 2014: 7).

In the present study we did not asses the time course of the markers in interest. Previous studies reported that in the absence of CKD, parathyroid hormone increases earlier than FGF23 when the estimated GFR decreases. The increase in FGF23 is closely associated with a decrease in $1.25(OH)_2D_3$ (Dhayat et al., 2016:20). In early DN though, we cannot claim weather this is also valid.

Finally, the present study did not asses the effect of therapy to FGF23. Yet, there are data that support the negative relation between the former and angiotensin-II receptor blocker therapy (Nowak et al., 2018:93).

## 5. Conclusion

FGF23 is related to early progression of diabetic nephropathy and to biomarkers of bone metabolism in stage III and IV of DM related chronic renal disease. The latter is not valid for patients with DM with normal renal function. FGF23 levels increase in diabetic nephropathy stage I and II DN, thus serving as a possible prognostic biomarker. However, since DN progression is a multifactorial process further research is needed to clarify its role as independent or as embedded in a prognostic model marker and as a therapeutic target.

## 6. Acknowledgments

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## 7. Conflicts of Interest

The authors declare no conflict of interest.

**Author Contributions:** Conceptualization, A.M.,T.D and D.G..; methodology, A.M,T.D,C.S,A.C..; formal analysis, T.A; investigation, A.M,T.A.; resources, A.M,T.A..; data curation, T.A..; writing—original draft preparation, T.A.,A.M..; writing—review and editing, T.A..; visualization, T.A; supervision, A.C.,C.S.,T.D, D.G.; project administration, A.C.

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